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Spatio-temporal modelling of stable isotopes in tree Mediterranean species (Quercus ilex L. and Pinus Halepensis Mill.): a climatic and ecophysiological view

Jorge del Castillo Díaz

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Spatio-temporal modelling of stable isotopes in tree Mediterranean species (*Quercus ilex* L. and *Pinus halepensis* Mill.): a climatic and ecophysiological view

PhD thesis *presented by* ***Jorge del Castillo Díaz*** *to pursue the Doctorate degree by the University of Lleida (Spain)*

The current work has been supervised by Dr Juan Pedro Ferrio Díaz (Dept. Crop and Forest Sciences, University of Lleida)

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Abstract

Trees hold important secrets that may be essential in order to face the unprecedented current environmental challenges. However, the information that we are able to obtain from them is still poorly understood, mainly because of the complexity of forest systems, from individuals to ecosystems. As in other environmental disciplines, to understand the information from trees it is indispensable to have a spatiotemporal, multi-scale and multidisciplinary approach. Current technological and scientific advances offer new research avenues to study forest ecophysiology and climatic inference from wood material. The basis of this thesis is to use a combination of modern tools such as stable isotopes, Geographical Information Systems (GIS), Point Process statistics to retrieve climatic and ecophysiological information from forests at different spatial and temporal scales. We focus on two typical coexisting Mediterranean species: holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill.). The thesis can be divided into two blocks working on different spatial (regional vs stand level) and temporal (annual or multiannual vs seasonal) scales. **At the regional scale**, we used two networks of carbon isotope discrimination ($\Delta^{13}C$) in tree-ring from Aleppo pine and holm oak in the northeastern part of the Iberian Peninsula to: **1)** generate carbon isotope landscapes (*isoscapes*) of each species, which correlated well with precipitation patterns, leading to annual precipitation maps that were successfully validated with real data; **2)** to evaluate the spatial and temporal variability of $\Delta^{13}C$, and its relationship with precipitation, radial growth (RG) and satellite vegetation index NDVI in Aleppo pine, showing that annual precipitation drives $\Delta^{13}C$, RG and NDVI, but the three variables hold complementary information. **At the local scale**, we focus on a mixed forest stand in which both species coexist. By combining water isotopes and point process statistics: **1)** we could interpret tree-to-tree interactions in terms of water use, and found that under severe drought there is hydrological niche segregation between pines and oaks; **2)** we explored the seasonal variations in water uptake in order to see whether competition patterns are dynamic, or niche partitioning is always present due to morphological differences like root depth. Results suggest that both cases are true. We conclude that increasing drought may decrease the room for complementarity between the two species in mixed forest stands, thus compromising the stability and sustainability of the system.

Resumen

Los árboles poseen secretos importantes que pueden ser esenciales para afrontar los actuales retos medioambientales. Sin embargo, la información que podemos extraer de ellos es aún difícil de interpretar debido a la complejidad de los sistemas forestales, desde los individuos a los ecosistemas. Como en otras disciplinas medioambientales, para entender la información de los árboles es necesaria una aproximación espaciotemporal, multiescala y multidisciplinar. Los actuales avances tecnológicos y científicos ofrecen nueva vías al conocimiento en ecofisiología forestal y la inferencia del clima a partir de la madera. La idea central de esta tesis es usar una combinación de herramientas modernas, tales como Sistemas de Información Geográfica (SIG), estadística de Procesos Puntuales, para extraer información de los bosques a diferentes escalas espaciotemporales. Nos centramos en dos especies mediterráneas: la encina (*Quercus ilex* L.) y el pino carrasco (*Pinus halepensis* Mill.). La tesis se divide en dos bloques centrados en distintas escalas: espacial (regional/ masa) y temporal (anual o multianual/ estacional). **A escala regional**, usamos dos redes de datos de discriminación isotópica del carbono ($\Delta^{13}C$) en anillos de crecimiento de pino y encina, provenientes del noreste de la Península Ibérica, para: **1)** generar paisajes de distribución isotópica (*isoscapes*) de carbono para cada especie, que se correlacionaron con los patrones de precipitación, derivando así mapas de precipitación anual que se validaron exitosamente con datos reales; **2)** evaluar la variabilidad espaciotemporal de la $\Delta^{13}C$ y sus relaciones con la precipitación, crecimiento radial (CR) e índice de vegetación (NDVI) para el pino, mostrando que la precipitación anual controla estas tres variables, aunque ofrecen información complementaria. **A escala local,** nos centramos en una masa mixta donde ambas especies coexisten. Combinando isótopos de agua y estadística de Procesos Puntuales: 1) interpretamos las interacciones árbol-árbol en el uso de agua, encontrando una segregación de nicho hidrológico entre pino y encina durante los periodos de sequía, 2) exploramos la variación estacional en los patrones de extracción de agua para determinar si las relaciones de competencia son dinámicas, o bien la segregación de nichos está siempre presente debido a diferencias morfológicas (p.ej. profundidad de raíces). Los resultados muestran que ambos casos son correctos. Concluimos que un aumento de la sequía reduciría las posibilidades de complementariedad entre las dos especies en masas mixtas, comprometiendo la estabilidad y sostenibilidad del sistema.

Resum

Els arbres amaguen secrets importants que poden ser essencials per enfrontar-se als actuals canvis ambientals sense precedents. Amb tot, la informació que en podem extreure encara és difícil d'interpretar degut a la complexitat dels sistemes forestals, des dels individus als ecosistemes. Com en altres disciplines ambientals, per entendre la informació dels arbres és necessària una aproximació multi-escala i multidisciplinària. Els actuals avenços tecnològics i científics ofereixen noves eines per l'estudi de la ecofisiologia forestal i la inferència climàtica a partir de la fusta. El fonament d'aquesta tesi és fer servir una combinació d'eines innovadores com ara isòtops estables, Sistemes d'Informació Geogràfica (SIG), o estadística de processos puntuals a fi d'obtenir informació climàtica i ecofisiològica dels boscos a diferents escales temporals i espacials. L'objecte d'estudi són dues espècies típicament mediterrànies: alzina (*Quercus ilex* L.) i pi blanc (*Pinus halepensis* Mill.). La tesi es pot dividir en dos blocs que treballen a diferents escales espacials (regional / massa) i temporals (anual-multianual / estacional). **A escala regional**, hem fet servir dues xarxes de dades de discriminació isotòpica de carboni ($\Delta^{13}C$) en anells d'arbres pi blanc i alzina al Nord-est de la Península Ibèrica, a fi de: **1)** generar paisatges de distribució isotòpica (*isoscapes*) per cada espècie, que es correlacionen amb els patrons de precipitació, duent a l'obtenció de mapes de precipitació anual que van ser exitosament validats amb dades reals; **2)** avaluar la variació espacial i temporal en $\Delta^{13}C$, i la seva relació amb la precipitació, creixement radial (CR) i els índexs de vegetació (NDVI) pel pi blanc, mostrant que la precipitació anual determina les tres variables, que tot i així ofereixen informació complementària. **A escala local**, ens centrem en una massa mixta on les dues espècies coexisteixen. Combinant isòtops d'aigua i estadística de processos puntuals: **1)** hem pogut interpretar interaccions arbre-arbre en l'ús de l'aigua, trobant que durant la sequera es dona una segregació de nínxol hidrològic entre pins i alzines; **2)** hem explorat les variacions estacionals en els patrons d'obtenció d'aigua a fi de definir si els patrons de competència són dinàmics, o bé la separació de nínxols està present en tot moment degut a diferències morfològiques (p.ex. profunditat d'arrel). Els resultats apunten a que tots dos casos es donen simultàniament. Concloem que un augment de la sequera pot reduir les possibilitats de complementarietat entre les dues espècies en masses mixtes, comprometent la estabilitat i sostenibilitat del sistema.

General Introduction

"The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge."- Stephen Hawking

Overview

Due to their large size and long live-span, trees constitute living archives that may hold the answer for some of the 21-century environmental questions derived from rising human populations and climate instability. Humans exploit the earth natural resources so rapidly that we are witnessing global biodiversity losses and pollution events capable of altering the earth´s geochemical cycles and climate. In this context, multi-scale and multidisciplinary tree research can provide critical environmental information for the future of the biosphere (Buchmann 2002; Leavitt et al. 2010).

On the one hand, trees, which are globally and densely distributed, are one of the most widespread high-resolution archives for predicting climate change. On the other, tree or community ecophysiology, i.e. forest responses to environmental change, is key not only to predict the future health of forest ecosystems, but also to understand the potential effects of vegetation on the earth system cycles (water, C, N, P) (Buchmann 2002). For instance, despite the natural ability of trees to sequester atmospheric CO_2 (the main driver of global warming), new evidences suggest that the Amazonian and Northern hemisphere forests have stopped sequestering CO_2 (Brienen et al. 2015), and this could lead to additional warming of the climate system (positive feedback). However, part of the large uncertainty of forest acting as either CO_2 reservoirs or source in the future resides in our inability to integrate tree information across separate spatiotemporal scales and research disciplines (Chave 2013). The main issue is that environmental changes at the global scale lead to changes on individuals, but also impose selective pressures upon populations, leading to alterations in the genetic, phenotypic, or species diversity (Chave 2013). Such ecological and evolutionary processes not only complicate predictions of forest performance on a global change scenario but also the understanding of the information retrieved from plant material. Part of the current lack of understanding of the role and responses of trees to global change has to do with:

1) the historically distant path between population biology and ecosystem sciences (Levin 1992)

2) the fact that we have not fully embraced the current technological revolution, which offers ever increasing possibilities for cross boundaries in research disciplines (Chave 2013).

Regarding the first, such a scientific divorce has perpetuated despite the fact that as early as 1805, Alexander von Humboldt, on his essay *the geography of the plants*, illustrated the significant of the spatiotemporal domain and its different scales for the understanding of plants. This included global altitudinal and latitudinal distributional vegetation patterns as well as ecosystems composition. Humboldt used incipient geographical information technology (GIS) (e.g. barometers to measure elevation and chronometers to measure longitudes) to understand the spatial interrelationships of the biotic and abiotic system (Smith 2013). However, not until recently, and specially propelled by the fast development of computational power and global monitoring system, has theoretical ecology considered much of the space-time domain (Bascompte & Sole 1995; Wainwright & Mulligan 2013). The revolution of computer sciences, geographical information systems (GIS) and environmental sensing brings an enormous potential for modeling plant terrestrial ecosystems at different spatio-temporal scales, opening new research avenues that not even Humboldt would have dreamed of. In his influential paper, Levin (1992) explained why patterns and scales are fundamental to study the spatio-temporal dimension of environmental processes, including ecosystem functioning (Bascompte & Sole 1995; McIntire & Fajardo 2009). Scales particularly applies to forest ecology, since it encompasses biogeochemical patterns at several orders of magnitude in time and space that might be controlled by a single process. For instance, how a tree functions and the nature of the local interactions with neighboring trees may have further implications on the structure and dynamics of the biogeography of forest at the global level (Osborne 2004). In essence, the problem is to bridge across very different spatial scales, from local to global (Chave 2013).

In addition to the aforementioned computational and observational revolution in plant ecology, there has been in parallel a biogeochemical revolution (Chave 2013). In particular, the application of stable isotopes is one of the most useful tools to understand plant processes at a range of scales. Scales that range from leaf-level physiological traits, such as stomatal conductance and photosynthesis (Farquhar & Sharkey 1982), to global patterns in plant responses to changes in climate and CO_2 (Diefendorfet al. 2010). In connection with the above, spatial models of stable isotopes (also known as *isoscapes*) have recently become a fascinating field of research on its own for many environmental studies at a wide range of scales, from local to global (See section 1.2.3).

In this context, the main aim of this thesis is to develop models of stable isotopes derived from spatial networks of trees at the regional and local scales in an effort to understand tree responses to their surrounding environment (biotic and abiotic). At the 1) regional scale, we developed techniques for the use of tree-ring datasets to build high-resolution climatic maps and to study tree-responses along climatic gradients; at the 2) stand scale, we used xylem water isotopes to understand the use of water between interacting neighboring trees.

As a case study for the application of these techniques, the thesis will focus on two widely extended and coexisting Mediterranean tree species, *Quercus ilex* and *Pinus halepensis,* which are representative of two different functional types dealing with the water scarcity typical of this biome. Despite that this thesis mainly employs stable isotopes and makes simultaneous use of the spatiotemporal dimensions to extract climatic and ecophisiological information from both species, these approaches were complemented with a range of additional environmental and physiological information (e.g. spectral indices, growth measurements, water potentials) that was integrated into the analysis.

Thesis structure

The thesis can be divided into two main blocks, which roughly correspond to the aforementioned regional and stand scales:

- **Block 1. Climatic and ecophysiological information in carbon isotopes of tree-rings at the regional scale.**

  The main aim of this block is to assess the usefulness of *Quercus ilex* and *Pinus halepensis* as tools for spatially-explicit climate inference (in particular precipitation). In addition, I will assess the responses to abiotic factors depending on the variations in space and time.

  - In **Chapter 1,** we used a spatial modeling method which included topographic variables, latitude and distance to water bodies to generate isoscapes (spatially-explicit models of isotopes, see next section) of $\Delta^{13}C$ derived from tree rings of *Quercus ilex* and *Pinus halepensis*. Such maps were then converted into annual precipitation models and evaluated from an ecophysiological perspective.

  - In **Chapter 2,** we confirmed that the main climatic factor controlling carbon isotope variability in tree rings of *Pinus halepensis* sampled along a strong spatiotemporal climatic gradient is mean annual precipitation. However, we also took advantage of this study to integrate stable isotopes information with other observational data such as tree-ring growth and spectral indices derived from satellites. Such variables also covariate with precipitation.

- **Block 2.** Local-scale spatial inter- and intra-specific tree to tree interactions in water uptake.

  The aim of this block is to evaluate plant water relations and rooting patterns of two coexisting species, as well as ecological processes such as competition, facilitation and niche segregation.

  - In **Chapter 3,** we developed a method to measure tree to tree inter- and intra-specific water-use interactions by employing water stable isotopes from xylem twigs in combination with point-process spatial statistics. As in previous chapters, we used *Quercus ilex* and *Pinus halepensis.*

  - In **Chapter 4,** we provided a closer insight on the dynamics of inter-specific hydrological niche segregation of *Quercus ilex* and *Pinus halepensis* along the seasonal cycle. We proved the existence of different inter-specific strategies in water-use in a Mediterranean environment. As in the previous chapter we used point process statistics, but complemented with other spatial approaches such as competition indices.

Stable Isotopes as an ecological and paleo-reconstruction tool

Stable isotopes - brief description

Isotopes are chemical species of the same element with different number of neutrons and, thus, with different atomic mass. For instance, atoms of carbon with the masses of 12, 13 and 14 (denoted as ^{12}C, ^{13}C and ^{14}C) all have the same amount of protons (6) but different amount of neutrons (6, 7 and 8, respectively). In this case, ^{14}C is a radioactive (non-stable) isotope that decays over time to become a stable, non-radioactive isotope (^{14}N). On the other hand, ^{12}C and ^{13}C are non-radioactive (stable) isotopes, meaning they do not decay over time, at least over geologic time scales. Thus, the natural abundance of stable isotopes depends on variables other than time. This is important because the science of stable isotopes focuses on how natural processes affect the relative abundance of each isotope with respect to the other; i.e. the ratio of the heavy isotope with respect to the lighter one (for example, $^{13}C/^{12}C$). Changes in such ratio are the result of biogeochemical processes involving kinetic, thermodynamic and nuclear effects, and thus quantifying fractionation (the effect of such processes) is a useful way to explore what is occurring within a system. For example, during photosynthesis the ratio of $^{13}C/^{12}C$ varies because the plant preferentially uses atmospheric ^{12}C over ^{13}C (known as isotopic discrimination) (Farquhar et al. 1989). This discrimination occurs because the lighter ^{12}C is energetically less expensive to the plant (it weights less and diffuses more easily into the plant compartments). Thus, the atmospheric C (carbon) pool is enriched in ^{13}C (heavier isotopic composition) compare to the terrestrial vegetation C pool, which is depleted in ^{13}C (lighter isotopic composition) because of photosynthesis is causing fractionation.

Given that the isotopic composition of materials varies throughout different pools within the earth system cycles and also at exceedingly small levels, we always compared any measured values to an international accepted standard and expressed in parts per thousand deviations from that standard by:

$$\delta (‰) = (R_{\text{sample}}/R_{\text{standard}} - 1) \text{ x } 1000 \qquad (1)$$

where R is the ratio of heavy-to-light (typically rare-to-abundant) isotope, R_{sample} is that ratio in the sample, and $R_{standard}$ is that in the standard (Table I). The different atomic mass of isotopes is usually quantified by mass spectrometry, but there are other methods such as laser absorption instruments.

Table I. Relative abundance of most common stable isotopes in plant ecology

Element	Isotope	Abundance (%)	Relative mass difference (%)	International Standard	Analytical error (‰)	Observe range in plants (‰)
Hydrogen	^{1}H	99.985	100	Vienna Standard	4 to 7	-300 to +20
	^{2}H	0.0155		Mean Ocean Water		
				(VSMOW)		
Carbon	^{12}C	98.892	8.3	Viena Pee Dee Belemnite	0.1	-35 to +5
	^{13}C	1.108		(VPDB)		
Nitrogen	^{14}N	99.635	7.1	Atmostpheric Nitrogen	0.2	-10 to +10
	^{15}N	0.365		(Air)		
Oxygen	^{16}O	99.759	12.5	VSMOW in water,	0.05 to 0.2	-15 to +35
	^{17}O	0.037	(^{18}O : ^{16}O)	generally VPDB in		
	^{18}O	0.204		CO2 or carbonate		

After: (Sulzman 2008)

Carbon stable isotopes in plant material

As mentioned above, carbon isotopes of terrestrial vegetation are depleted in ^{13}C as compared to atmospheric CO_2. In C_3 plants, this is because of two fractionation processes: (*a*) diffusion of CO_2 through stomata, which reduces $\delta^{13}C$ by 4.4‰, and (*b*) the enzymatic process of carboxylation, which further reduces $\delta^{13}C$ by 27‰ (note that for C4 and CAMs different photosynthetic processes operate leading to different ^{13}C discrimination, for full review see Marshall et al. (2007)). Farquhar, O'Leary, & Berry (1982) developed the well-known model on the $\delta^{13}C$ discrimination of C_3 plants during photosynthesis:

$$\Delta_A = a + (b - a)\, c_i/c_a \qquad (2)$$

where *a* is diffusive fractionation (-4.4‰), *b* is enzymatic fractionation by Rubisco (-27‰), and c_i/c_a is the concentration of CO_2 in the intercellular air space divided by concentration outside the leaf, i.e. atmospheric CO_2 . Photosynthetic fractionation thus is affected by changes in atmospheric CO_2 concentrations (c_a), for instance by the recent increase from the burning of fossil fuels, but also by changes in the substomatic chamber or intercellular (c_i) CO_2 concentrations. The substomatic chamber has a CO_2 input rate (g_s) regulated by the stomata and an output rate (A) regulated by the CO_2 assimilation by the Rubisco (the carboxylation enzyme). Changes in the input (g_s) and output (A) of this model (i.e. in the intercellular CO_2 balance of the leaf) are controlled by environmental variables such as light, temperature, water and nutrients availability, etc. Additionally, given that water-use efficiency (WUE - ratio of net photosynthesis to transpiration, A/E) is also controlled by intercellular CO_2 concentration, the $\delta^{13}C$ of phosynthates (including plant sugars and tissues such as wood or leaves) provides a reliable index of water-use efficiency (Farquhar and Francis 1984). The formula of $\delta^{13}C$ in plants can be expressed as:

$$\delta^{13}C_{plant} = \delta^{13}C_{atmospheric} - a - (b - a)\ c_i/c_a \qquad (3)$$

where $\delta^{13}C_{atmospheric}$ has a current value of -8‰ becoming more negative each year (0.02 to 0.03‰/year). In addition, this value changes seasonally with relative ^{13}C depletion during winter and enrichment during summer, particularly in the northern hemisphere. Also, it changes vertically in closed canopies due to near-soil CO_2 accumulation from respiration. Therefore, we need to consider $\delta^{13}C_{atmospheric}$ variations when assessing $\delta^{13}C$ variations of plant material in distantly separated samples (either in time or in space) (Gessler et al. 2014).

To avoid the $\delta^{13}C$ spatiotemporal changes from the CO_2 source, many physiological studies use the carbon isotope discrimination to ensure that only plant processes are studied:

$$\Delta^{13}C = (\delta^{13}C_{atmostpheric} - \delta^{13}C_{plant})/\ (1 - (\delta^{13}C_{plant}/1000)) \qquad (4)$$

However, because the $\delta^{13}C_{atmospheric}$ variation is almost non-existing at the studied scales, we assume that changes in $\delta^{13}C_{plant}$ are mainly controlled by changes in c_i/c_a, which are in turn controlled by both g_s and A.

- *$\Delta^{13}C$ linked to stomatal conductance*

Lowered c_i/c_a ratios by stomatal closure (lower input) mean that Rubisco has less CO_2 available to fix. Because Rubisco preferentially uses the lighter carbon isotope ($^{12}CO_2$) for photosynthesis, the plant is then forced to use (or cannot discriminate against) the heavier isotope ($^{13}CO_2$), thus enriching the plant material with ^{13}C. Strong stomatal regulation, which leads to partial or total stomatal closure, is typical of plant species in water-limited environments to prevent desiccation. Such regulation leads to large variations in the discrimination of $\delta^{13}C$ by Rubisco, which then gets imprinted in plant material. However, $\delta^{13}C$ variability related to stomatal regulation may also happen not only in dry environments. For instance, mean annual precipitation (P) was found to control $\Delta^{13}C$ in the leaf organic matter of C_3 plants around the globe (Schulze et al 1998; Diefendorf et al., 2010; Kohn, 2010) , and in the tree rings of a wide range of species (Korol et al. 1999; McCarroll & Loader 2004; Miller et al. 2001; Stewart et al. 1995; Warren et al. 2001), suggesting in many cases stronger control in the dry environments and less in wet environments (the ones approaching $P/E \approx 1$) (Warren et al. 2001). The major role of water in driving the balance of leaf gas-exchange in modern plants and the global trends in ecosystem-scale primary productivity can be understood through the study of $\Delta^{13}C$ (Diefendorf et al., 2010). This is because $\Delta^{13}C$ is strongly controlled by stomatal conductance, a key land surface attribute that controls both transpiration and photosynthesis. Moreover, stomatal conductance responds not only to water availability but to water demand too; thus other variables such as vapor pressure deficit, air relative humidity and potential evapotranspiration may cause variations in $\Delta^{13}C$ (Ferrio & Voltas 2005). Similarly, stomatal conductance is affected by the resistance produced by the water-transporting-material within the plant, related e.g. to wood anatomical density (Klein 2014); as a consequence, species with alternative water-use strategies may show different $\Delta^{13}C$ (Miller et al. 2001).

- *$\Delta^{13}C$ linked to C assimilation*

On the other hand, $\Delta^{13}C$ and c_i/c_a can also be affected by assimilation (output in the substomatic chamber). For instance, in wet environments, maximal stomatal aperture yields an internal CO_2 concentration closer to the atmospheric concentration, and Rubisco can thus discriminate against ^{13}C, assimilating more carbon depleted in ^{13}C. Still, under such non-water-limited environments for photosynthesis, an improvement in other limiting conditions, like temperature (Miller et al. 2001; Schulze et al. 2014; Körner et al. 1991), light and nutrients availability (Schulze et al. 2014; Warren et al. 2001), oxygen partial pressure (Körner et al. 1991), etc, will lead to increases in the CO_2 assimilation rate by Rubisco (A), therefore also decreasing c_i/c_a; this will also force the plant to use more ^{13}C for photosynthesis thus decreasing the $\Delta^{13}C$ of plant assimilates. It is important to note though that changes in $\Delta^{13}C$ by increased assimilation are more strongly noticed when (c_i) is lower as a consequence of stomatal limitation, because changes in c_i are larger in magnitude (Miller et al. 2001). This is also true for c_i changes by stomatal regulation (see below).

- *Other factors contributing to changes in $\delta^{13}C$ of plant material*

Stomatal diffusion and assimilation rates often operate in coordination. For instance, lower water availability may lead to a decrease in both, the A and g_s, thus dampening the response of $\Delta^{13}C$ to the environment as well as the realized range of variation in c_i/c_a (Cernusak et al. 2013; Wong et al. 1979). In fact, such coordination is very likely one of the main factors for a constant $\Delta^{13}C$ and c_i/c_a found in tree species living along a strong precipitation gradient from ~1700 to ~300 mm in northern Australia (Cernusak et al. 2011; Schulze et al. 1998). However, the mechanism controlling such coordination are not clear yet (Cernusak et al. 2013).

Similarly, when studying spatial variations of the $\Delta^{13}C$ and c_i/c_a, it is important to note that other processes can modulate the response to the environment, like an adjustment of the ratio of leaf area to water-conducting tissue (Miller et al. 2001), or changes in altitudinal and latitudinal atmospheric to internal pressure (Körner et al. 1991). Such physiological adjustment or plasticity can be due to genotypic diversity. Therefore, environmental factors can modify $\Delta^{13}C$, but internal plant physiology, which varies between and within species, may also amplify or constrain the response (Cernusak et al. 2013).

Thus, carbon isotopes can be used to retrodict the environment that affected the plant during the time of tissue formation (leaves, tree rings, etc.) as well as to study differences in species- or genotype-specific physiological responses (Cernusak et al. 2013; Schulze et al. 2014; Voltas et al. 2008). Despite that the water status of the plant is crucial during the formation of plant tissue (growing period) and affects changes in $\Delta^{13}C$, other factors during growing period may also affect $\Delta^{13}C$ variability, such as high/low temperatures, leaf nitrogen or specific leaf area (Schulze et al. 2014). It is also important to note that $\Delta^{13}C$ in plant material may also reflect conditions during the wet rather than the dry period when there is no growth (Stewart et al. 1995; Schulze et al. 2014).

Furthermore, in the study of $\Delta^{13}C$ plant material such as tree rings additional sources of variation unrelated to environmental factors may be derived from fractionation processes taking place during the mobilization of photosynthetic assimilates from the leaf to final produce a tree-ring (Gessler et al. 2014). For instance, the tree may use remobilized stored carbohydrates (primarily starch) for the construction of tree rings. As a consequence, the tree ring may have a signal from when the starch was produced, possibly many months or even years earlier (Gessler et al. 2014).

- *$\Delta^{13}C$ and its link to plant performance*

In addition to the above, $\Delta^{13}C$ variability also may reflects changes in plant performance. In fact, given that assimilation (A) saturates as intercellular CO_2 concentrations (c_i) increases at high g_s, $\Delta^{13}C$ provides a non-linear measure of plant performance in terms of carbon assimilation (Miller et al. 2001). As mentioned earlier, decreases in $\Delta^{13}C$ reflect either a decrease in g_s or increases in assimilation rates, and both may reflect an opposite effect on plant performance. Increased carbon may result from increased g_s raising c_i and $\Delta^{13}C$ with a fixed photosynthetic capacity, or increased photosynthetis with a fixed g_s decreasing c_i and $\Delta^{13}C$ (Miller et al. 2001).

Water isotopes

Water is an essential element for life and this includes plants. Two molecules of water are split into $4H^+$ and O_2 during photosynthesis, acting as primary electron donors for Photosystem II, and water is also directly involved in CO_2 fixation in the Calvin cycle, as well as in many subsequent metabolic conversions, see e.g. Taiz & Zeiger (2010). Furthermore, water is used by plants as a solvent, a transport medium, a coolant or as a structural component giving turgor to the cells. Typically, C_3 plants spend as much as 500 g of water through transpiration for each gram of dry weight produced (Taiz and Zeiger 2010). Hence, it is not surprising that water controls almost every imaginable plant function as well as the global distribution of plant species. In addition, the physical scale of the soil water availability affecting plant community composition may range from the geographic to the highly local (Silvertown et al. 2015). In regions where water scarcity predominates plants have developed different strategies to survive. This includes being more efficient in their water use or investing in parts of the plant (like a larger and deeper root system) to have a competitive advantage over other species (See chapter 3).

However, inter- and intra-specific tree-water interactions are not well understood because of the difficulty of tracking water movement through the water cycle. This is particularly true in the soil-atmosphere interface, which is mainly controlled by plant water use (uptake, storage, transport) and evapotranspiration (lose) (Dawson 1996). In this context, water stable isotopes offer a great opportunity to study the ecological, physiological and environmental variables that control the water balance of different forest ecosystems. This was, until recently, a rather obscured scientific subject, as belowground plant-to-plant interactions are complex and difficult to monitor with conventional methods of root measurements. However, during the 1990s, the study of isotopic ratios of deuterium/hydrogen (δD) and oxygen-18/ oxygen-16 ($\delta^{18}O$) in xylem water helped in determining the sources of water used by plants, and therefore plant rooting depths and patterns (Ehleringer & Dawson 1992; Dawson 1996; Filella & Peñuelas 2003; Walker & Richardson 1991). This was possible mainly because of two facts: 1) the isotopic signal of the xylem water equals that of the source water in the soils, without significant fractionation processes in between (Dawson & Ehleringer 1991); 2) there is a natural variability in the isotopic composition of potential water sources, e.g. with different soil depths. For example, the soil water within the soil profile often displays a degree of evaporation, increasing as it gets closer to the top of the soil where higher temperatures, sun radiation, wind, etc., accelerate the evaporation process. This evaporation gradient also produces an isotopic gradient because the lighter isotopes evaporate to the atmosphere faster than the heavy ones, thus creating a fractionation process in the water isotopes (Barnes & Allison 1984) (Figure 1). The above allows, through the isotopic characterization of the soil, to monitor the depth at which the plants extract water.

In order to understand plant water extraction using water stable isotopes, it is necessary to point out that changes in water phase (under non-equilibrium conditions) produce fractionation. So as said, evaporation leads to lighter water escaping as vapor gas (isotopic depletion), leaving the heavier isotopes behind in the pool of liquid water (isotopic enrichment). Contrary, condensation and precipitation lead to exactly the opposite effect; that is, isotopic depletion of the water vapor as the heavier isotopes fall out (rain out) first, with the consequent enrichment of the drops of liquid water (Bowen 2010). Soil and ground water, the main source of water for plants, comes mainly from precipitation, which can be of different origin; for instance, convective (recycled) precipitation or oceanic weather fronts. Both types of precipitation have a different isotopic imprint as their original pool is also different (terrestrial versus oceanic) (Figure 1). In addition, both precipitations may contribute differently to the recharge of the soil and ground water available to plant communities. In the Iberian Peninsula, where this thesis is based, recharge is mainly provided by Atlantic fronts (see Chapter 1 and Figure 1). As a consequence, the water found in the tree-xylem may tend to have the isotopic value of Atlantic fronts. However, recycle water from convective storms may also contribute to ground-soil recharge during some parts of the year, mixing with the Atlantic water, thus making the tracking of the source water in the xylem harder to evaluate. Similarly, the plant may extract water at different depths, thus providing a mix of isotopic signals (Figure 1). It is for this reason that the isotopic signature of xylem water needs to be evaluated along the growing season, and compared to the water at different depths within the soil profile and the aquifer (Dawson 1996).

The degree of evaporation of sampled water with respect to the source water can be assessed by using the global or local meteorological water line; that is, the relationship between the δ^2H and the δ^{18}O that exist for different parts of the world (Craig 1961) (Figure 2):

$$\delta^2H = 8\times\delta^{18}O + 10 \qquad (5)$$

This global variability is produced by changes in the concentration of different isotopes in the primary input source, precipitation, along seasonal, latitudinal and elevational temperature gradients (Dansgaard 1964). Another variable, the Deuterium excess, (*D-excess* = δ^2H − 8×δ^{18}O; figure 2), denotes the divergence from the meteoric water line, and is sensitive to the conditions of evaporation of water from a surface, particularly near-surface temperature (Dansgaard 1964) but more importantly to relative humidity (RH) (Pfahl & Sodemann 2014); *D-excess* can help us in quantifying the degree of evaporation of water as well as the possible sources of precipitation (Figure 1). It has become irreplaceable as a way to track the hydrological and atmospheric systems, with a wide range of applications such as paleoclimatology, ecology, forensic sciences, criminology, etc. (Bowen 2010).

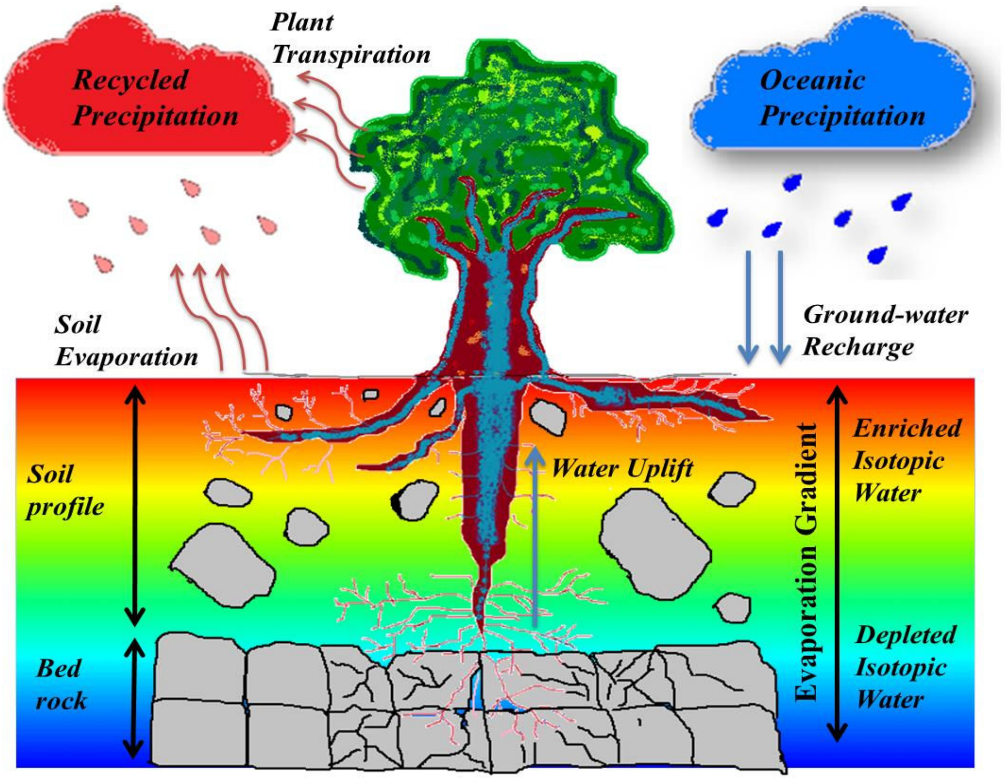

Figure 1. Part of the water cycle tracked by using water stable isotopes.

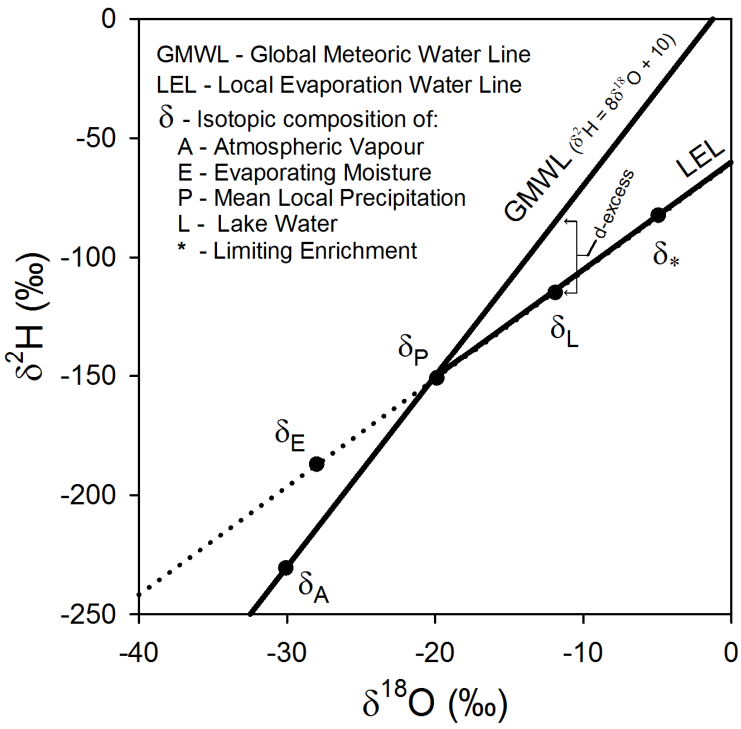

Figure 3. Generalized δH^2 vs δO^{18} plot showing global meteoric water line and local evaporation line. Adapted from Gibson, Edwards, & Bursey (1993).

Spatio-temporal monitoring and modeling of the environment

Spatiotemporal modeling

A spatiotemporal model is a mathematical explanation of a dynamic system in which space is explicitly introduced (Bascompte & Sole 1995). Modeling, especially through extrapolation in time and space (mainly as predictive tool) can help us in reducing the complexity of a system by focusing only on the most relevant components for that system (Wainwright & Mulligan 2013). Environmental modeling is a powerful tool to integrate environmental processes (theory of how things work) and patterns (observation of the system) at different spatio-temporal scales. However, certain systems are too complex to be broken into manageable parts. For instance, and related to this thesis, this is the case for the spatial distribution of precipitation in the Mediterranean region, in which a complex interaction among multiple, and often unpredictable, components makes it a real challenge to model it; the effect of the abrupt topography on precipitation and the convective origin of a substantial part of the accumulated precipitation are good examples of the difficulties faced by climate modelers in this region. Similarly, modeling vegetation is challenging because it is constantly changing in space and time. Part of that difficulty derives from the fact that abiotic factors such as climate control part of that change, which introduces stochasticity into the system. In addition, plant dispersal, for instance through wind, and subsequent colonization, are surely subjected to random mechanisms too. As a consequence, when modeling the spatial structure and dynamics of vegetation, the random dynamic of climate and other environmental, biotic and ecologicalvariables must be considered.

At the fine scale, processes that occur within a forest system (known as forest dynamics), such as tree-to-tree interaction for resources, can be explored through the study of the forest spatial configuration (Comas & Mateu 2007). The dimensions and the characteristics of a single tree determine its relationships with their neighbors. Empirical studies have frequently exposed that spatial and size configuration of trees in a community carry the fingerprint of growth, competition and habitat heterogeneity (Ford 1975, Chen et al. 2004). In this context, modern forestry research requires a multidisciplinary approach, for which forest ecology, landscape ecology and related spatial statistical methods become increasingly important (Stoyan & Penttinen 2000).

As mentioned in the introduction, an emergent field of study, known as *isoscapes* (from isotope landscapes) and focus on building spatial models of continuous observations of stable isotopic ratios that evolve over time, is providing innovative methods to the understanding of the biochemical cycles as well as many environmental and ecological processes on earth (West et al. 2010). *Isoscapes* integrate information across a range of different scales in time and space in grid-based models built through geostatistical tools from GIS packages (Bowen 2010). Questions being addressed include plant and animal ecology, geology, atmospheric sciences, anthropology, microbiology, climate change, paleoclimatology, forensic sciences and more (West et al. 2010). Isoscapes, thus, offers the opportunity to explore biochemical spatial patterns and flows within systems in a precise way.

Geographic Information Systems and Remote Sensing

Monitoring and modeling the environment has become much easier due to the development of two sister technologies, Remote Sensing and Geographic Information Systems (GIS). Whereas Remote Sensing allows airborne monitoring of the earth through satellites, planes, drones, etc., with sensors capable of capturing radiation at a wide range of wavelengths (see next section for monitoring vegetation), GIS helps in the post-processing and analysis of these data and any other georeferenced spatio-temporal data. As its name indicates, GIS is a computer-based system designed to manage and model geographic information (Coppock, J. T., and Rhind 1991). Through a system of layers of geographical information of any kind (for instance, topographic, environmental, ecological, sociological, economic, etc.) it is possible to store, integrate, edit, analyze or display such information in many forms. The first GIS software in the world was developed in 1960 by Dr. Roger Tomilson, a visionary geographer who was able to solve complex problems related to the land capability of the rural Canada. He mapped information about soils, land use, forestry, wildlife, recreation, saving the Canadian government large amounts of time and money (Coppock & Rhind 1991). Since then, GIS, which is a byproduct of computer revolution, offers endless possibilities in spatiotemporal data analysis and modeling, just 'as far as the mind can go' (Mark Mulligan 2006, personal communication).

GIS analysis includes geostatistical algorithms to deal with spatiotemporal datasets. Typically different methods for spatial interpolation of data, which generates spatial information in those areas in space where information is missing, are part of geostatistical packages in GIS. From a single network of points containing some type of information we can generate spatially-continue layers of information; in other words, a spatial model displayed as a map containing some real and some modeled information. The principle behind geostatistical models is that of spatial autocorrelation; when a spatial process (e.g. precipitation) influences values of a variable (e.g. $\delta^{13}C$ values in plants), then values from nearby locations are likely to be more similar than those from locations that are widely separated in space. There are many types of interpolation methods, including kriging, inverse distance weight, closest neighborhood, etc. In this thesis, I will concentrate in the multiple regression method, which allows incorporating a process-based, more mechanistic approach into spatial interpolation. For example, if we are able to model the response of a given variable to different topographical variables, we can create more realistic environmental maps, thus, better models, than by simple distance interpolation.

Normalized vegetation index (NDVI)

Vegetation indices are radiometric estimates of the amount of photosynthetically active radiation (PAR) absorbed by the pigments (including chlorophyll and carotenoids) of green leaves as an energy source for photosynthesis. A sensor (in the space or ground) can gather both the reflected solar radiation from the plant, in order to quantify a proportion of PAR that is used by plants during photosynthesis (e.g. we might be interested in the red band, between 0.6 and 0.7 μm) and the low-energy radiation that harms plants by increasing their internal heat (e.g. the near-infrared band, between 0.74 and 1.1 μm). The difference between the two reflected radiations provides a very distinctive signature of vegetation when compared to other earth surface materials (Tucker & Sellers 1986) (Figure 3). One of the most typically used indices is the normalized difference radiation index (NDVI), which is calculated as:

$$NDVI = \frac{(Red - \ Near\ infrared)}{(Red\ +\ Near\ infrared)}$$

After linking remotely sensed vegetation greenness to atmospheric CO_2 changes in 1986, theoretical work by Tucker & Sellers (1986) attractively showed that absorption was related to photosynthetic activity, stomatal resistance and evapotranspiration. In this pioneer work, the authors clarified the issue that the remote sensors provide some indication of chlorophyll density, which is in turn related to photosynthetic and transpiration rates, rather than just an indicator of the photosynthetic surface (leaf area index or biomass). In this regard, a series of articles during the early 1980s by Compton J. Tucker, as well as many subsequent studies (see references in chapter 2), demonstrated that NDVI generated from modern satellite sensors can be used to map land cover and monitor global-scale vegetation changes in productivity, physiological activity, and other processes like desertification.

However, the complexity of canopies in terms of light absorption and radiative transfer (scattering, diffusivity, absorption, etc.) also imprints a signal in vegetation indices, associated to the density and health of canopy, leaf orientation, angle of the satellite with respect to the sun, etc. This can make the information contained in the vegetation indexes hard to interpret and not purely physiological. Furthermore, other problems related to atmospheric, soil, leaf litter water content, color of the soil, shadow of the trees, sun-sensor geometry etc., can affect the reflected radiation by vegetation, thus distorting the final signal collected by the sensors (Tucker & Sellers 1986; Morton et al. 2014). In spite of this, seasonal and interannual variations in NDVI have been linked to net primary production (NPP), leaf area index, leaf chlorophyll content etc., and usually correlate well with other environmental variables (See chapter 2 for references). Nowadays, a global coverage dataset (Global Inventory Modeling and Mapping Studies, GIMMS) is the most widely employed data source for medium-term vegetation change studies, particularly at the global or regional scale. The data included in GIMMS is derived from the Advanced Very High Resolution Radiometer (AVHRR), launched by the National Oceanic and Atmospheric Administration (NOAA) in 1979

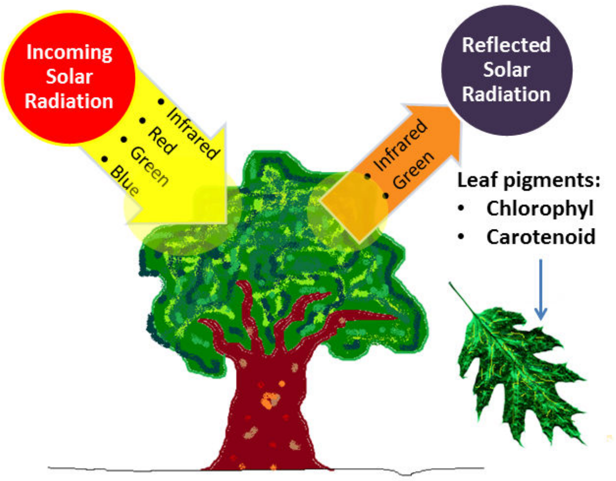

Figure 3. The sensors (mounted on satellite, drones, or fix to the ground) measure the difference between solar radiation that is less reflected (Red) and most reflected (Infrared) by vegetation.

A point-process model is one of the tools developed by the discipline of point-pattern analysis. Point-process models simulate spatial point patterns with known properties to be compared with observed patterns (Wiegand & Moloney 2014). As its name indicates the point-process model tries to identify the processes that might be behind a particular point spatial pattern within a spatial observation window. The distribution of points in space may depend on some form of stochastic mechanism (Diggle 2003), and point-process models try to characterize the spatial patterns as accurately as possible by using appropriate statistical techniques (Wiegand & Moloney 2014). Ecologists have studied spatial patterns to look for underlying biological and ecological processes and to identify whether the spatial scale at which they are operating fits spatially-related scientific theories (Comas & Mateu 2007). For instance, we may find that in densely areas of a forest, growth and survival rates might be lower than in less dense areas, and we may then hypothesize that this is a consequence of strong competition between neighboring trees; in this context, point processes allows to statistically test for that.

In point-process statistics we may evaluate ecological objects within an observation window considering different types of information. If only spatial coordinates are taken into account, this is commonly referred to as 'point-pattern data'. If we have only one type of object, we call it univariate point pattern, and if we have two or more types of objects (e.g., two or more different species of trees), we call it bivariate or multivariate point pattern respectively (Wiegand & Moloney 2014). In addition to the coordinate attributes of the ecological object or points in the plane, other attributes can be considered in this sort of spatial analysis. They are referred as 'marks', which can be qualitative (e.g. dead *vs.* living trees) or quantitative (e.g. height, basal area, trunk size, etc. of a tree). Such marks can be modeled together with the spatial coordinates to provide information about the processes behind the actual spatial configuration of the points, as well as to reveal the processes behind the observed values of the marks (See Chapter 3).

Forestry applications

Two of the first publications employing ideas from point-process statistics in the field of forestry are the book by Konig (1835) and the article by Svedberg (1922). Since then, point-process statistics has grown in forest sciences more than in any other scientific discipline (references in Stoyan & Penttinen, 2000). A forest stand is the result of previous land use, complex ecological processes and practical forestry, thus, the spatial pattern formed by tree distribution may be hard to model (Comas & Mateu 2007). This is particularly true for the fine-scale spatial distributions of trees, something that still remains poorly understood (Condit et al. 2000). The application of point-process statistics helps to study spatial tree patterns and to contrast statistical and ecological hypotheses with empirical data (Stoyan & Penttinen 2000).

One important aspect of point-process statistic in ecology is that we can understand how things work from the point of view of the ecological object (or individual) (Stoyan & Penttinen 2000), which in forestry is known as single-tree modeling of whole forest. This provides an excellent opportunity to create spatially-explicit models of plant-to-plant interaction. Such kind of models have often being neglected, despite they may hold important information regarding ecosystem functioning (Rascher et al. 2012). For instance, inter-tree competition is often thought of as one of the primary drivers of forest-stand dynamics (Ruiz-Benito et al. 2013), and the density and identity of neighbors within a stand are considered important aspects for tree mortality (Olano et al. 2009; Ruiz-Benito et al. 2013), tree regeneration (Comas 2009), growth (Comas et al. 2013), etc.

Objectives

Objectives

i. To model precipitation at the regional scale with carbon isotope discrimination from the tree-rings of *Quercus ilex* L. and *Pinus halepenesis* Mill.

ii. To integrate into tree-ring isoscapes topographical variables, assessing the use of regression-interpolation methods currently in use for climate data modeling.

iii. To determine whether the $\Delta^{13}C$ in the tree-rings of *Pinus halepensis* is mainly controlled by stomatal processes both at the spatial and temporal level, contrasting isotopic data with tree-ring growth and satellite derived NDVI.

iv. To assess the existence of hydraulic niche segregation between co-existing *Pinus halepensis* and *Quercus ilex*, combining water isotopes with measurements of tree growth and physiological variables.

v. To evaluate tree-to-tree below ground interactions at the inter and intra-specific level in terms of water extraction patterns related to seasonal dynamics, by combining stable isotopes with a set of tools for spatial analysis.

vi. To understand the implications for future forest dynamics of the different water use extraction patterns and strategies to cope with drought in pines and oaks.

Methods

Materials and Methods (overview)

This thesis studies forest systems at to different spatial scales (local vs regional) as well as temporal scales (seasonal vs multiannual). We focus on two of the most representative tree species of the Western Mediterranean basin, the Aleppo pine (*Pinus halepensis Mill.*) and the holm oak (*Quercus ilex L.*) which coexist in a wide range of Mediterranean environments. As mentioned earlier, the objective is to integrate the technology of stable isotopes (water and C in the wood) with modern ways of spatial modeling in order to unveil important biological, ecological, physiological and environmental aspects concerning both species.

At the regional scale:

In chaper 1, a methodology to develop precipitation maps from carbon isotope discrimination ($\Delta^{13}C$) of tree rings by (1) producing high-resolution ^{13}C-isoscapes using multiple linear regression analysis, which incorporates geographical variables as predictors of isotopic records, combined with geostatistical interpolation of the errors (Ninyerola et al. 2006), and (2) applying causal relationships between precipitation and $\Delta^{13}C$ to convert ^{13}C-isoscapes into precipitation maps. Precipitation maps for the study area were derived from isotope networks of two Mediterranean tree species, Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.), which are known to provide complementary information on seasonal precipitation. Isoscapes where generated using ArcGIS 10.0 (ESRI, Redlands, USA) and Miramon Miramon V6.1 package.

Altogether, we used 44 sampling locations for *Q. ilex* and 38 for *P. halepensis*, respectively, with 15 locations being common to both species. The compiled $\delta^{13}C$ records represented available number of pooled tree rings (24 ± 2.0 years for *Q. ilex*, 17 ± 2.8 years for *P. halepensis*) and covered different temporal ranges for the period 1975–2008. Monthly precipitation data for the sampling sites were obtained from the Digital Climatic Atlas of the Iberian Peninsula (http://www.opengis.uab.es/wms/iberia/mms/index.htm) (*reference precipitation*, representative of the period 1950–99) and used in model for error estimation and model validation.

In chapter 2 we evaluated not only the spatial but also the temporal variability of the isotopic discrimination $\Delta^{13}C$, and its relationship with precipitation, radial growth (RG) and satellite vegetation index NDVI. We used a network of seven localities of tree-rings from Pinus halepensis in the northeastern part of the Iberian Peninsula. We looked into the biennial tree-ring variability for a period of 25 years and compared that to climatic, radial growth and NDVI time series. The isotopic analysis to estimate the $\delta^{13}C$ from the wood were analysed in mass spectrometer (Chapter 2) at the university of Davis (California), see chapter 2. We used simple Pearson correlation analysis to find relationships at the spatial and temporal level with all the mentioned variables MAP $\Delta^{13}C$, NDVI and RG.

At the local scale:

In chapter 3 and 4 we sought to developed a methodology to understand water extraction patterns in mixed forest of *Q. ilex* and *P. halepensis*. We explored the use of water through the isotopic analysis of water from twigs, trunks and ground along a year of drought (6 sampling campaings), in which trees were likely to be at its limit of tolerance. The study was conducted in a mixed stand (area = 888 m^2) with a total of 33 *Q. ilex* and 78 *P. halepensis* and them were sampled. To understand the interactions inter and intra-specific between individuals at the stand level, we have employed the analysis of the marked point patterns of oaks and pines, we used the mark correlation function to describe the spatial structure of marks (e.g. $\delta^{18}O$ and $\delta^{2}H$; the isotopic values of water in the twigs) associated to each tree location. Mark-correlation spatial analysis can also unveil whether water extraction patterns were related to any point process or particular spatial configuration.

Water isotope ratios of hydrogen and oxygen of the xylem and soil were determined using a Picarro Water Analizer L2130-*i* (Picarro Inc., Santa Clara, California), and expressed in delta (δ) notation (‰) relative to V-SMOW (i.e. isotopic composition of oxygen, $\delta^{18}O$, and hydrogen, $\delta^{2}H$). Tree position for spatial analysis was determined using a high resolution GPS technology (GeoExplorer 6000 Series Handheld, Trimble Navigation Limited, California, USA). We also used other spatial interpolation methods with GIS to estimate spatial tree densities to derived tree ecophysiological information.

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Chapter I

"Todo viaje, aunque tenga 1000 leguas, comienza con un solo paso". **Lao-tsé (570 aC-490 aC) Filósofo chino.**

Isoscapes of tree-ring carbon-13 perform like meteorological networks in predicting regional precipitation patterns

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Abstract

[1] Stable isotopes in tree rings provide climatic information with annual resolution dating back for centuries or even millennia. However, deriving spatially explicit climate models from isotope networks remains challenging. Here we propose a methodology to model regional precipitation from carbon isotope discrimination ($\Delta^{13}C$) in tree rings by (1) building regional spatial models of $\Delta^{13}C$ (*isoscapes*), and (2) deriving precipitation maps from $\Delta^{13}C$-isoscapes, taking advantage of the response of $\Delta^{13}C$ to precipitation in seasonally-dry climates. As a case study, we modeled the spatial distribution of mean annual precipitation (MAP) in the northeastern Iberian Peninsula, a region with complex topography and climate (MAP=303-1086 mm). We compiled wood $\Delta^{13}C$ data for two Mediterranean species that exhibit complementary responses to seasonal precipitation (*Pinus halepensis* Mill., *N*=38; *Quercus ilex* L.; *N*=44; pooling period: 1975-2008). By combining multiple regression and geostatistical interpolation, we generated one $\Delta^{13}C$-isoscape for each species. A spatial model of MAP was then built as the sum of two complementary maps of seasonal precipitation, each one derived from the corresponding $\Delta^{13}C$-isoscape (September–November from *Q. ilex*; December–August from *P. halepensis*). Our approach showed a predictive power for MAP (RMSE=84 mm) nearly identical to that obtained by interpolating data directly from a similarly dense network of meteorological stations (RMSE=80-83 mm, *N*=65), being only outperformed when using a much denser meteorological network (RMSE=56-57 mm, *N*=340). This method offers new avenues for modeling spatial variability of past precipitation, exploiting the large amount of information currently available from tree-ring networks.

Introduction

[2] There is a current need to develop precipitation models with high spatial resolution in order to understand past climate and delineate future scenarios of global change [*Brayshaw et al.*, 2011; *IPCC*, 2007]. This, however, is particularly challenging in drought-prone areas where precipitation regimes are extremely complex and accurate predictions usually require geographically dense networks of instrumental observation [*González-Hidalgo et al.*, 2011; *New et al.*, 2001]. Since long-term coverage of instrumental meteorological records (i.e. longer than a few decades) is often unavailable, proxies for precipitation are required to complement or expand instrumentally derived information.

[3] In this context, carbon isotope ratios ($^{13}C/^{12}C$) in different plant tissues can be used as a proxy for precipitation, particularly in seasonally dry climates [*Aguilera et al.*, 2009; *Diefendorf et al.*, 2010; *Ferrio and Voltas*, 2005; *Klein et al.*, 2005; *Kohn*, 2010; *Leavitt et al.*, 2007; *McCarroll and Loader*, 2004; *Warren et al.*, 2001]. Specifically, stable isotopes in tree rings provide climatic information with annual or seasonal resolution dating back for centuries or even millennia, and thus a growing interest in the spatial and temporal dependence of climate processes have resulted in a burst of tree-ring isotope networks worldwide [*Kagawa and Leavitt*, 2010; *Leavitt et al.*, 2007; 2010; *Schubert and Jahren*, 2011; *Sidorova et al.*, 2010; *Treydte et al.*, 2007]. However, although preliminary attempts to infer climatic trends using spatially explicit models of isotope distribution (*isoscapes*; [*Bowen*, 2010; *West et al.*, 2010]) have provided promising results [*Aguilera et al.*, 2009; *Leavitt et al.*, 2007], the development of robust methodologies to interpolate existing data remains essential to derive accurate regional estimates from tree-ring networks [*Büntgen et al.*, 2010; *Frank et al.*, 2008; *Leavitt et al.*, 2010; *Treydte et al.*, 2007]. Given that climate variables can be spatially modeled using geographic variables in areas with complex topography [*Agnew and Palutikof*, 2000; *Ninyerola et al.*, 2000; *Perry and Hollis*, 2005; *Sánchez Palomares et al.*, 1999], we argue that the same approach can be applied to tree-ring isotope networks in order to reconstruct spatial patterns of precipitation.

[4] Therefore, the main goal of this paper is to propose a methodology to develop precipitation maps from carbon isotope discrimination ($\Delta^{13}C$) of tree rings by (1) producing $\Delta^{13}C$-isoscapes with high spatial resolution using multiple linear regression (MLR) analysis, which incorporates geographical variables as predictors of isotopic records, combined with geostatistical interpolation, and (2) applying causal relationships between precipitation and $\Delta^{13}C$ to convert $\Delta^{13}C$-isoscapes into precipitation maps. As a case study, we modeled the spatial distribution of mean annual precipitation (MAP) in the northeastern Iberian Peninsula, a region with strong spatial variability for this variable. Precipitation maps were derived from isotope networks of two mostly co-occurring Mediterranean tree species, *P. halepensis* and *Q. ilex*, which are known to provide complementary information on seasonal precipitation [*Aguilera et al.*, 2012; *Ferrio et al.*, 2003]. After comparing the performance of isoscape-derived spatial models with those derived from meteorological records, the potential and

limitations for modeling spatial variability in precipitation from tree-ring isotope networks are discussed.

Materials and Methods

Study area and data compilation

[5] The study focused on the northeast area of the Iberian Peninsula, in the western Mediterranean basin (Fig. 1). The area is characterized by a complex topography and the interaction of African subtropical, North Atlantic and Mediterranean climate systems [*Gimeno et al.*, 2010; *Rodó et al.*, 1997]. MAP in the area ranges from *ca.* 300 mm to over 1000 mm. MAP at the sampling sites ranged from 391 to 965 mm. Precipitation generally show a bimodal seasonal distribution, with two maxima located in spring and autumn (see Fig. S1).

[6] Data on carbon isotope composition ($\delta^{13}C$) in whole wood from Holm oak (*Q. ilex* L.) and Aleppo pine (*P. halepensis* Mill.) were compiled from previous studies [*Aguilera et al.*, 2009; 2012; *Ferrio et al.*, 2003; *Ferrio and Voltas*, 2005]. Altogether, we used 44 sampling locations for *Q. ilex* and 38 for *P. halepensis*, respectively, with 15 locations being common to both species (Fig. 1b). Since our study focused on spatial variability, data compilation aimed at maximizing spatial resolution, being relatively flexible with temporal accuracy, as suggested in previous works when dealing with a limited number of continuous instrumental records [*AEMET-IM*, 2011; *Ninyerola et al.*, 2000; 2007]. Hence, in most cases compiled $\delta^{13}C$ data was determined from wood cores in which tree rings from several years were pooled together for analysis (24 ± 2.0 years for *Q. ilex*, 17 ± 2.8 years for *P. halepensis*), and covering different (yet widely overlapping) temporal ranges for the period 1975–2008. Precipitation data for the sampling sites were obtained from the Digital Climatic Atlas of the Iberian Peninsula, which provides a 200-m gridded reference precipitation, representative of monthly averages for the period 1950-1999 (data available at http://www.opengis.uab.es/wms/iberia/mms/index.htm); for details on the methodology used to generate the Atlas see [*Ninyerola et al.*, 2007]).

[7] Carbon isotope discrimination ($\Delta^{13}C$) was calculated from the $\delta^{13}C$ of samples and the $\delta^{13}C$ of the atmospheric CO_2 ($\delta^{13}C_{atm}$) [*Farquhar and Richards*, 1984]:

$$\Delta^{13}C = (\delta^{13}C_{atm} - \delta^{13}C) / (1 + \delta^{13}C / 1000) \qquad [1]$$

[8] $\delta^{13}C_{atm}$ was inferred by interpolating a range of data from Antarctic ice-core records [*Francey et al.,* 1999; *Indermühle et al.*, 1999; *Leuenberger et al.*, 1992], together with modern data from two Antarctic stations (Halley Bay and Palmer Station) of the CU-INSTAAR/NOAA-CMDL network for atmospheric CO2 measurements, as first described in [*Ferrio et al.*, 2005] (smoothed $\delta^{13}C_{atm}$ curve from 16100 BCE to present, available at http://web.udl.es/usuaris/x3845331/AIRCO2_LOESS.xls). Estimated $\delta^{13}C_{atm}$ for the time period represented in each sample ranged from -7.7 to -8.0‰.

Generation of multiple linear regression models to describe Δ13C from geographic variables

[9] Stepwise regression analysis was used to determine the best-fit model describing the spatial variability of $\Delta^{13}C$ based on geographic data consisting of 28 predictor variables (see section 2.3 for details). A forward selection procedure was used by which variables were progressively added provided they made a significant contribution to the model based on F-statistic values. Threshold F-statistics required for a variable to enter or stay in the model were set to 0.10 (F-to-enter) and 0.15 (F-to-remove), respectively. In this way, a multiple linear regression (MLR) model was built for each species predicting $\Delta^{13}C$ from geographic variables.

Candidate variables for MLR models

[10] A set of 28 independent predictor variables with known climatic relevance in the area was selected. Altitude, latitude, terrain curvature and aspect were directly derived or calculated from a 90-m resolution Digital Elevation Model (http://opengis.uab.es/wms/world/) using ArcGIS 10.0 (ESRI, Redlands, USA). Distances to different water bodies (Atlantic Ocean, Cantabrian Sea and Mediterranean Sea) and also the minimum distance to all seas were computed as indicators of continentality. We calculated both Euclidian (linear) and cost distances (i.e. the "cost" of traveling from one point to another, considering geographic barriers), which in turn were transformed into logarithmic and quadratic distances. Cost distance was calculated by (1) reclassifying a Digital Elevation Model (DEM) into a simple raster (also known as cost surface) by giving a subjective weight to different intervals of

altitude (see below), and (2) calculating, for each cell, the least accumulative cost distance over a cost surface to the water bodies. In this way, we generated a raster with the lowest total distance from a target cell to the nearest sea. Although we created several cost distance rasters with different weights, we opted for the one that provided the best correlation with $\Delta^{13}C$ in both species. DEM reclassification into a cost surface was done as follows: every 100 m until 2000 m (starting at 0 m) a linear increment of 1 m. However, from 2000 to 3500 m, we provided a greater weight ([2000 - 2500 = 30 m] and [2500 - 3500 = 50 m]). This was done in order to take into account a likely strong rain-shadow effect of high mountain peaks over maritime air masses. All of the above were calculated with the Cost Distance Tool of ArcGIS 10.0.

Generation of $\Delta^{13}C$-isoscapes from MLR and residual interpolation

[11] The *b* coefficients of the regression equations and the raster matrices of the independent (geographic) variables were implemented in a raster calculator tool in order to produce a 90-m resolution raster layer of $\Delta^{13}C$ for each species. The $\Delta^{13}C$-isoscapes derived from MLR models (hereafter, MLR-isoscapes) may yield biased estimates in areas underrepresented by sampling, as these have less weight in the regression model. To overcome this potential limitation, we applied an error interpolation method, which has been widely used in combination with MLR to model spatial patterns in climate from meteorological networks [*Agnew and Palutikof*, 2000; *Ninyerola et al.*, 2000; 2007; *Perry and Hollis*, 2005]. Briefly, the residuals (observed-predicted) from the MLR-isoscapes were spatially interpolated using the inverse distance weighting method to generate a residual layer (see supplementary Fig. S2). By adding this layer to the corresponding MLR-isoscapes, we ended up with a residual-interpolated $\Delta^{13}C$-isoscapes for each species (hereafter, RI-isoscapes). The addition of the residual layer was intended to correct for spatial trends, not originally accounted for by the MLR model, which potentially could have originated from the existence of spatial correlation in the distribution of errors. Finally, maps were imported to the Miramon V6.1 package (http://www.creaf.uab.es/miramon/index.htm) in order to add a mask of the areas not suitable for the studied species (topo-climatic suitability index<0.2; http://www.opengis.uab.cat/IdoneitatPI/).

Validation of $\Delta^{13}C$-isoscapes

[12] The predictive capability of the MLR- and RI-isoscapes was tested through a leave-one-out cross-validation process. Briefly, we fixed the selected input variables according to the best stepwise regression model, and generated a series of models (as many as samples) using all samples except one used for validation. The cross-validated root mean square error (RMSE) was calculated as

$$\mathrm{RMSE} = \sqrt{\sum \left(Y_{\mathrm{meas}} - Y_{\mathrm{mod}}\right)^2 / (N-1)} \qquad [2]$$

where Y_{meas} and Y_{mod} are, respectively, the measured and modelled Δ^{13}C values of the test samples not included in calibration during each iteration, and N is the number of samples. For each test sample, predictive error was determined before and after applying residual interpolation.

Deriving a map of annual precipitation from Δ^{13}C-isoscapes

[13] To take advantage of the differential seasonal responses of Δ^{13}C to precipitation in *P. halepensis* and *Q. ilex* [*Aguilera et al.*, 2012; *Ferrio et al.*, 2003], layers of MAP were generated by combining Δ^{13}C-isoscapes from both species. We built a family of 12 bi-specific models in which precipitation was predicted for subsets of consecutive months from Δ^{13}C in one species and for the remaining months in the other. Adding the predictions from each species provided an estimate of MAP for each sampling point. Since *P. halepensis* offered the best mono-specific model to estimate annual precipitation from carbon isotope discrimination, this model served as starting point for a process aimed at identifying the optimal bi-specific annual precipitation model by predicting two complementary sets of consecutive months from each of the two species. In the first instance, we used the best monthly precipitation model (highest R^2, October) of *Q. ilex* to provide a monthly estimate of precipitation to complement the estimate for the rest of the year obtained from *P. halepensis*. A leave-one-out cross-validation process was applied to this particular month combination using those sampling sites (15) in which both species were present as validation data (Fig. 2). The cross-validation RMSE was calculated as described for Δ^{13}C-isoscape models (Equation 2). Progressively, additional months predicted by *Q. ilex* instead of *P. halepensis* were added, either the previous or the following to the one initially included, retaining the month combination with lowest RMSE. We ended up with 12 annual models, the last one being an annual precipitation

model where only *Q. ilex* was used. Out of the 12 models built, we decided which combination of months using carbon isotope discrimination of both species provided the lowest RMSE value, and thus the best predictive annual precipitation model. To avoid confusions, RMSE values at the model selection step will be referred to hereafter as $RMSE_{reg}$. Finally, to generate a spatial model of MAP, the regression coefficients for each species relating reference precipitation to $\Delta^{13}C$ were applied to the corresponding $\Delta^{13}C$-isoscapes using a raster calculator tool.

Validation of spatial models of precipitation

[14] We evaluated the strength of our model predictions by direct comparison with an independent dataset of long-term averages of MAP from 573 climatic stations, available for the period 1971–2000 (MAP=303-1086 mm) [*AEMET-IM*, 2011]. To this end, observed and predicted data were compared and the coefficient of determination (R^2), slope and RMSE were calculated. To avoid confusions, RMSE values determined to validate MAP predictions will be referred to hereafter as $RMSE_{map}$. The coefficient of variation (CV, expressed in %) of model predictions was then calculated as the quotient between RMSE and the mean of the set of meteorological stations used for validation. Spatial patterns in model performance were assessed by interpolating relative errors (% of observed values) using the inverse distance weighting method. As a reference for our model statistics, we applied the same procedure used to generate $\Delta^{13}C$-isoscapes to model annual precipitation directly from the aforementioned network of climatic stations. We generated a family of models to assess the potential role of seasonal precipitation distribution and sampling density in defining model performance. Thus, we modeled annual precipitation either directly or as the sum of two seasonal models (as done in $\Delta^{13}C$-isoscapes), and compared models generated with a high sampling density (60% of randomly selected stations, following *Ninyerola et al.* [2007]; $N = 340$) with models with low sampling density, generated with a subset of 65 weather stations having the lowest Euclidian distance to the corresponding tree-ring sampling sites.

Results

Isoscape modeling of $\Delta^{13}C$

[15] Species-specific multiple linear models predicting Δ^{13}C from geographic variables showed a similar linear fit, with $R^2 = 0.56$ ($N = 44$) for *Q. ilex* and $R^2 = 0.48$ (N = 38) for *P. halepensis*. For *Q. ilex,* variables entering the model were (1) logarithmic cost distance to all seas, (2) latitude and (3) altitude, in that order of significance (Fig. 3a). For *P. halepensis*, selected variables were (1) latitude, (2) Euclidian distance to all seas and (3) altitude, in that order of significance (Fig. 3b). Further details of the fitted models are given in Table 1.

[16] Modeled values for *Q. ilex* ranged from 16.9‰ to 21.2‰ (range of calibration samples: 16.8-19.9‰), having the highest values in the coastal strip (MLR-isoscape, Fig.3c), which highlights the strong influence of continentality (distance to all seas) in the model (Table 1). A latitudinal trend was also observed, with lower values to the south, and the effect of altitude was particularly visible in the abrupt northern river valleys of the Pyrenees. Overall, a similar pattern was observed in the *P. halepensis* model (MLR-isoscape, Fig. 3d); however, since the weights of each variable in this model were similar (Table 1), the effect of continentality was not as strong as for *Q. ilex*, and the latitudinal gradient was clearer. Predicted values of Δ^{13}C for *P. halepensis* ranged from 15.0‰ to 18.9‰ (range of calibration samples: 15.4-18.0‰). Validation statistics for Δ^{13}C-isoscape models are provided in Table 2.

Predicting MAP from $\Delta^{13}C$-isoscapes using a bi-specific model

[17] The lowest $RMSE_{reg}$ (78.3 mm) in predicting MAP from Δ^{13}C was found using *Q. ilex* to infer precipitation from September to October, and *P. halepensis* for December to August (Fig. 2). However, the next best-fitting month combination provided almost identical quality (i.e. accuracy and precision; $RMSE_{reg}$=78.6 mm) by predicting the whole autumn season (September–November) from *Q. ilex* and the rest of year from *P. halepensis*. From a climatological point of view, it seemed sensible to have the final combination of months grouped by seasons, so the latter model was finally adopted, as follows:

$$P_{sept-nov} = 46.4 \times \Delta^{13}C_{Quercus} - 640 \qquad R^2=0.41, N=44, P<0.001 \qquad [3]$$

$$P_{dec-aug} = 106.7 \times \Delta^{13}C_{Pinus} - 1402 \qquad R^2=0.63, N=38, P<0.001 \qquad [4]$$

$$MAP = P_{sept-nov} + P_{dec-aug} \quad [5]$$

[18] Comparing predicted MAP with observed precipitation (Table 2), we found higher predictive ability using the RI-isoscapes ($RMSE_{map} = 84$ mm) than the MLR-isoscapes ($RMSE_{map} = 102$ mm) (Table 2). Before applying a residual correction, models calibrated with instrumental data performed similarly to the MLR-isoscapes, regardless of sampling density ($RMSE_{map} = 97–111$ mm) (Table 2). After residual correction, models based on meteorological data showed similar results to RI-isoscapes when station density was comparable to that of the tree-ring network (N=65, $RMSE_{map}$=80-83 mm), but showed higher predictive ability when using a high-density sampling network (N=340, $RMSE_{map}$ = 56-57 mm) (Table 2). In all cases, modeling spatial patterns in MAP from annual climatic data or as the sum of two seasonal values (i.e. emulating $\Delta^{13}C$-derived models) gave almost identical results. The predicted distribution of MAP generally agreed with the observed precipitation patterns in the area (Fig 4a, as compared to Fig. 1b). Nevertheless, the spatial patterns of predictive errors revealed some areas with substantial overestimations (>20%) (Fig. 4b).

Discussion

How good is a $\Delta^{13}C$-isoscape model at predicting MAP?

[19] Multiple linear models based on geographic variables explained about 50% of $\Delta^{13}C$ in tree-rings, an outcome comparable to that reported when modeling $\Delta^{13}C$ directly from climatic variables (e.g. [*Aguilera et al.*, 2009; *Ferrio et al.*, 2003; *Sidorova et al.*, 2010; *Treydte et al.*, 2007; *Warren et al.*, 2001]). This supports our initial assumption that $\Delta^{13}C$ can be spatially modeled in the same way as the climate variables that have a strong influence on it (e.g. precipitation). Furthermore, applying a transfer function to derive mean annual precipitation from bi-specific $\Delta^{13}C$, we were able to generate spatial maps of annual precipitation, which in turn were validated with independent data from meteorological stations. At this step, we tested two kinds of isoscapes: those derived exclusively from the multiple linear models represented in Fig.1 (MLR-isoscapes), and those in which an additional residual correction was applied (RI-isoscapes). Despite having a relatively sparse tree-ring network (0.5×10^{-3} sites km^{-2}), the predictive ability obtained with the RI-isoscape (RMSE= 84 mm; CV=16.4%, see Table 2) is comparable to that of other studies modeling

precipitation from much denser meteorological networks e.g. for the whole Iberian Peninsula (3.5×10^{-3} stations km^{-2}; RMSE = 137 mm; [*Ninyerola et al.*, 2007]; CV>19.5%, calculated for an average MAP < 700 mm, according to Spanish Meteorological Agency [*AEMET-IM*, 2011]), or for the different river basins included in the study region (5.8×10^{-3} stations km^{-2}; RMSE = 67–147 mm [*Sánchez Palomares et al.*, 1999]). Similarly, the performance of isoscape-derived models was nearly identical to that found for models based on a meteorological network with a spatial distribution resembling that of our sampling sites (low-density meteorological network; 0.8×10^{-3} stations km^{-2}; CV=15.9-16.5%, see Table 2). Only our reference models calibrated with a high-density network of weather stations (4.3×10^{-3} stations km^{-2}) performed better than the RI-isoscapes in predicting MAP, and this only after applying residual interpolation (33% lower RMSE, CV=11.1%). It should be noted here that the slight differences between the temporal range covered by $\Delta^{13}C$ samples (1975-2008) and the meteorological data used for validation (1971-2000, [*AEMET-IM*, 2011]) might have artificially decreased the predictive accuracy of the isoscape models. Despite this, our results indicate that it is possible to obtain reasonable spatial predictions of precipitation from tree-ring $\Delta^{13}C$ networks, with comparable accuracy to that reached using meteorological networks, thus providing a unique opportunity to validate global climate models, well beyond the oldest instrumental records.

Combined use of pines and oaks to reconstruct MAP

[20] One of the advantages of this approach is that it relies on well known physiological responses of different species, taking advantage of their different behavior to build more precise regional spatial models. In our case, for instance, the two species showed a complementary response to seasonal precipitation, in agreement with previous works [*Aguilera et al.*, 2012; *Ferrio et al.*, 2003]. This was observed not only in the relationship between $\Delta^{13}C$ and monthly precipitation (Fig. 2), but also in the different set of geographic variables defining precipitation (Fig. 3, Table 1). For instance, $\Delta^{13}C$ in *Q. ilex* is mainly sensitive to autumn precipitation [*Aguilera et al.*, 2012; *Ferrio et al.*, 2003], while continentality appears to be a key geographic factor defining $\Delta^{13}C$ spatial variability for this species. In this regard, autumn–winter precipitation in this region is caused either by the long-distance transport of moisture within the tropical–subtropical North Atlantic corridor or by Mediterranean cyclogenesis and, thus, is mainly driven by maritime air masses [*Millán et al.*, 2005], in other words, continentality plays a major role in defining spatial distribution of

precipitation. In contrast, spring and summer precipitation is strongly affected by convective episodes [*Gimeno et al.*, 2010] and, thus, continentality has less influence over the spatial distribution of precipitation during this period. Accordingly, *P. halepensis*, more sensitive to spring–summer precipitation, shows a more balanced contribution of geographic variables explaining $\Delta^{13}C$ than *Q. ilex*, with latitude being the most influential. Modeling spatial distribution of monthly precipitation in the area, *Ninyerola et al.* [2000] also found that continentality played the most significant role in early autumn, while the greatest influence of latitude was during spring–summer. This trend is further confirmed in our reference precipitation models, showing a major role of continentality for autumn precipitation, while latitude takes first place for the rest of the year. Finally, in both species altitude showed a positive effect on $\Delta^{13}C$. However, this effect was relatively weak when compared to observed (positive) altitudinal trends in MAP in the area ([*Ninyerola et al.*, 2000], Table 1). This could be due to other environmental variables, such as temperature or atmospheric pressure, exerting negative effects on $\Delta^{13}C$ along altitudinal gradients [*Kohn*, 2010; *Körner et al.*, 1991]. As a result, although both $\Delta^{13}C$ and MAP show an overall positive response to altitude, they do not share the same kind of linear response, and this may be one of the causes for the observed underestimation of MAP in mountain areas. In this regard, combining $\Delta^{13}C$ with other tree-ring proxies (e.g. oxygen isotopes, wood density), more sensitive to temperature, may help to better define the role of different geographic variables in precipitation.

Residual interpolation as the bottleneck for model performance

[21] According to our results, it is at the interpolation step where sampling density plays a major role at improving the quality of spatial inferences. Whereas all MLR models showed a similar predictive ability, error interpolation caused a much bigger improvement in the high-density meteorological network (43% reduction in RMSE) as compared to the low-density meteorological network (27% reduction in RMSE) or the isoscapes-based model (17% reduction in RMSE). Most likely, interpolating errors in sparse or uneven sampling networks could result in a single point value influencing a large area, thus leading to error propagation. For instance, we can point at two zones with large overestimation errors (40.5–41.5ºN, 0.5–1.5ºW; 42.0–42.5ºN, 1.5–2.0ºW, see Fig. 3b), which correspond to marginal areas considering our sampling network. A third area (41.5–42.0ºN, 0.0–1.0ºE) corresponds to a semi-arid area of the mid Ebro Valley (MAP = 300–400 mm). This zone is strongly deforested and the nearest available sampling sites are located in slightly wetter areas. Consequently, MAP in this zone falls below the range covered by our sampling locations (391-965 mm), which may

have lead to an overestimation by the model. Nevertheless, although having a denser tree-ring sampling network would have improved model predictions ([*Agnew and Palutikof*, 2000], this work), our results indeed suggest that correcting for residuals in relatively low-density networks is still worthwhile.

[22] Besides sampling density, the relatively small effect of interpolation on isoscapes-derived models could also be attributed to the fact that we did not use meteorological data for correction, but Δ^{13}C values instead. Paradoxically, residual interpolation of Δ^{13}C caused a greater improvement in the prediction of MAP than on Δ^{13}C itself: although residual interpolation increased notably the R^2 derived from the leave-one-out cross validation for Δ^{13}C (18.5-22.6% higher, see Table 2), relative changes in RMSE for Δ^{13}C predictions were smaller than those observed for MAP (6.6% and 4.4% reduction in RMSE, for $\Delta^{13}C_{Quercus}$ and $\Delta^{13}C_{Pinus}$, respectively). It should be noted, however, that given the low density of the sampling network, the cross-validation procedure might have underestimated the increase in predictive power of the RI-isoscapes, due the strong effect of excluding the most isolated sites from the interpolation (see error maps in Fig. S2). Hence, these RMSE values are not directly comparable to those obtained through validation with an independent set of samples, as was the case for MAP. Nevertheless, we cannot rule out compensation effects resulting from the combination of Δ^{13}C from both species. Indeed, considering the 15 sites where Δ^{13}C values for both species were available (N=15), residual interpolation caused a higher decrease in RMSE (20.9%) for site-averaged Δ^{13}C values than for each individual species (1.9% and 17.9%, for $\Delta^{13}C_{Quercus}$ and $\Delta^{13}C_{Pinus}$, respectively). This further confirms our findings that combining data from species with different growing requirements it is possible to obtain a more robust prediction of MAP than with mono-specific models.

Limitations and future prospects

[23] Our approach opens an encouraging field of research, suggesting that continuous climate layers, either annually or multi-annually resolved, can be derived from tree-ring isotope networks, even in areas with complex topography. Physiological responses to climate are species-dependent, but this should not be seen as a constrain for this approach, since similarly strong relationships between Δ^{13}C and different climate variables have been reported for many species around the world, and thus the method is potentially extensible to other regions of study [*Büntgen et al.*, 2010; *Frank et al.*, 2008; *Leavitt et al.*, 2007; 2010; *Sidorova et al.*,

2010; *Treydte et al.*, 2007; *Warren et al.*, 2001]. Even when comparing unrelated species (e.g. conifers and angiosperms) or contrasting functional types (evergreen and deciduous) some common patterns for $\delta^{13}C$ in tree-rings have been found both at the regional scale (this work, but also [*Reynolds-Henne et al.*, 2007; *Treydte et al.*, 2007]) and at the global scale [*Schubert and Jahren*, 2011]. In any case, as in other dendroclimatic approaches, species-specific calibrations are needed to determine which climatic variables and during which period of the year can be reflected in wood $\Delta^{13}C$. However, unlike classical dendroclimatology, which usually deals with site-specific calibrations, $\Delta^{13}C$-isoscapes are likely to work better with multi-site calibrations. On the other hand, although examining spatial trends over wider areas might be challenging due to species- or site-specific responses, these can provide additional climatic information. For example, [*Treydte et al.*, 2007] found time-dependent spatial trends in the relationship between $\delta^{13}C$ and climatic variables across European tree-ring chronologies, which might be related to the differential response of the species used, but also to changing effects of climate forcing mechanisms in different areas. In this regard, combining the geographic information provided by isoscape models with climate-response functions of stable isotopes in tree rings, it might be possible to go one step forward in the application of tree-ring signals as palaeoenvironmental proxies, by providing feedback to global circulation models.

[24] According to the results presented, exploring past precipitation patterns using tree-ring networks seems a feasible task, allowing upscaling of paleoclimate inferences for climate model data comparisons. One limitation of this approach is its dependence on sampling density, which might be limited for very long tree-ring chronologies. Nevertheless, even using a relatively low sampling density we can capture the forcing factors defining precipitation using a topo-geographic model. Thus, besides getting insight into spatial patterns of precipitation, changes over time in the role of geographic variables explaining tree-ring $\Delta^{13}C$ for different species could be interpreted in terms of varying relevance of forcing factors affecting precipitation. Likewise, given that vegetation productivity in semi-arid regions depends mainly on seasonal rainfall patterns [*Guttal and Jayaprakash*, 2007], combining information from species with differential seasonal responses (e.g. pines and oaks) it is likely to provide a useful insight into past ecosystem dynamics.

[25] In addition to tree-ring networks, the same methodology is potentially applicable to other spatially explicit paleoenvironmental records (e.g. lake sediments, speleotherms). But whatever the proxy, the large-scale forcing factors imprinting a spatial climate signal must be

shared throughout the sampling network, otherwise climatic patterns might be too complex to be explained by geographical variables alone. The methodology is thus particularly suitable at regional scales, where it can complement with high spatial resolution data the outcome of global circulation models.

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Figures and Tables

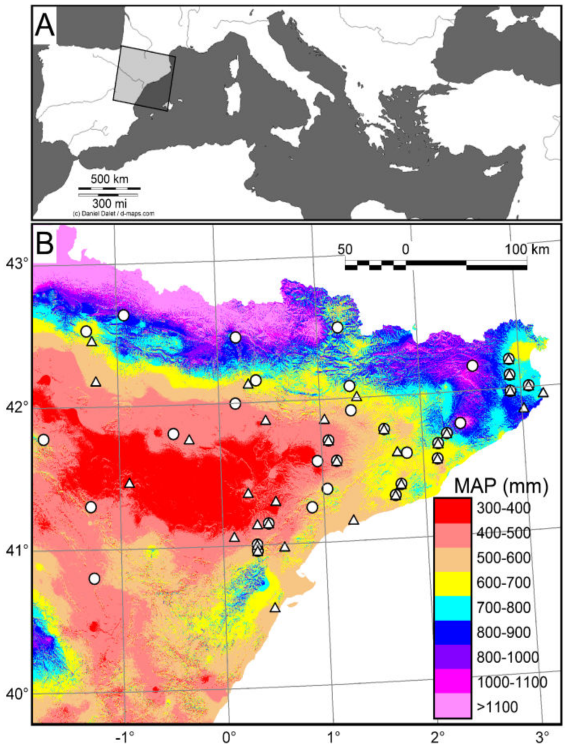

Fig. 1 Study area and sampling sites. (A) Location of the study area. (B) Mean annual precipitation (MAP) in the area (source: [*Ninyerola et al.*, 2007]) and location of sampling sites. Circles, *Quercus ilex*; triangles, *Pinus halepensis.*

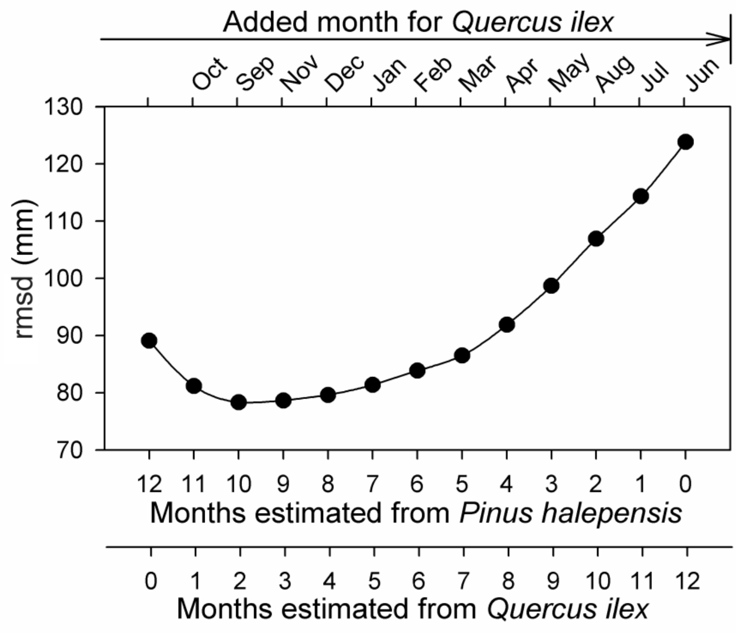

Fig. 2. Root mean square error (RMSE) of a leave-one-out cross-validation procedure aimed at selecting the best combination of months estimating mean annual precipitation from carbon isotope discrimination of *Pinus halepensis* and *Quercus ilex*. RMSE was determined using a subset of sampling sites (15) common to both species as validation data (see section 2.3 for details).

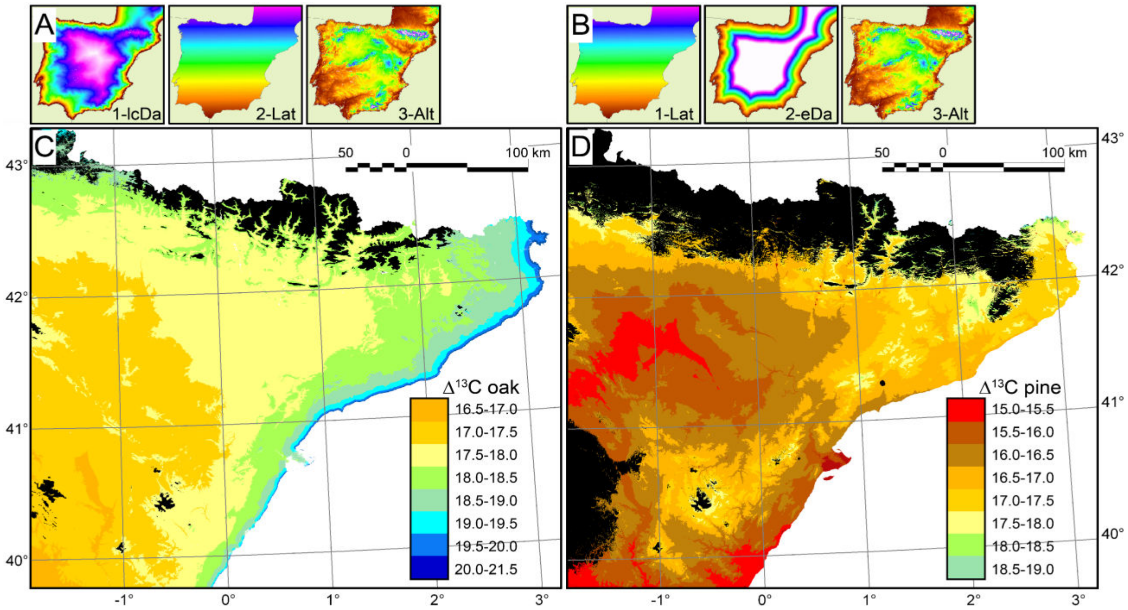

Fig. 3. Main steps in the generation of Δ^{13}C-isoscapes. (A, B) Raster images of the geographical variables chosen in the step-wise multiple regression, in order of significance (1–3), for *Quercus ilex* and *Pinus halepensis*, respectively (lcDa, logarithmic cost distance to all seas; eDa; Euclidian distance to all seas; Lat, latitude; Alt, altitude). (C, D) Δ^{13}C-isoscapes of *Q. ilex* and *P. halepensis* respectively, derived after implementing the *b* coefficients into a raster calculator. Areas not suitable for each species (topo-climatic suitability index<0.2; http://www.opengis.uab.cat/IdoneitatPI/index.html) are masked in black. Further details on the models are provided in Table 1.

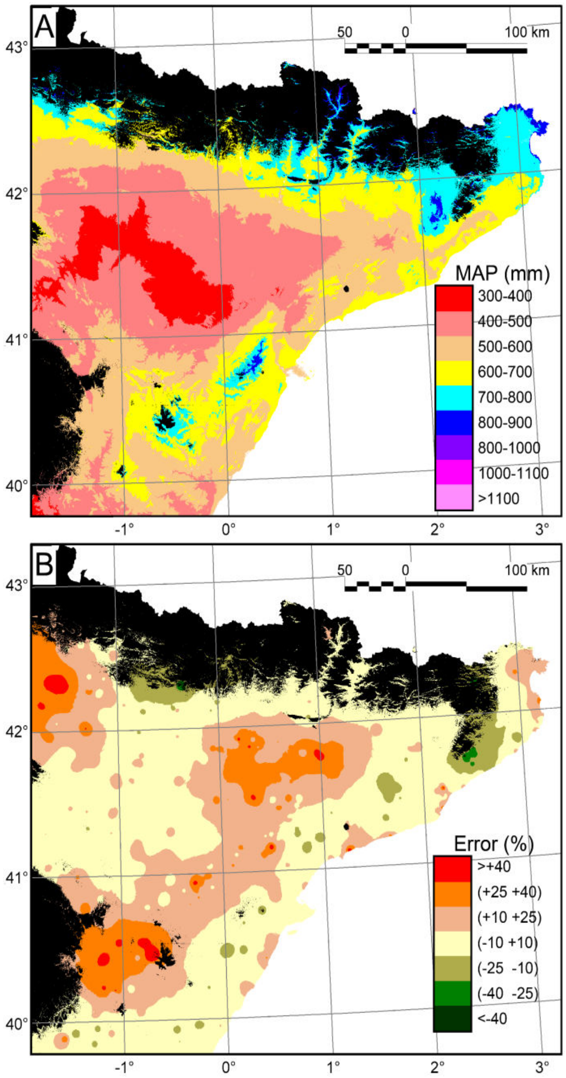

Fig.4. Modeled mean annual precipitation (MAP) and associated error map. (A) MAP modeled from residual-interpolated Δ^{13}C-isoscapes. (B) Spatial trends of relative errors in predicted MAP ((predicted-observed)/observed, in %). Areas not suitable for both species are masked in black (topo-climatic suitability index<0.2; http://www.opengis.uab.cat/IdoneitatPI/index.html).

Table 1. Calibration statistics of step-wise multiple regression models of spatial distribution of carbon isotope discrimination, and for spatial models of precipitation generated from instrumental records[a].

Calibration network	Modeled variable	N	Regression coefficients, order of entry and increase in R^2								Total R^2
			Y_0	Latitude	Altitude	lcDa	eDm	eDc	eDa	cDc	
Tree-ring sampling sites	$\Delta^{13}C_{Quercus}$	44	5.1	4.16E-06	1.25E-03	-1.35					0.56
				(2) 0.05	(3) 0.07	(1) 0.44					
	$\Delta^{13}C_{Pinus}$	38	-23.5	8.76E-06	1.63E-03				-1.15E-05		0.46
				(1) 0.18	(3) 0.12				(2) 0.16		
Weather stations (Low-density)	$P_{sept\text{-}nov}$	65	-2202	5.21E-04	5.78E-02				-7.81E-04		0.52
				(2) 0.28	(3) 0.03				(1) 0.20		
	$P_{dec\text{-}aug}$	65	-6606	1.44E-03	1.66E-01			9.20E-04			0.61
				(1) 0.33	(3) 0.08			(2) 0.19			
	MAP	65	-8704	1.91E-03	1.75E-01			1.33E-03			0.57
				(1) 0.28	(2) 0.25			(3) 0.05			
Weather stations (High-density)	$P_{sept\text{-}nov}$	340	-539	1.69E-04	6.68E-02				-7.27E-04	-4.89E-05	0.56
				(2) 0.08	(3) 0.06				(1) 0.39	(4) 0.027	
	$P_{dec\text{-}aug}$	340	-2199	5.97E-04	2.93E-01		-1.10E-03			-2.12E-04	0.53
				(1) 0.07	(3) 0.23		(2) 0.12			(4) 0.11	
	MAP	340	-2533	7.30E-04	3.77E-01		-1.69E-03			-3.13E-04	0.54
				(2) 0.25	(3) 0.16		(1) 0.11			(4) 0.12	

Table 2. Validation statistics of spatial models of carbon isotope discrimination ($\Delta^{13}C$, in ‰) and mean annual precipitation (MAP, in mm)[a].

Calibration network	Modeled variable	N_{cal}	N_{val}	Mean ±SD	MLR				MLR + RI			
					RMSE	CV	R^2	b	RMSE	CV	R^2	b
Tree-ring sampling sites	$\Delta^{13}C_{Quercus}$	43	44[b]	18.1 ±0.68	0.53	2.9	0.41	0.85	0.49	2.7	0.51	0.81
	$\Delta^{13}C_{Pinus}$	37	38[b]	16.8 ±0.72	0.58	3.2	0.36	0.86	0.56	3.1	0.42	0.83
	$P_{sept-nov}$ ($\Delta^{13}C_{Quercus}$) + $P_{dec-aug}$ ($\Delta^{13}C_{Pinus}$)	44 38	573	512 ±141	101	19.7	0.48	0.47	84	16.4	0.65	0.62
Weather stations (Low-density)	$P_{sept-nov}$ + $P_{dec-aug}$	65	508	504 ±138	111	22.0	0.40	0.53	83	16.5	0.64	0.74
	MAP				111	22.1	0.38	0.48	80	15.9	0.67	0.68
Weather stations (High-density)	$P_{sept-nov}$ + $P_{dec-aug}$	340	233	503 ±132	97	19.3	0.44	0.48	56	11.1	0.82	0.84
	MAP				100	19.9	0.49	0.51	57	11.3	0.82	0.84

[a] $P_{sept-nov}$, precipitation during autumn months; $P_{dec-aug}$, precipitation during the rest of the year. $\Delta^{13}C_{Quercus}$ and $\Delta^{13}C_{Pinus}$, carbon isotope discrimination of *Quercus ilex* and *Pinus halepensis*, respectively. MLR and MLR+RI, regression models before and after residual interpolation, respectively. N_{cal} and N_{val}, number of samples used for calibration and validation, respectively. Mean ±SD, mean and standard deviation of the set of stations used for validation; RMSE and CV, root mean square deviation and coefficient of variation (in %) of model predictions, respectively; R^2 and b, coefficient of determination and slope of the regression line between observed and predicted values, respectively.

[b] For $\Delta^{13}C$, validation statistics are derived from a leave-one-out cross validation.

Supporting Material

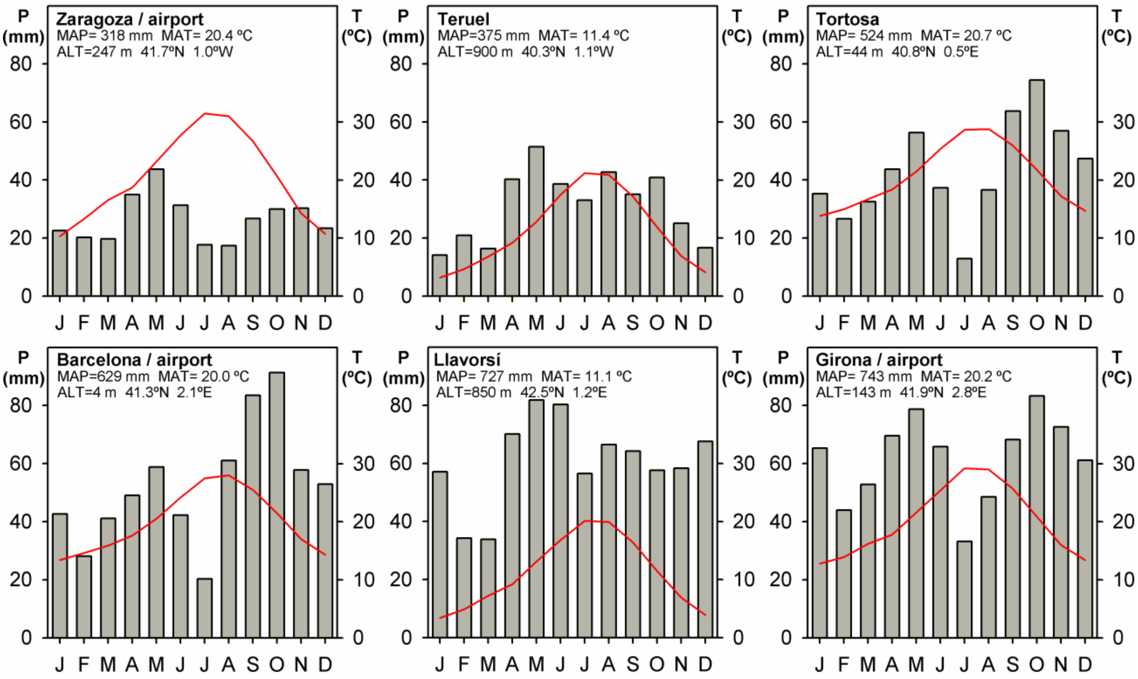

Fig S1. Climographs of a selection of weather stations, representative of the climatic variability in the region. MAP, mean annual precipitation; MAT, mean annual temperature; ALT, altitude above sea level. Source: State Meteorological Agency of Spain.

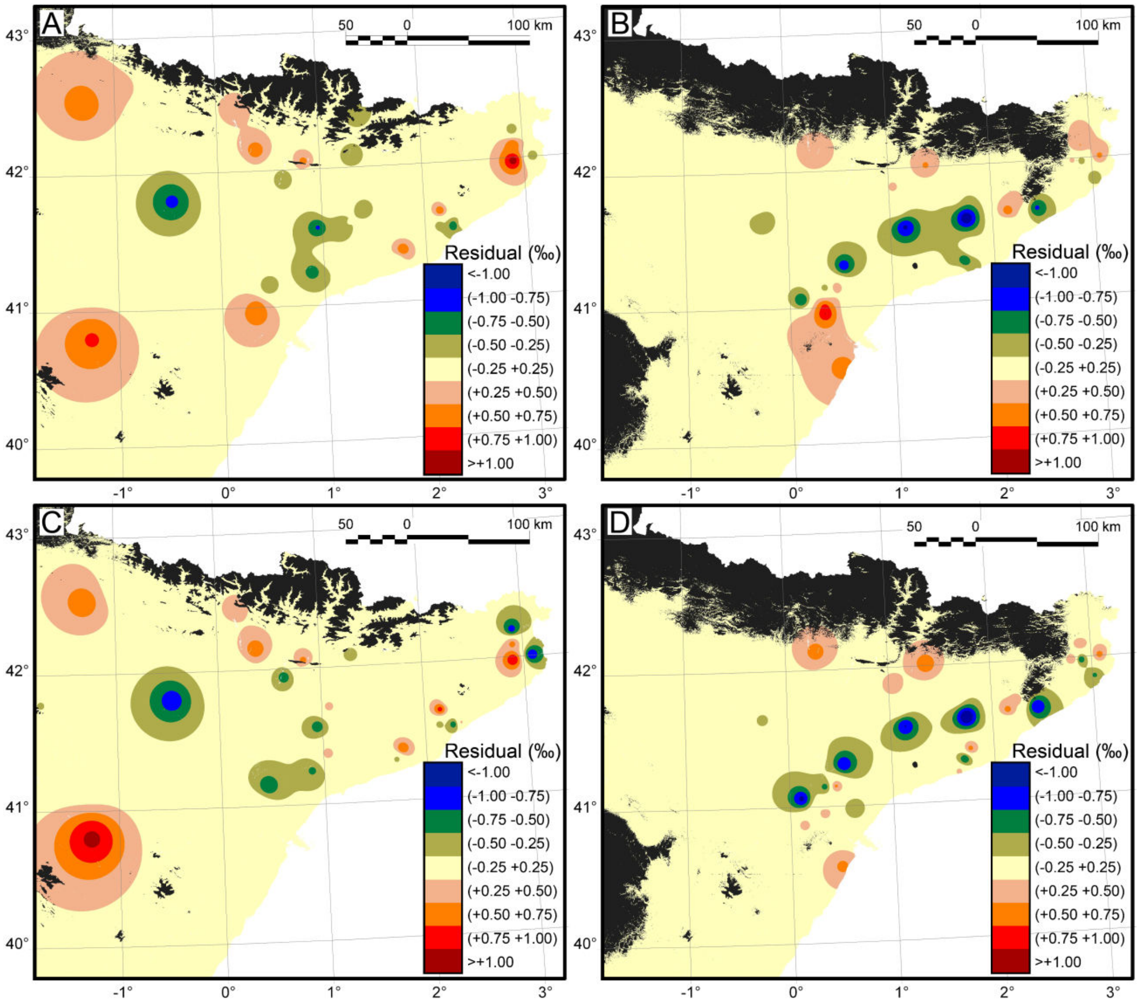

Fig S2. Interpolated residual maps (observed-predicted) for Δ^{13}C. (A,B) Residual interpolation layer of the multiple linear regression models of Δ^{13}C (MLR-isoscapes) displayed in Fig. 3. These layers were added to the MLR-isoscapes to generate the residual-interpolated isoscapes (RI-isoscapes). (C,D) interpolation of residuals resulting from the leave-one-out cross-validation,.used to estimate the predictive capability for Δ^{13}C of the RI-isoscapes (see Section 2.5 for details). (A, C) *Quercus ilex*; (B, D) *Pinus halepensis*.

Chapter II

Example of tree-ring cores of *Pinus halepensis* used to build the tree-ring chronologies for each site. Numbers indicate provenance regions (or adaptive units).

> *"The reason birds can fly and we can't is simply because they have perfect faith, for to have faith is to have wings."* **J.M. Barrie**, ***The Little White Bird***

Carbon isotope discrimination, radial growth, and NDVI share spatiotemporal responses to precipitation in Aleppo pine

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Key message. A common pattern in Aleppo pine $\Delta^{13}C$ responses to both spatial and temporal variability in precipitation was observed, with a general agreement between NDVI, $\Delta^{13}C$ and growth that confirms precipitation as key environmental driver.

Abstract

The aim of this study was to assess the spatio-temporal variability of carbon isotope discrimination ($\Delta^{13}C$) records and its relationship with radial growth (RG) and Normalized Difference Vegetation Index (NDVI) data using a tree-ring network of Aleppo pine (*Pinus halepensis* Mill.) in the eastern part of the Iberian Peninsula. For this purpose, we collected a biennial time series of $\Delta^{13}C$ (1949–1998), together with mean annual precipitation, tree-ring width, and remote sensing (NDVI) data for seven locations along a precipitation gradient. We evaluated how intra-site correlations between variables changed across locations, and how inter-site (or spatial) correlations changed across years. We found that correlations between $\Delta^{13}C$ and precipitation were higher in dry than in wet sites, in agreement with previous studies. Mean RG and NDVI were good indicators of site-specific $\Delta^{13}C$ sensitivity to precipitation. The strongest spatial associations between $\Delta^{13}C$ and precipitation were also found during the driest biennia. However, spatial correlations were strongly affected by carry-over effects of extreme events. Overall, we found a good agreement between $\Delta^{13}C$, NDVI, and RG, although they showed different response patterns to precipitation. We suggest that the combination of these proxies may be useful for monitoring changes in water-use efficiency and productivity at the regional level.

Keywords: carbon isotopes, tree rings, vegetation indices, water-use efficiency, dendroecology

Introduction

Tree rings are extraordinary repositories of climate information. The rising number of tree-ring networks available worldwide can offer new insights on the spatial variability of climate at regional scales (Treydte et al. 2007; Leavitt et al. 2008; del Castillo et al. 2013). Such kind of spatiotemporal information may be relevant to bridge the existing knowledge gap in climate dynamics between large-scale global circulation models and instrumental records of limited geographical coverage (Brayshaw et al. 2011). However, tree sensitivity to climate may vary in time and space for different reasons, including phenotypic plasticity and genetic variability (e.g. Voltas et al. 2008; de Luis et al. 2013), individual life history (Hereş et al. 2012; Voltas et al. 2013), and community structure or local environmental conditions (e.g. Martín-Benito et al. 2011; Moreno-Gutiérrez 2012), among others. Hence, in order to maximise the information retrieved from tree-ring networks, there is a need to explore the environmental drivers underlying spatiotemporal vegetation responses (Treydte et al. 2007; Maseyk et al. 2011; de Luis et al. 2013; del Castillo et al. 2013).

The dendrochronological archive of carbon isotope discrimination ($\Delta^{13}C$) tracks the balance between assimilation rate and stomatal conductance (or intrinsic water-use efficiency; Farquhar and Richards 1984), thus aiding at characterizing tree physiological status (Korol et al. 1999; Ferrio et al. 2003). However, complementary physiological information is needed to disentangle the role of photosynthetic potential, canopy structure and stomatal limitations in determining $\Delta^{13}C$ (Martín-Benito et al. 2011; Moreno-Gutiérrez et al. 2012). In this regard, vegetation indices constitute another type of regional-scale ecological record (Kaufmann 2004; Beck et al. 2013), being now available over a sufficiently long time span as to complement tree-ring records with useful ecophysiological information. In particular, an index of vegetation greenness such as the Normalized Difference Vegetation Index (NDVI) provides estimates of canopy photosynthetic capacity at different spatial scales through its correlation to both leaf area index (Gamon et al. 1995; Myneni et al. 1997) and the fraction of photosynthetically active radiation absorbed by ecosystems (Gamon et al. 1995).

Previous studies have reported strong positive correlations between summer NDVI and tree-ring width (e.g. Kaufmann 2004; Wang et al. 2004; Beck et al. 2013). Most often summer NDVI appears as the only period of the year related to growth in temperate climates, pointing to the time in which climate exerts the greatest effect on tree-ring width (Kaufmann et al. 2008; Leavitt et al. 2008). However, annual NDVI can be, in some cases, a better integrator of ecosystem productivity, incorporating additional vegetation features such as early spring activity or changes in phenology (Alcaraz-Segura et al. 2008). Thus, the combination of NDVI and tree-ring width, together with climatic data, may offer a better understanding of the biophysical drivers underlying changes in $\Delta^{13}C$ of tree rings.

The main objective of this study was to understand the relationship between climate and tree sensitivity for stable carbon isotopes in *Pinus halepensis* Mill., a widespread, drought-avoidant circum-Mediterranean species, and whether this relationship is subject to variability in time and space. We hypothesised that: 1) the sensitivity of tree-ring $\Delta^{13}C$ to precipitation, as a major biophysical factor modulating ecosystem functioning in Mediterranean climates, would be highly variable in both time and space, hence revealing contrasting tree performances across precipitation gradients; and 2) since NDVI and radial growth are also influenced by precipitation dynamics in drought-prone environments, they must correlate with $\Delta^{13}C$ to a varying degree depending on the particular restrictions imposed by precipitation on tree functioning. To test these hypotheses, we evaluated the spatio-temporal variability of $\Delta^{13}C$ derived from tree rings of *P. halepensis* and its connection to precipitation, NDVI and tree-ring width records.

Materials and Methods

Study area

The study area is located in the Northeastern Iberian Peninsula, Western Mediterranean basin (Fig. 1). The region is dominated by a Mediterranean climate, with warm and dry summers and mild winters, but also includes continental areas with cool and dry winters, and more humid zones with less seasonality (Table 1). Seven sites were chosen along an annual precipitation gradient ranging from 376 to 835 mm (mean = 562 mm, period 1949–1998; Table 1). Each site was selected to be representative of a distinct eco-geographic region according to the classification of *P. halepensis* provenances (or adaptive units) in Spain (Gil et al. 1996). The provenances represented in this study were: no. 1 (Alta Catalunya; temperate humid Mediterranean); no. 2 (Catalunya Litoral; warm sub-dry Mediterranean maritime); nos. 3, 4, and 5 (Catalunya Interior, Bárdenas-Ribagorza, and Ibérico Aragonés; warm sub-dry sub-Mediterranean); and nos. 6 and 15 (Monegros-Depresión del Ebro and Bética Meridional; warm dry sub-Mediterranean).

Sampling strategy and development of tree-ring chronologies

For each site, we sampled wood cores (5 mm in diameter) at 1.30 m from the south side of 7 to 11 dominant individuals (Table 1). Samples were oven-dried at 60ºC for 48 h and their outermost part removed with a scalpel for tree-ring dating. Tree-ring width was measured with a binocular microscope coupled to a PC with the program TSAP v. 3.0 (Frank Rinn, Heidelberg). We assessed the quality of the chronologies with COFECHA through calculation of the Expressed Population Signal (*EPS*) statistic (Holmes 1983):

$$EPS = \frac{N \times R}{N \times R + (1 - R)} \qquad [1]$$

where *N* is the number of individuals and *R* is the mean inter-series correlation. All chronologies reached the threshold of *EPS* = 0.85, ranging from 0.853 in Girona to 0.955 in Lanaja (Table 1).

After cross-dating, standardised ring-width chronologies were built for each site with ARSTAN (Cook and Holmes 1986). First, a double-detrending step was performed for each tree-ring series. The residuals of the first detrending (best-fit curve, linear or exponential) were fitted with a cubic smoothing spline of 50% frequency cut-off of 32 years. This resulted in a standardised tree-ring series for each individual, in the form of:

$$TRW_i = RG_i / F_i \qquad [2]$$

where TRW_i, RG_i, and F_i stand for indexed tree-ring width, measured ring width (or radial growth), and fitted ring width, respectively, at year *i*. Subsequently, each series was modelled as a stationary autoregressive process, resulting in a 'residual' index chronology. Finally, a composite autoregressive model across all tree-ring series was added to each 'residual' chronology, resulting in the 'arstan' chronology (Cook and Holmes 1986). All subsequent analyses were performed using 'arstan' chronologies.

Carbon isotope discrimination in tree rings

For stable isotope analysis, we pooled samples across individual trees and every 2 years (from 1949–1950 to 1997–1998) at each site. This decision stemmed from the need to balance the recovery of high-frequency variability in the isotopic signal against analytical cost. For the sake of comparison, we checked the loss in annual variability of TRW_i associated with a hypothetical biennial analysis of tree-ring series, which ranged from 23.4% in Rasquera to 40.0% in Lanaja (mean = 33.1%). This indicates that approximately two-thirds of the original TRW_i signal had been retained if using this pooling procedure.

After pooling, samples were milled (IKA-A10) to a fine powder. To minimize juvenile effects, we omitted the first 20 years of cambial age (Loader et al. 2010). We used intact wood tissue for carbon isotope analyses (i.e., without any chemical pre-treatment) since whole wood provides more consistent relationships with climatic variables for this species compared with particular wood fractions (e.g., holocellulose; Ferrio and Voltas 2005). The $^{13}C/^{12}C$ ratios of wood samples were determined by mass spectrometry and the results expressed as isotopic composition ($\delta^{13}C$) relative to the international standard Vienna PeeDee Belemnite (VPDB).

To take into account temporal changes in the isotope composition of atmospheric CO_2 ($\delta^{13}C_{atm}$), carbon isotope discrimination ($\Delta^{13}C$) was calculated (Farquhar and Richards 1984):

$$\Delta^{13}C = (\delta^{13}C_{atm} - \delta^{13}C_{plant}) / (1 + \delta^{13}C_{plant}) \qquad [3]$$

$\delta^{13}C_{atm}$ was inferred by interpolating data from two Antarctic stations (Halley Bay and Palmer Station) of the CU-INSTAAR/NOAA-CMDL network for atmospheric CO_2 measurements, as described in Ferrio et al. (2005). Estimated $\delta^{13}C_{atm}$ for the time period represented in each sample ranged from -6.9 to -7.9‰.

Meteorological data

Monthly values of temperature and precipitation for the study period (1949–1998) were obtained from the Instituto Nacional de Meteorología and the Confederación Hidrográfica del Ebro. Wherever the altitude of the sampling site exceeded that of the meteorological station, we applied a 0.6ºC decrease in temperature every 100 m and a 8% precipitation increment per 100 m, except for July and August, when precipitation is mostly convective and not related to altitude (Gandullo 1994). A seasonality index (SI) was calculated following Walsh and Lawler (1981):

$$SI_i = \frac{1}{R_i} \sum_{n=1}^{n=12} \left| X_{in} - \frac{R_i}{12} \right| \qquad [4]$$

where R_i is the total annual precipitation for year i and X_{in} is the monthly precipitation for month n. According to this index, sites with SI = 0.60–0.79 are classified as 'seasonal', whereas those with SI = 0.80–0.99 are classified as 'markedly seasonal with a long dry season'.

Remote sensing data

Time series of NDVI were obtained from the Global Inventory Modeling and Mapping Studies (GIMMS) dataset covering the period 1982–1998 at a biweekly temporal resolution (University of Maryland, 2004; available at http://glcf.umd.edu/data/gimms/). The NDVI data has a 9 × 9 km spatial resolution, and we collected a single pixel representative of each forest stand of our study sites. To compensate for the limited spatial resolution, we selected the pixels in order to maximize the fractional area covered by forest stands, and checked the seasonal variation of the index to confirm that the temporal spectra was typical of conifer forests. Biweekly NDVI data were recalculated as annual and seasonal mean NDVI for further temporal analysis (January to March = winter; April to June = spring; July to September = summer; October to December = autumn). For the analysis of spatial signals in tree-ring width and $\Delta^{13}C$, we used the site mean NDVI for the period 1982–1998.

Data analysis

Annually (for RG and NDVI) or biennially resolved (for $\Delta^{13}C$) data were subjected to analysis of variance, with site and time included as factors in the model. Relationships between climate and physiological variables were assessed using simple Pearson correlations (r), either across the annual (or biennial, when involving $\Delta^{13}C$) chronology for each site or across long-term means of all sites (hereafter, intra-site and inter-site analyses, respectively). We then related the r values of the relationship between $\Delta^{13}C$ and precipitation at the site level to the site means and coefficients of variation (CVs) of precipitation, $\Delta^{13}C$, and NDVI. This was done to identify possible factors underlying the varying strength of this relationship. By definition, mean values of tree-ring width indices (TRW_i) fall around unity at all sites; thus, for inter-site analyses we used mean values of RG for the period 1949–1998 obtained as the average of the median growth of each tree to minimize the effect of extreme years. On the other hand, we used indexed values (TRW_i) in all calculations involving temporal variability in radial growth (i.e., intra-site correlations and inter-annual coefficients of variation) to avoid artifacts due to age trends.

Results

Variability and relationships between tree growth, $\Delta^{13}C$ and NDVI

Radial growth (RG) varied significantly among sites from 0.8 ±0.19 mm (Riba-roja) to 2.1 ±0.35 mm (Girona), with a mean of 1.1 ±0.17 mm. Besides, there were significant differences in Δ^{13}C among sites, ranging from 15.6 ±0.12 ‰ in Riba-roja to 17.3 ±0.11 ‰ in El Grado, with a mean value of 16.6 ±0.28 ‰. Intra-site correlations (N=25) between indexed tree-ring widths (TRW_i) and Δ^{13}C values were positive and significant for three (out of seven) sites (Purchena, Riba-roja and Rasquera), whereas a marginally significant (p = 0.07) positive correlation was found between RG and Δ^{13}C at the spatial (i.e., inter-site) level (Table 2).

Annual NDVI values varied significantly among sites from 0.34 ±0.082 (Purchena) to 0.63 ±0.045 (Girona), with a mean of 0.44 ±0.040. We found positive temporal (i.e., intra-site) associations between biennial Δ^{13}C and NDVI (N=9), but they were significant only at three sites (Rasquera, El Grado and Girona) (Table 2). Similarly, we found a significant positive inter-site correlation between mean annual NDVI and long-term mean Δ^{13}C (Table 2). Intra-site correlations between annual NDVI and TRW_i (N=17) were positive and significant at two sites (Purchena and Riba-roja), and a significant positive correlation was also observed across sites between mean annual NDVI and mean RG (Table 2).

Generally TRW_i showed stronger correlations with summer NDVI (correlation coefficients, r, ranging from 0.35 in Valderrobres to 0.84 in Riba-roja) than with annual or other seasonal NDVI, except for Girona and Lanaja, which exhibited negligible correlations with summer NDVI (r = 0.02 for Girona; r = 0.05 for Lanaja). Correlations between Δ^{13}C and summer NDVI were stronger than with other NDVI values at three of the driest sites (r = 0.61, 0.62 and 0.74 in Riba-roja, Lanaja and Rasquera, respectively), weaker at the wettest sites (r = 0.40 and 0.70 for El Grado and Girona, respectively), and similar in Purchena and Valderrobres.

Climate factors determining tree growth, $\Delta^{13}C$, and NDVI

Overall, RG was positively related to annual precipitation both at the temporal (intra-site, using TRW_i, N=50) and spatial (inter-site, using mean RG, N=7) levels (Table 2). Nevertheless, temporal correlations were only significant at four sites. Precipitation seasonality was significantly correlated with TRW_i only at the driest site (Purchena) (Table 2). Temperature usually showed slightly weaker correlations with TRW_i than precipitation, being negatively related to TRW_i at four sites but positively correlated at the wettest site (Girona) (Table 2). RG did not show significant temperature dependence at the spatial level.

We found strong positive correlations between precipitation and $\Delta^{13}C$ at five sites (biennial records, *N*=25), but this relationship was not significant at the wettest sites (El Grado and Girona) (Table 2). Precipitation also showed a strong positive inter-site correlation with $\Delta^{13}C$ ($r = 0.90$; $P < 0.01$). In contrast, no significant correlations were found between temperature and $\Delta^{13}C$, and SI was significantly and negatively correlated with $\Delta^{13}C$ only at the two extremes of the precipitation gradient (Purchena and Girona) (Table 2).

We only found a significant intra-site correlation between annual NDVI and precipitation at Riba-roja (*N*=17, Table 2). In contrast, NDVI showed a strong positive inter-site correlation with precipitation. For temperature, we only found a significant negative correlation with annual NDVI at one site (Valderrobres), while SI showed a strong negative correlation with annual NDVI at both extremes of the precipitation gradient (Purchena and Girona) (Table 2). Annual precipitation showed higher correlations with summer NDVI than with other seasonal NDVI values at all sites, except in Lanaja and Girona. Nevertheless, correlations with summer NDVI were only significant at the two driest sites ($r = 0.49$ and $r = 0.57$ in Purchena and Riba-roja, respectively). However, annual NDVI showed tighter correlations with temperature and SI than seasonal NDVI values.

General trends in the response of $\Delta^{13}C$ to precipitation across sites

The best precipitation model accounting for Δ^{13}C variability involved a log fitting to the complete dataset ($r^2 = 0.59$, $N = 175$, $P < 0.001$; Fig. 2). Still, a linear model fitted equally well Δ^{13}C records if values above 800 mm were not considered ($r^2 = 0.60$, $N = 155$, $P < 0.001$; Fig. 2). A similar result was observed for the relationship between long-term site means of Δ^{13}C and annual precipitation (period 1949–1998), which was best explained using a log model ($r^2 = 0.89$, $N = 7$, $P < 0.01$, not shown).

Site-dependent temporal responses of $\Delta^{13}C$ to precipitation

A number of potential variables underlying the temporal sensitivity of Δ^{13}C to precipitation were investigated by correlation analysis. We found a significant negative correlation between *r* values of Δ^{13}C *vs*. precipitation ($N = 25$) and mean site precipitation (Fig. 3a). However, the relationship between *r* values of Δ^{13}C *vs*. precipitation and CVs of precipitation (instead of mean site values) was not significant (Fig. 3b). Conversely, a significant positive association was found between *r* values of Δ^{13}C *vs*. precipitation and CVs of Δ^{13}C (Figs. 3c, d), whereas the relationship involving Δ^{13}C mean records was not significant. There was a strong negative association between site *r* values and RG means, but no significant trend with CV of TRW_i (Figs. 3e, f). We also observed a significant negative correlation with site means of annual NDVI, and a positive correlation with CV of annual NDVI (Figs. 3g, h). We did not find any significant correlation with mean or CV values of either temperature or SI (results not shown).

Time-dependent spatial responses of $\Delta^{13}C$ to precipitation

In order to explore whether the observed long-term spatial relationship between Δ^{13}C and precipitation was consistent over time, this relationship was evaluated for 25 biennia independently for the period 1949–1998. Only a marginally significant ($P < 0.10$) negative trend was detected with both mean precipitation and mean Δ^{13}C (results not shown). Two clear outliers, corresponding to the biennia 1985–1986 and 1987–1988, were detected, which presented rather low *r* values (Fig. 4, crosses). For both biennia, we found that precipitation in the precedent biennium 1983–1984 (the driest in the period 1949–1998) was better linked to Δ^{13}C than the precipitation of the actual years (Fig. 4, triangles). By excluding these outliers, both mean precipitation and mean Δ^{13}C showed significant negative correlations with *r* values of Δ^{13}C *vs*. precipitation (r = -0.51 and r = -0.45, respectively). We did not find significant correlations with the remaining variables.

Discussion

Site-specific responses of Δ^{13}C to precipitation

By combining biennial data from seven sites we observed a saturation point of Δ^{13}C around 800 mm, above which Δ^{13}C was hardly sensitive to annual precipitation. The log function describing the relationship between both variables is almost identical to that reported for *P. halepensis* by Ferrio et al. (2003) using a 25-year tree-ring pool ($\Delta^{13}C = 4.6 + 1.9 \times \ln(P)$; r^2 = 0.59; $P < 0.001$). In line with this overall trend, our results also indicate that the association between Δ^{13}C and precipitation is stronger at sites where the mean annual precipitation is lower and weaker where it is higher (Fig. 3a). A lower sensitivity of Δ^{13}C to precipitation as water availability increases has been reported for *P. halepensis* (Klein et al. 2005; Maseyk et al. 2011) and other conifers (e.g. Korol et al. 1999; Warren et al. 2001). When water becomes less limiting, site-specific factors such as soil properties (Korol et al. 1999; Treydte et al. 2007) or stand attributes (e.g., canopy height or density) (Fernandez et al. 2007; Moreno-Gutiérrez et al. 2012) tend to blur the relationship between Δ^{13}C and precipitation. These factors may also affect the Δ^{13}C signal in drier sites; however, under such conditions precipitation is still the most limiting factor for tree performance, at least for drought-avoidant species such as *P. halepensis* (Ferrio et al. 2003; Ferrio & Voltas 2005; Del Castillo et al. 2013). Additionally, Δ^{13}C can be also affected by the yearly pattern of rainfall distribution (see e.g. Korol et al. 1999). In this regard, the negative correlation between SI and Δ^{13}C at the wettest site suggests that precipitation distribution may have a stronger effect than total annual precipitation under near-optimal conditions.

Is the spatial response of Δ^{13}C to precipitation consistent over time?

The analysis of the spatial relationship between Δ^{13}C and precipitation for 2-year periods pointed to tighter associations in dry than in wetter biennia (Fig. 4a). However, spatial responses showed erratic variations through time. Different physiological processes may obscure the spatial dependence of Δ^{13}C on precipitation, with an expected stronger effect over short time periods than for long-term site-specific signals. For instance, year to year carry-over effects may imprint a significant isotopic signature lasting for 2 or more years (see e.g., Sarris et al. 2013). After particularly extreme years, $\Delta^{13}C$ of subsequent years may correlate well with the environmental conditions of the event year. In this regard, we observed exceptional carry-over effects in the biennia 1985–1986 and 1987–1988 (Fig. 4), in which the spatial $\Delta^{13}C$ variation was better explained by the precipitation occurring in the biennium 1983–1984 (the driest of the entire record). Alternatively, water stress can lead to limited carbon loading in the phloem, as well as to readjustments of leaf area, reducing wood production and causing an uncoupling of leaf and tree-ring signals (Cernusak et al. 2013; Voltas et al. 2013).

Cross-links between physiological responses and tree growth

NDVI was highly correlated with precipitation across sites (Table 2, r = 0.94), in agreement with previous studies reconstructing spatial patterns of precipitation from annual NDVI in the Iberian Peninsula (Immerzeel et al. 2009). At the temporal level, summer NDVI correlated better with $\Delta^{13}C$ at three drought-prone sites, whereas correlations with annual NDVI were stronger for the two wettest sites. Overall, these relationships were weaker than that obtained across sites, although these results are not conclusive due to the limited number of records and the loss of temporal variability due to tree-ring pooling (N=9 and N=7, for temporal and spatial correlations, respectively).

Correlations between TRW_i and either NDVI or $\Delta^{13}C$ were generally stronger at drier sites. Tree productivity, in terms of radial growth, seed or foliage production, has already been linked to growing season-integrated NDVI in oak trees (Wang et al. 2004). This explains the observed link to annual NDVI in *P. halepensis*, which can grow all year around if conditions are favourable. In this regard, tree-ring growth can be strongly limited by water availability in Mediterranean environments, which explains the tight correlation with both $\Delta^{13}C$ and summer NDVI at dry sites. The negative correlation between TRW_i and temperature at four sites can be interpreted as a response to increasing evaporative demand (Ferrio and Voltas 2005; de Luis et al. 2013; Maseyk et al. 2011). On the contrary, a positive correlation with temperature at the wettest site (Girona) agrees with previous observations reporting on growth limitations associated with winter cold (de Luis et al. 2013). Hence, where $\Delta^{13}C$ is responsive to variations in precipitation, $\Delta^{13}C$, TRW_i, and summer NDVI share a strong common signal. In contrast, the link between $\Delta^{13}C$ and annual NDVI, as a surrogate of productivity, tends to be stronger at the wettest environments.

Remote sensing and carbon isotopes as complementary proxies for water-use efficiency

An interesting outcome of this study is that NDVI correlated well with both $\Delta^{13}C$ and TRW at the spatial level, with a number of significant relationships also emerging at the temporal level. Given the strong spatial agreement between NDVI and $\Delta^{13}C$, the combination of tree-ring $\Delta^{13}C$ networks (*isoscapes*, see, e.g., Leavitt et al. 2008; del Castillo et al. 2013) and high-resolution NDVI data may allow to spatially model historical stand attributes, including productivity or water-use efficiency (Leavitt et al. 2008; Beck et al. 2013). Furthermore, NDVI appears as a good proxy for site-specific sensitivity of $\Delta^{13}C$ to precipitation, and could be used to pre-select potentially sensitive tree-ring sampling sites for paleoenvironmental research. Nevertheless, we still could observe site-specific deviations in NDVI not reflected in precipitation and $\Delta^{13}C$ (Fig. 3, Fig. 5). Besides potential differences in stand attributes affecting NDVI, Aleppo pine is a thermophilous species with polycyclic growth (see e.g. de Luis et al. 2013) that, due to extended phenology, may produce comparatively denser canopies in warm (Riba-roja) than in cold (El Grado) sites, but having little effect on $\Delta^{13}C$ (Figs. 3 and 5). Additionally, NDVI tends to increase at a faster rate than $\Delta^{13}C$ (Fig. 5), more likely due to changes in leaf area rather than changes in leaf chlorophyll content, since the latter would have an opposite effect on $\Delta^{13}C$: leaves with higher photosynthetic activity would show higher water-use efficiency and, thus, lower $\Delta^{13}C$ (Farquhar and Richards 1984; Cernusak et al. 2013). Still, both chlorophyll content and leaf area tend to decrease under drought conditions in *P. halepensis* (Baquedano and Castillo 2006) and, thus, NDVI might hold a signal from both variables, particularly in drought-prone environments (Pasquato 2013). Hence, the poor agreement between $\Delta^{13}C$ and NDVI at the temporal scale in the driest sites could be the result of a greater relevance of the chlorophyll content signal controlling NDVI in water-limited environments.

Conclusions

Our results confirm precipitation as a key driver of variations in tree growth, water-use efficiency, and vegetation greenness for Aleppo pine. We observed a broad common pattern in the response of $\Delta^{13}C$ to both spatial and temporal variability in precipitation, showing a saturation response of $\Delta^{13}C$ when water becomes less limiting. In this regard, inter-site differences in the sensitivity of $\Delta^{13}C$ to precipitation are mainly linked to the frequency of water-limiting conditions in the time series. The general agreement between NDVI, $\Delta^{13}C$ and tree growth opens the possibility to integrate information from tree-ring networks and satellite data to monitor changes in water-use efficiency and productivity at regional scales.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

Study idea by J.P.F.; all authors designed the research, analysed the data, interpreted the results and wrote the paper.

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Figures and Tables

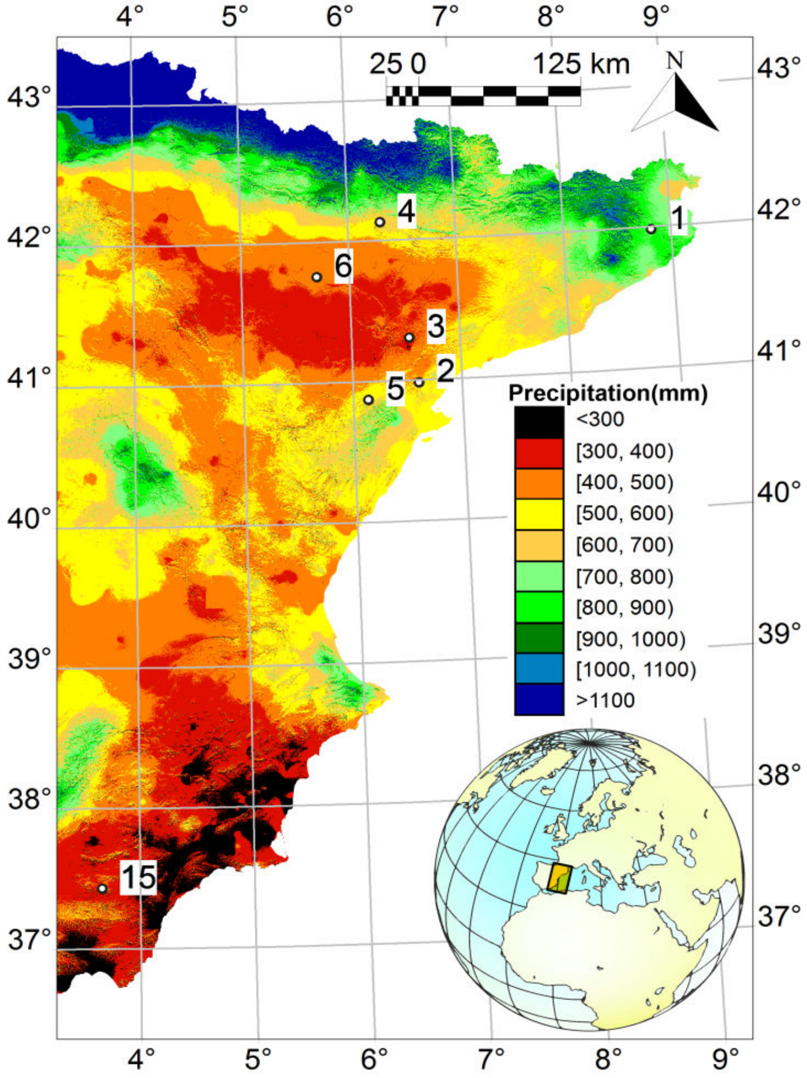

Fig. 1 Map of the study area and sampling sites, depicting mean annual precipitation according to the Digital Climatic Atlas of the Iberian Peninsula (http://www.opengis.uab.es/wms/iberia/mms/index.htm). Numbers correspond to provenance regions as indicated in Table 1.

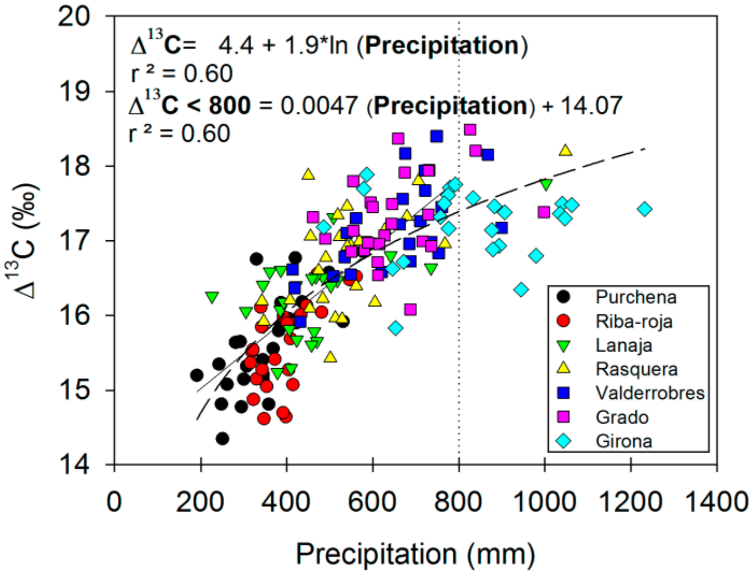

Fig. 2 Scatterplot depicting linear (below 800 mm precipitation) and log relationships between annual precipitation and carbon isotope discrimination ($\Delta^{13}C$) (biennial records; period 1949-1998) at seven sampling sites. Dotted vertical line indicates the approximate threshold for a linear response.

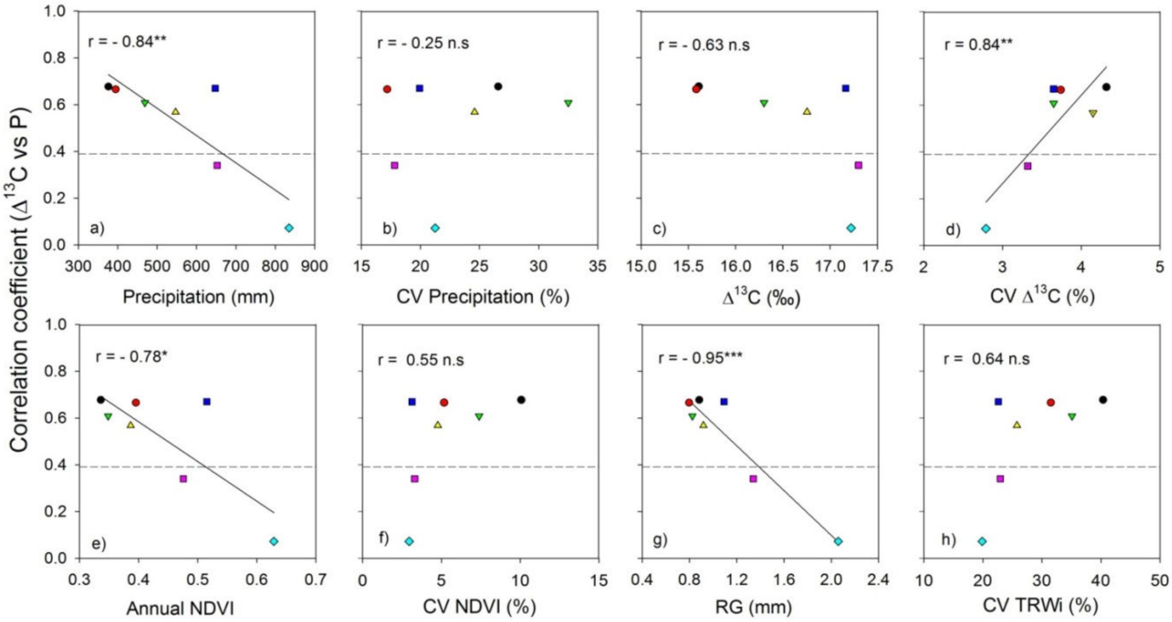

Fig. 3 Intra-site correlation coefficients (*r*) of the relationship between time-series of annual precipitation and carbon isotope discrimination (Δ^{13}C) (biennial records; period 1949-1998, *N*=25) as a function of: a) long-term mean annual precipitation; b) inter-annual coefficient of variation (CV) of precipitation; c) long-term mean Δ^{13}C; d) inter-biennial CV of Δ^{13}C; e) mean annual NDVI (1982–1998); f) inter-annual CV of NDVI; g) long-term mean of radial growth (RG); and h) inter-annual CV of tree-ring width indices (TRW_i). Dashed lines indicate the threshold value for significance ($P < 0.05$).

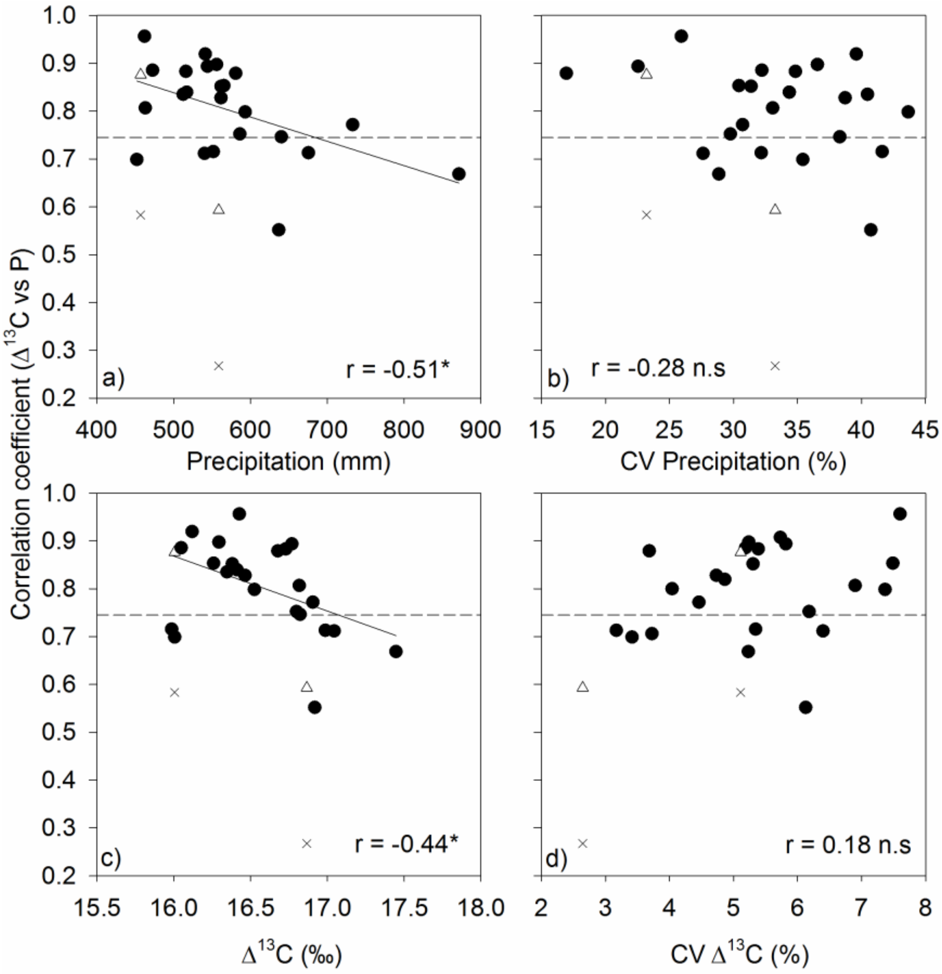

Fig. 4 Inter-site correlation coefficients (*r*) of the relationship between annual precipitation and carbon isotope discrimination (Δ^{13}C) (biennial records; period 1949-1998, N=7) and: a) mean annual precipitation for each biennium; b) inter-site coefficient of variation (CV) of mean annual precipitation; c) biennial Δ^{13}C; and d) inter-site CV of Δ^{13}C. Dashed lines indicate the threshold value for significance ($P < 0.05$). Crosses indicate outliers showing weak correlations with current precipitation (biennia 1985-1986 and 1987-1988), but better correlated with precipitation during the driest biennium (1983-1984, correlations shown with open triangles). See text for details.

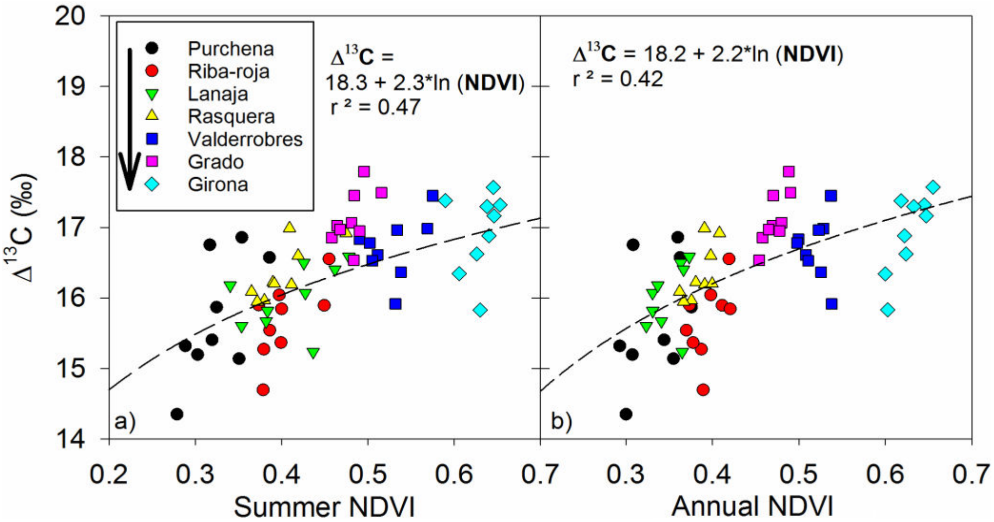

Fig. 5 Regression plots illustrating the log relationship between Normalized Difference Vegetation Index (NDVI) and carbon isotope discrimination (Δ^{13}C) across seven sampling sites at a biennial scale (period 1949–1998): a) annual NDVI; and b) summer NDVI. The arrow indicates the increasing precipitation gradient among study sites.

Table 1. Main eco-geographic and climatic characteristics of the seven sites included in this work, together with the chronology statistics.

Site description					Climate			Soil	Chronology		
Prov.	Site	Latitude	Longitude	Alt. (m)	P (mm)	T (ºC)	SI	Group	*N*	EPS	Period
1	Girona	42º01'	03º00'E	190	835	14.0	0.66	Haploxerept	8	0.85	1948-1999
2	Rasquera	41º01'	00º36'E	180	547	15.3	0.82	Xerorthent	8	0.93	1926-1999
3	Riba-roja	41º20'	00º30'E	80	395	15.1	0.73	Torriorthent	11	0.95	1936-1999
4	El Grado	42º09'	00º15'E	625	652	12.6	0.60	Haploxerept	8	0.94	1928-2000
5	Valderrobres	40º53'	00º12'E	630	648	13.9	0.77	Haploxeralf	10	0.94	1938-1999
6	Lanaja	41º50'	00º32'W	380	469	13.1	0.74	Xerorthent	11	0.96	1884-1999
15	Purchena	37º26'	02º20'W	900	376	16.6	0.81	Torriorthent	7	0.93	1919-1999

Prov., provenance region, following Gil et al. (1996); Alt., altitude; P, mean annual precipitation; T, mean annual temperature. SI, seasonality index, according to Walsh and Lawler (1981); *N*, number of trees; EPS, expressed population signal. Soil groups according to USDA Soil Taxonomy (Soil Survey Staff 2010).

Table 2. Pearson correlation coefficients between physiological parameters (carbon isotope discrimination [$\Delta^{13}C$], indexed tree-ring width [TRW_i], and mean annual Normalized Difference Vegetation Index [NDVI]) involving annual or biennial (for correlations with $\Delta^{13}C$) records (period 1949–1998) at the site level (upper section). Pearson correlation coefficients between physiological parameters ($\Delta^{13}C$, TRW_i, and NDVI) and climatic records of annual precipitation (P), mean annual temperature (T), and precipitation seasonality index (SI) at the site level (lower section). *N*, number of common observations. The last column shows inter-site correlations of long-term means (1949–1998, $N = 7$) involving the same variables, except for tree-ring width, in which original values of radial growth (RG) were used instead of TRW_i. *$P < 0.05$; *$P < 0.01$; ***$P < 0.001$.

Variables	***N***	**Purchena**	**Riba-roja**	**Lanaja**	**Rasquera**	**Valderrobres**	**El Grado**	**Girona**	**Inter-site**
TRW_i vs $\Delta^{13}C$	25	**0.61****	**0.58****	0.36	**0.42***	0.27	-0.04	0.03	0.67
NDVI vs $\Delta^{13}C$	9	0.44	0.52	0.42	**0.70***	-0.03	**0.81****	**0.81****	**0.75 ***
NDVI vs TRW_i	17	**0.58***	**0.69****	0.21	0.27	0.19	0.42	-0.16	**0.91****
P vs TRW_i	50	**0.61*****	**0.39****	**0.36***	0.09	0.07	**0.42****	0.23	**0.91****
T vs TRW_i	50[1]	**-0.37***	0.03	**-0.30***	**-0.33***	-0.08	**-0.36****	**0.30***	-0.34
SI vs TRW_i	50	**-0.33***	-0.16	-0.21	-0.14	0.22	0.02	-0.06	-0.62
P vs $\Delta^{13}C$	25	**0.68*****	**0.64*****	**0.61****	**0.58****	**0.67*****	0.34	0.07	**0.90****
T vs $\Delta^{13}C$	25[1]	0.11	0.29	-0.24	0.18	0.28	-0.04	0.33	-0.67
SI vs $\Delta^{13}C$	25	**-0.55****	-0.15	-0.16	0.05	0.04	-0.20	**-0.44***	-0.52
P vs NDVI	17	0.35	**0.51***	-0.17	-0.02	0.16	0.05	-0.07	**0.94****
T vs NDVI	17	0.37	0.40	0.24	-0.12	**-0.60***	0.10	0.33	-0.41
SI vs NDVI	17	**-0.61****	-0.20	0.07	0.03	-0.11	0.13	**-0.62****	-0.56

[1] For Purchena, due to missing temperature data, $N = 41$ years (for TRW_i) and $N = 21$ biennia (for $\Delta^{13}C$).

Chapter III

> “We kill all the caterpillars, then complain there are no butterflies.”
> John Marsden, *The Dead of Night*

Point processes statistics of stable isotopes: analysing water uptake patterns in a mixed stand of Aleppo pine and Holm oak

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Abstract

Aim of study: Understanding inter- and intra-specific competition for water is crucial in drought-prone environments. However, little is known about the spatial interdependencies for water uptake among individuals in mixed stands. The aim of this work was to compare water uptake patterns during a drought episode in two common Mediterranean tree species, *Quercus ilex* L. and *Pinus halepensis* Mill., using the isotope composition of xylem water ($\delta^{18}O$, $\delta^{2}H$)[1] as hydrological marker.

Area of study: The study was performed in a mixed stand, sampling a total of 33 oaks and 78 pines (plot area= 888 m^2). We tested the hypothesis that both species uptake water differentially along the soil profile, thus showing different levels of tree-to-tree interdependency, depending on whether neighbouring trees belong to one species or the other.

Material and Methods: We used pair-correlation functions to study intra-specific point-tree configurations and the bivariate pair correlation function to analyse the inter-specific spatial configuration. Moreover, the isotopic composition of xylem water was analysed as a mark point pattern.

Main results: Values for *Q. ilex* ($\delta^{18}O$= -5.3 ±0.2‰, $\delta^{2}H$=-54.3±0.7‰) were significantly lower than for *P. halepensis* ($\delta^{18}O$= -1.2±0.2‰, $\delta^{2}H$ = -25.1±0.8‰), pointing to a greater contribution of deeper soil layers for water uptake by *Q. ilex*. *Research highlights:* Point-process analyses revealed spatial intra-specific dependencies among neighbouring pines, showing neither oak-oak nor oak-pine interactions. This supports niche segregation for water uptake between the two species.

Keywords: Cross-pair correlation function, Deuterium, Mark correlation function, Oxygen-18, Point patterns, Xylem.

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[1] **Abbreviations:** $\delta^{18}O$, oxygen isotope composition; $\delta^{2}H$, hydrogen isotope composition; BA, basal area.

Introduction

In Mediterranean climates, the temporal coupling of heat and drought stress, and the existence of nutrient-deficient soils have been major evolutionary forces shaping plant communities (Herrera, 1992; Mooney & Dunn, 1970). Examples of adaptive strategies include extensive root systems, evergreen foliage to compensate for nutrient scarcity and to permit year-round production, or water-saving mechanisms such as leaf sclerophylly or a very sensitive stomatal regulation (Rundel, 1988; Zavala *et al.*, 2000). As a result, dominant tree species in Mediterranean ecosystems are either evergreen sclerophyllous or conifers adapted to the scarcity of water resources. Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill.) are representative of each one of these functional types, being extensively found in the Mediterranean basin. Although *P. halepensis* and *Q. ilex* are well adapted to seasonally-dry areas, the particular strategies followed by each species differ considerably (Ferrio *et al.*, 2003; Zavala *et al.*, 2000; Baquedano & Castillo, 2006; Del Castillo *et al.*, 2013). On the one hand, *P. halepensis* is a typical drought-avoiding species that relies on water saving mostly through stomatal closure (see e.g. Borghetti *et al.*, 1998; Voltas *et al.*, 2008), but it may also act as an opportunistic species when conditions are favourable (Nicault *et al.*, 2001; Klein *et al.*, 2005). On the other hand, *Q. ilex* is a slow growing species with a considerable stomatal regulation (at least when compared to other species of the same genus, see e.g. Damesin *et al.*, 1998; Infante *et al.*, 1999), but combined with drought-tolerance mechanisms such as osmotic and elastic adjustments (Terradas & Savé 1992; Sala & Tenhunen 1994). The existence of tolerance mechanisms, together with a deeper root system, typical of evergreen schlerophyllous (Canadell *et al.*, 1996), leads to a more effective water uptake of evergreen oaks as compared to pines (Valentini et al. 1992; Klein et al. 2013). Despite their functional differences, both species often form mixed stands that are widely distributed in the Eastern Iberian Peninsula. In this context, understanding interactions among individuals in pine-oak mixed stands is crucial not only to interpret current species distribution, but also to foresee future vegetation scenarios (Zavala *et al.*, 2007).

A considerable number of studies have shown how inter- and intra-specific competition affects individual growth and stand dynamics under water-limited conditions (Gracia *et al.*, 1996; Moreno-Gutiérrez *et al.*, 2011; Zavala *et al.*, 2007). However, although soil water availability and water uptake patterns are likely to play a major role in shaping the composition of mixed-species stands, information on the use of water resources at inter- and intra-specific levels is still limited (Filella & Peñuelas, 2003a; Moreira *et al.*, 2003; Brooks *et al.*, 2006). In this regard, the analysis of the isotopic compositions of oxygen and hydrogen ($\delta^{18}O$ and $\delta^{2}H$) in xylem sap presents a great potential to characterise water movement along the soil-plant-atmosphere continuum, particularly in arid and semi-arid environments (Dawson *et al.*, 1993; Dawson & Simonin, 2011; Ferrio *et al.*, 2005). During the dry season, evaporation causes a decreasing trend in soil water $\delta^{18}O$ and $\delta^{2}H$ with soil depth (Filella & Peñuelas, 2003b; Moreno-Gutiérrez *et al.*, 2012b). Thus, by comparing observed trends along the soil profile with data on xylem water, stable isotopes can reveal differential water uptake patterns in co-existing Mediterranean species (Armas *et al.*, 2010; Máguas *et al.*, 2011; Filella & Peñuelas, 2003a; Filella & Peñuelas, 2003b; Moreno-Gutiérrez *et al.*, 2012b). However, studies so far compared isotopic records averaged over a representative sample of trees per species (Máguas *et al.*, 2011; Filella & Peñuelas, 2003b; Moreno-Gutiérrez *et al.*, 2012b), or focussed on the interaction between selected individuals (Armas *et al.*, 2010; Filella & Peñuelas, 2003a), but a stand-level based study of inter-individual interactions in water uptake is still lacking.

Forest science has applied numerous statistical methods belonging to point processes (Stoyan & Penttinen, 2000; Diggle, 2003; Illian *et al.*, 2008) to tackle ecological questions (for a review, see Comas & Mateu, 2007). This includes, for instance, the study of the spatial structure of pure and mixed forest stands (Moeur, 1993; Pélissier, 1998; Mateu *et al.*, 1998), the distribution and severity of infected trees (Shaw *et al.*, 2005), and the space-time modelling of forest dynamics (Renshaw *et al.*, 2009; Comas, 2009). Here we propose the use of marked point process tools to analyse the isotopic composition of xylem water as a mark associated to each tree position. The resulting marked point pattern represents the spatial structure of water uptake for each tree species. The analysis of such marked point configurations may be valuable to interpret spatial inter- and intra-specific dependencies (e.g. competition, facilitation) for water uptake dynamics underlying particular tree performances in water-limited environments. As a case-study to show the potential of this method, we analysed the spatial inter- and intra-specific interactions for water uptake under drought for a pine-oak mixed Mediterranean forest based on individual tree $\delta^{18}O$ and $\delta^{2}H$ records of xylem water. Due to their deeper root system, oaks are likely to extract water from soil layers not accessible for the pines. We hypothesize that, under drought conditions, the two species might not directly compete for the same water pools in the soil, thus showing a functional niche segregation. Accordingly, we would expect different levels of tree-to-tree interdependency, depending on whether neighbouring trees belong to one species or the other.

Materials and methods

Study area

The study area is a forest stand located in the Montsant mountain range (41^{o} 19' 47.3" N, 0º 50' 2.6" E, 750 m a.s.l), in the northeast of the Iberian Peninsula. The climate in the region is Mediterranean temperate with continental tendency, with a mean annual precipitation of 517 mm and mean annual temperature of 12.3 °C. It is characterized by a dry and a relatively warm summer (mean summer precipitation of 89.5 mm, mean average temperature of 20.9 °C; averaged data of the two nearest meteorological stations with a long-term record (period 1970-2000), *El Vilosell* and *Bisbal de Falset*, each located at aprox. 11 km distance from the sampling site (AEMET-IM, 2011)). Year-to-year climate variability at this location is high with extreme drought events occurring every few years, a typical feature of the Mediterranean climate. The forest stand is a dense woodland community co-dominated by two typical Mediterranean trees, Holm oak and Aleppo pine. It is the result of natural regeneration of pine and oak in a former agricultural terrace which, according to the age of some individuals, was abandoned about 80 years ago. Understory vegetation is relatively scarce and current regeneration from both species is rare; thus, only adult pines and oaks with diameter at breast height (dbh) above 10 cm were included in the study. Stand density (dbh>10 cm) was 560 and 863 stems/ha for oak and pine respectively. We considered stem clumps of *Q. ilex* that seemingly derived from the same stool as single individuals. These sprout clumps were relatively common (about 1/3 of individuals), making stool density of *Q. ilex* considerably lower than stem density (370 stools/ha).

The rectangular plot area (24 x 37 m) had a strong slope (15-22%) facing west (X-axis), together with a gentle slope (3-7%) facing south (Y-axis). According to USDA soil taxonomy (Soil Survey Staff, 2010), soil is a loamy-skeletal, carbonatic, termic, active calcic pachic haploxeroll, with soil depths ranging from *ca.* 50 cm in the lower parts of the plot to *ca.* 20 cm in the upper part of the plot.

Sample collection and tree mapping

Field sampling took place on the 9th September 2011, at the end of an exceptionally dry, but moderately warm, summer (summer precipitation of 23 mm, mean summer temperature of 21.5 °C, data from *Ulldemollins*, a recently established automatic meteorological station, only 3.5 km away from the site),) (Fig. 1). We sampled sun-exposed twigs from the 33 oaks and 78 pines with dbh>10 cm. After removing the bark and phloem, the xylem of the twigs was placed into cap vials, immediately frozen in dry ice and kept until water extraction using a cryogenic vacuum distillation line (Dawson et al., 1993). Six soil samples were also collected from the topsoil layer (2-10 cm, A horizon) and from 5 cm above the maximum soil depth (subsoil, up to 19-33 cm depending on the sample, B horizon), and similarly handled for water extraction. Water isotope ratios of hydrogen and oxygen of the xylem and soil water were determined using a Picarro Water Analizer L2130-*i* (Picarro Inc., Santa Clara, California). They were expressed in delta (δ) notation (‰) relative to V-SMOW (i.e. isotopic composition of oxygen, $\delta^{18}O$, and hydrogen, $\delta^{2}H$). Raw values were calibrated against three internal laboratory references (calibrated against IAEA standards VSMOW2, SLAP2 and GISP). Overall uncertainty (determined as the standard error of repeated analyses (N=20) of a reference sample not included in the calibration) was 0.05‰ and 0.17‰, for $\delta^{18}O$ and $\delta^{2}H$, respectively. The potential presence of organic contaminants was checked using the post-processing software Picarro ChemCorrect 1.2.0, giving in all cases negative results.

Tree position for spatial analysis was determined using a high resolution GPS technology (GeoExplorer 6000 Series Handheld, Trimble Navigation Limited, California, USA) with spatial error inferior to 20 cm for latitude and longitude and to 40 cm for altitude. Tree coordinates were re-checked in the field with the aid of a measuring tape.

Analysis of covariance

Isotope data ($\delta^{18}O$ and $\delta^{2}H$) and one tree dendrometric characteristic (individual basal area, BA) were subjected to mixed model analysis of covariance (ANCOVA) considering a fixed effect for species (pine, oak) and the variation along the X and Y axes of the two-dimensional space (covariates), allowing for heterogeneity of regression slopes at the species level. This was done to check for (possible) differential systematic variation in the response variables following X and Y directions, i.e. anisotropic effects. We also allowed for heterogeneity of residual variances at the species level, which was checked by means of log likelihood ratio tests. For the difference between two nested models (homocedastic and heterocedastic), minus two times the log likelihood ratio follows, under the null hypothesis, asymptotically a χ^2 distribution with one degree of freedom (difference in the number of variance components; Verbeke and Molenberghs, 2000).

Spatial Statistics

To analyse the spatial structure of *Q. ilex* and *P. halepensis*, we used spatial correlation functions derived from point process theory. A spatial point process is a stochastic mechanism that generates a countable set of events x_i in a bounded region A (see, for instance, Diggle, 2003). Any sequence of events, which can be seen as points on a given region, can be explained by point process theory, and one of the most common applications is the study of point occurrences in the Euclidean plane (e.g. individual trees in a forest stand) (Stoyan & Penttinen, 2000).

Spatial correlation functions

To study the spatial structure of trees (point locations) we used the pair correlation function (Illian *et al.,* 2008), an estimator of which can be obtained as

$$\hat{g}(r) = \frac{1}{2\pi\hat{\lambda}^2|A|} \sum_{(x_1,x_2)\in\varphi}^{\neq} \frac{\kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|)} \qquad (1)$$

for a forest stand A with area $|A|$, where φ is the observed point pattern, $\hat{\lambda}$ is an estimator of the point intensity, $\kappa(\cdot)$ is the Epanechnikov kernel function, $\sum^{\neq}$ stands for the summation over all pairs such that $x_1 \neq x_2$ and $e(\cdot)$ is the Ripley's factor (Ripley, 1976) to correct for edge effects, for a given inter-distance r between points (trees). Broadly speaking, this function indicates point inhibition (i.e. repulsion) when $g(r) < 1$, $g(r) = 1$ denotes the Poisson case (i.e. a random point process) with no interaction between points, whilst $g(r) > 1$ implies point clustering, for any $r > 0$.

To analyse the bivariate point pattern of *Q. ilex* and *P. halepensis* we used the partial or cross-pair correlation functions, $g_{12}(r)$ (Illian *et al.,* 2008). This correlation function is a bivariate derivation of the pair correlation function to study the spatial dependencies of point classes for bivariate point patterns. The interpretation of $g_{12}(r)$ is similar to that of $g(r)$. It indicates point-type inhibition when $g_{12}(r) < 1$, $g_{12}(r) = 1$ is the Poisson case (i.e. point types are independently distributed from each other), whilst $g_{12}(r) > 1$ implies point-type clustering. An estimator of this function can be defined as

$$\hat{g}_{12}(r) = \frac{1}{2\pi\hat{\lambda}_1\hat{\lambda}_2|A|}\sum_{x_1\in\varphi_1, x_2\in\varphi_2}\frac{\kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|)}, \qquad (2)$$

where φ_s and $\hat{\lambda}_s$ are the point pattern and the point intensity of the point class $s = 1,2$, respectively. Note that $\varphi_{12} = \varphi_1 \cup \varphi_2$, i.e. the bivariate point pattern.

To analyse the marked point patterns of oaks and pines, we used the mark correlation function $k_m(r)$ (Stoyan & Stoyan, 1994). This function is a mark counterpart of the pair correlation function that accounts for the spatial correlation of marks (characteristics) associated to each tree. Specifically, this function describes the spatial structure of marks (e.g. tree basal area, stable isotope composition) associated to each tree location, and an estimator of this function can be written via

$$\hat{k}_m(r) = \frac{1}{2\pi\hat{\lambda}^2|A|}\sum_{(x_1,m_1),(x_2,m_2)\in\varphi_m}^{\neq}\frac{m_1 m_2 \kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|)\hat{g}(r)\mu^2} \qquad (3)$$

where φ_m is a marked point pattern, μ^2 is the expectation of $m_1 m_2$ and m_1 is the mark value for tree 1 (say). This function denotes independence between marks when $k_m(r) = 1$, $k_m(r) > 1$ indicates positive mark correlation, whilst $k_m(r) < 1$ implies mark inhibition for all $r > 0$. Because the spatial correlation function is defined for positive marks, we scaled the original stable isotope composition values as to avoid negative values, noting that a change in mark scale does not affect the resulting estimators. In particular, we subtracted the minimum negative values of the resulting isotopic composition to each tree record. Therefore, the new resulting scale is defined for Real positive numbers starting from zero (minimum negative isotopic value of the original dataset). Finally, to study the correlation between species (qualitative mark) with regard to tree quantitative characteristic (basal area, stable isotopic compositions) we adopted a derivation of the mark correlation function initially proposed by Penttinen *et al.* (1992). Here the point pattern consists of two distinct tree species together with a mark associated to each tree position. In fact, two marginal processes are present over the same underlying point configuration, one as a bivariate (qualitative) point process and another as a marked (quantitative) point process. Penttinen *et al.* (1992) defined an estimator of this cross-mark correlation function through

$$\hat{k}_m^{12}(r) = \frac{1}{2\pi\hat{\lambda}_1\ \hat{\lambda}_2|A|} \sum_{(x_1,m_1)\in\varphi_{m_1},(x_2,m_2)\in\varphi_{m_2}} \frac{m_1 m_2 \kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|)\hat{g}_{12}(r)\hat{\mu}_{12}^2} \qquad (4)$$

where φ_{m_1} is the marked point pattern for class 1 (say), and $\hat{\mu}_{12}$ is an estimator of μ_{12}, is the expectation of $m_1 m_2$ (marks from classes 1 and 2). The interpretation of $\hat{k}_m^{12}(r)$ is similar to that of $k_m(r)$ and $g_{12}(r)$. It indicates spatial positive correlations for the spatial pattern when $\hat{k}_m^{12}(r) > 1$, $\hat{k}_m^{12}(r) = 1$ is the Poisson case, whilst $\hat{k}_m^{12}(r) < 1$ implies negative dependencies. For the Epanechnikov kernel function, we chose the bandwidth to be equal to $c/\sqrt{\lambda}$, where typically $c = 0.1 - 0.2$, (here $c = 0.2$) as suggested by Stoyan & Stoyan (1994).

For each kind of spatial correlation function, we tested for spatial independence following a Monte Carlo approach based on the random simulation of (marked) point patterns from the null hypothesis (Poisson). We simulated 199 (marked) point patterns under the null hypothesis of spatial independence, and for each one, an estimator of one of the correlation functions defined above was obtained. These set of functions were then compared with the resulting estimator of this correlation function for the point pattern under analysis. Under this test, we rejected the null hypothesis (spatial independence) if the resulting estimator of this correlation function lay outside the fifth largest and/or smallest envelope values obtained from the set of simulated functions with an exact significant level of $\alpha = 2 \times 5/(199 + 1) = 0.05$. Tests for each (marked) point pattern considered here are defined as follows. For the point patterns of oaks and pines analysed separately we tested against spatial point independence based on the random simulation of Poisson point configurations (see for instance, Stoyan & Stoyan, 1994). Under the bivariate point pattern (i.e. the point patterns of both species together) we considered two approaches, *random labelling* and *random superposition* (see Illian *et al.*, 2008). Testing for spatial independence of marked point patterns of oaks and pines, respectively, was based on the *random marking* approach (Illian *et al.,* 2008). Finally, for the point pattern consisting of both tree species together with a mark associated to each tree position we assumed *random labelling* of tree species over the fixed point positions, and then over this new bivariate point pattern, we considered a random marking approach to generate independent bivariate marked point configurations (see, Penttinen et al.,1992).

For the statistical analysis of point patterns, we considered the computational implementation in the statistical package Spatstat for the R statistical environment (R Development Core Team, 2007).

Results

δ^{18}O and δ^{2}H in soil water and xylem sap

The analysis of isotopic compositions of water extracted from soil samples showed a decreasing trend along the soil profile. In particular, the topsoil was significantly more enriched (δ^{18}O = 0.2±1.2 ‰; δ^{2}H = 34.6 ± 3.8 ‰) than the subsoil (δ^{18}O = -3.0 ± 2.4 ‰; δ^{2}H = -45.4 ± 8.5 ‰) (P=0.019 and P=0.035 for δ^{18}O and δ^{2}H, respectively; two-tailed, paired *t*-test). In addition, the variability found among soil samples taken in the subsoil (ranging from 19 to 33 cm depending on digging point) was mostly explained by soil depth (δ^{18}O= 20.3–2.2×depth(m), R^2=0.85, P=0.02; δ^{2}H= 3.1–0.52×depth(m), R^2=0.67, P=0.07). Conversely, we did not find any significant correlation between the isotopic composition of soil water and X or Y coordinates, neither for the topsoil nor for the subsoil. The range of values of xylem water for each species (δ^{18}O=-7.4 to +3.0‰ and δ^{2}H=-61.5 to -7.0‰ in pines; δ^{18}O=-8.1 to -2.6‰ and δ^{2}H=-67.8 to -41.6‰ in oaks) was comparable, although in some cases exceeded the range observed in soil samples, particularly for δ^{2}H (δ^{18}O=-5.7 to +1.7 ‰; δ^{2}H=-55.7 to -28.5‰).

ANCOVA of BA, δ^{18}O and δ^{2}H

ANCOVAs revealed significant differences between pines and oaks (-1.2 ± 0.18‰ and -5.3 ± 0.15‰, respectively, for δ^{18}O; -25.1 ± 0.78‰ and -54.3 ± 0.66‰, respectively, for δ^{2}H), in addition to a progressive increase of both isotopes along the X axis that was significantly higher for *Q. ilex* (0.054‰ m^{-1} and 0.155‰ m^{-1}, for pines and oaks, respectively, for δ^{18}O; 0.248 ‰ m^{-1} and 0.610 ‰ m^{-1}, for pines and oaks, respectively, for δ^{2}H) (test of unequal slopes; Table 1). These results suggest the existence of spatial anisotropic effects for the xylem water isotopic compositions of oxygen and hydrogen.

Spatial distribution of pines and oaks

Figure 2 shows the bivariate point pattern of *Q. ilex* and *P. halepensis* together with the resulting pair correlation functions (Eq. 1) and cross-pair correlation function (Eq. 2), and their respective fifth-largest and smallest envelope values based on 199 point configurations based on the null hypotheses, i.e. random labelling and Poisson point randomizations. Results suggest that both point configurations were at random, i.e. trees from the same species were independently located from each other, and that trees from distinct species were located at random from each other, thereby pointing to spatial independence between these two species in terms of individual tree location.

Spatial analysis of BA, $\delta^{18}O$ and $\delta^{2}H$

The spatial locations of *Q. ilex* and *P. halepensis*, along with individual tree BA in the area of study, are shown in Figure 3a. Visual inspection of bivariate marked point patterns did not provide much information about the spatial dependence of these two species. The resulting estimators of the mark correlation function (Eq. 3) for tree BA (Figure 3c and d) suggested that only the spatial structure of *P. halepensis* showed dependence, whereas no correlation was observed for *Q. ilex*. In particular, tree BA had negative correlation effects for *P. halepensis* as this empirical function lies down the lower envelope. There was evidence that trees at distances of less than two meters had smaller tree sizes than they should have under the hypothesis of random marking. Moreover, the resulting cross-mark correlation function (Eq. 4) for tree BA for both tree species (Figure 3b) suggested no inter-specific BA spatial correlation. Thus, tree size for a given species did not depend on the presence of the other species.

Visual inspection of the mark point pattern of *Q. ilex* and *P. halepensis* for the isotopic compositions of oxygen and hydrogen indicated increasingly higher values along the X-axis, suggesting the presence of anisotropic effects; this result was especially noticeable for *Q. ilex* (figure not included). This is in full agreement with the results obtained in the ANCOVAs. Because we observed directional components in the X-axis, i.e. anisotropic mark effects, and these effects affected the resulting estimated correlation functions, which are defined for isotropic (marked) point patterns, we considered a correction for such effects. Since the related point patterns were isotropic and only the mark component showed anisotropic effects, the use of inhomogeneous (anisotropic) versions of the point correlation functions based on a (non)parametric estimate of the point intensity (see, for instances, Law *et al.,* 2009) were not of applicability. In the case of anisotropy in the marginal distribution of marks, these anisotropic effects should be incorporated in the mark correlation function by allowing this distribution to vary along this directional component. This could be done by assuming the mark expectation (say) to vary along this directional component. However, this approach is not trivial and few studies (if any) have corrected anisotropy for the mark component. Therefore, we adopted an easier procedure to correct mark anisotropic effects by considering the residuals of these variables (marks) after assuming a deterministic, species-dependent linear trend through this X-axis in accordance with the outcome of the ANOVAs. These residuals were also scaled to avoid negative values. The resulting marked point pattern of residuals is not affected by the linear trend and therefore ensures isotropy.

Figure 4 shows the resulting mark point pattern for $\delta^{18}O$ residuals and the resulting mark correlation function, highlighting that water uptake strategies for *P. halepensis* were dependent on the spatial tree configuration. Particularly, pine trees had similar water uptake patterns at short inter-trees distances, and neighbouring trees at distances less than 4 meters tended to obtain more superficial (i.e. closer to topsoil values) water. Non-significant spatial dependencies were obtained for *Q. ilex*. In contrast, the cross-mark correlation function for this isotope showed spatial independence between both tree species (see Figure 4b). Thus, species-specific water extraction strategies did not depend on the presence of the other species.

As expected, comparable results to those of $\delta^{18}O$ were obtained for the spatial structure of $\delta^{2}H$ residuals (Fig. 5) since both isotopes were highly correlated. For *P. halepensis*, $\delta^{2}H$ residuals had spatial dependencies at short inter-tree distances (<4 meters), while for *Q. ilex* we assumed that the isotopic composition were spatially uncorrelated.

Discussion

Do Aleppo pine and Holm oak occupy different niches for water uptake?

Marked point process statistics are valuable techniques to evaluate and describe forest systems (see, amongst others, Stoyan & Penttinen, 2000; Comas & Mateu, 2007). Here, we considered these spatial tools to disentangle competition effects for water at inter- and intra-specific levels in a water-limited environment. Our results highlight the existence of clear niche segregation between the Mediterranean trees *P. halepensis* and

Q. ilex. Firstly, and using classical analysis of covariance procedures, we found that the stable isotope composition of xylem water (both $\delta^{18}O$ and $\delta^{2}H$) was on average significantly higher in Aleppo pine than in Holm oak. Decreasing trends in soil water $\delta^{18}O$ and $\delta^{2}H$ were also observed with soil depth, confirming the existence of an evaporative gradient in the soil. An increasing trend in xylem water $\delta^{18}O$ and $\delta^{2}H$ was also observed along the X dimension of the experimental plot, which agrees with decreasing soil depth following this direction, hence favoring higher water evaporation. However, this trend was steeper for *Q. ilex*, suggesting that this species had comparatively better access to deep soil layers than *P. halepensis* with increasing soil depth. Comparing the $\delta^{18}O$ and $\delta^{2}H$ of xylem water with the soil profile, we may first conclude that Holm oak takes up more water from deeper soil layers than Aleppo pine after a long drought period, as would be expected according to the deeper root system of evergreen schlerophyllous, as compared to pines (Canadell *et al.*, 1996). Besides overall inter-specific differences, the study of tree-tree interactions using mark correlation functions confirmed the existence of two separate niches for water uptake. Firstly, we observed a significant spatial dependencies of neighbouring pines (inter-tree distances of less than 4 m.) to obtain water from upper soil layers (i.e. higher $\delta^{18}O$ and $\delta^{2}H$ of xylem water), and an uncorrelated spatial configuration for oaks (see Figures 4-5). In contrast, when looking at the inter-specific relationships (i.e. the effect of neighbours from the opposite species), we did not find any clear pattern for either competition or facilitation. Thus, there are interdependencies among neighbour pines, but not among neighbours of different species. This is in agreement with the niche segregation hypothesis, further supporting the idea that oaks are able to get water from deeper soil layers that may be less accessible for pines. In this regard, the depleted isotopic values observed in the xylem of oaks are typical of deep soil water, showing no signs of evaporative enrichment. The postulated access to non-evaporated deep soil layers, with high water availability, would also explain the lack of spatial interaction among oak individuals: under such conditions, competition for water resources is scarce.

Similarly, the mark correlation function for BA of pines (Figure 3d) suggested the existence of competitive inhibition for growth at distances below 2 m. In direct contrast, tree BA was distributed at random for oaks and oaks-pines spatial structures, respectively. This points out that long-term growth in Aleppo pine is more strongly affected by competition than it is in the case of Holm oak, in agreement with its greater plasticity in radial growth (Ferrio et al., 2003; Zavala et al., 2000). Nevertheless, what remains intriguing is the fact that Aleppo pine, a more sensitive species to competition, does not show a clear response in terms of growth in the presence of Holm oak. In our case, observed BA responses may reflect the shade-intolerant character of Aleppo pine, but also they could be a result of increasing competition for water resources, or (most likely) a combination of both factors (Zavala *et al.*, 2000).

In any case, it is likely that the competitive effect of Holm oak trees on individuals of Aleppo pine was much lower than if neighbor trees were from the same species. Particular reasons for this may be two-fold. On the one hand, and regarding competition for water resources, the observed evidences of distinct water uptake patterns for the two species may explain the lack of interaction, even when water resources are limiting (see e.g. Klein *et al.* 2013). On the other hand, the much faster height growth of pines may ensure them attaining a dominant position in terms of light interception, as compared to oaks (Zavala *et al.*, 2000). Conversely, the shade-tolerant nature and conservative growth of the evergreen oak would cause a lack of negative response to this sort of dominance. Nevertheless, due to the existence of a relatively open canopy, it is more likely that growth patterns were dominated by water limitation, at least for the case of the most shade-intolerant species.

Alternative sources of variation for stable isotopes in xylem water

In this study we initially assumed that differences in xylem water would reflect distinct water uptake patterns originating from contrasting contributions of soil layers.

However, whereas interspecific differences can be easily explained by the uptake of water from different depths, the observed increase in $\delta^{18}O$ and $\delta^{2}H$ in neighbouring trees, particularly in pines, is less straightforward. The presence of close neighbours can be interpreted as a local increase in stand density, and indeed more positive values in $\delta^{18}O$ of xylem water of Aleppo pine have been reported when comparing a densely afforested stand (770 trees ha^{-1}) with an open woodland (20 trees ha^{-1}) (Moreno-Gutiérrez *et al.*, 2012a). The direct interpretation is that closer trees tend to use more water from upper soil layers, although there is no clear physiological reason for this behaviour. As pointed out by Moreno-Gutiérrez *et al.* (2012a), one possibility is that the presence of close neighbours increased shadowing, thus reducing soil evaporation and keeping more water available in upper soil layer, which is generally enriched as compared to deeper soil. Nevertheless, since the enrichment of upper soil water is caused by evaporation, the water available in the upper soil of dense stands is likely to be less enriched than that of a more exposed soil surface, thus having an opposite effect. In addition, a similar effect would have been expected in response to shadowing caused by oaks, and this is not supported by our data. An alternative explanation may come from evaporation processes occurring in the branch (Dawson & Ehleringer, 1993): when transpiration rates are drastically reduced, e.g. during drought periods or in a cold winter, water in the branches has a longer turnover time and may show progressive evaporation, or partially mix with enriched phloem water. In this regard, it is likely that neighbour trees competing for a limited water source would show higher restrictions in transpiration than those trees having fewer neighbours, thus becoming more prone to branch evaporation. Branch evaporation, in turn, would increase the proportion of heavy isotopes in xylem water, due to the faster evaporation of the light isotopes, and thus could explain the higher $\delta^{18}O$ and $\delta^{2}H$ observed in trees with close neighbours. This would also explain the stronger neighbour effect in pine as compared to oak, since the former is a water-saving species, with a more sensitive stomatal response (Ferrio *et al.*, 2003; Zavala *et al.*, 2000). However, again certain effect of the presence of oaks over pine isotope composition would have been expected, since the effective water uptake of oaks would also decrease water available for the pine, pushing the reduction of transpiration. Nevertheless, although we do not have direct measurements of tree transpiration in our site, previous studies on *P. halepensis* have shown that late-summer transpiration at the leaf level may still account for *ca.* 20% of maximum values (Klein et al. 2005; Baquedano & Castillo 2006; 2007), and a similar proportion has been observed in whole-tree transpiration (Raz Yaseef *et al.* 2010; Klein *et al.* 2013, 2014). Similarly, studies on Ponderosa pine have shown that even during summer drought the trees do not cease transpiration and can still use a significant proportion of water from upper soil layers (Fernández *et al.* 2008). Altogether, both current isotope evidence and previous works on *P. halepensis* suggest that the observed inter- and intra-specific differences could reflect the use of distinct water pools in the soil, although the underlying causes still require further clarification.

Conclusions

Although results from our case study are not totally conclusive, the application of point-process statistical tools has allowed us to go beyond the comparison of inter and intra-specific (non-spatial) differences in water uptake, thereby revealing complex spatial dependencies in the use of water. In particular, our study indicates complementary water uptake patterns between Aleppo pine and Holm oak during the dry season, showing intra-specific competition among neighbour pines, but neither facilitation nor competition between individuals of different species. These results, however, might not be extrapolated to any pine-oak mixed stands, since root development might be affected by the history of the stand (e.g. whether oaks are seedlings or sprouts) and the different degree of dominance of each species. However, it should be noted that competition for water resources can be dynamic, mainly modulated by water availability (see e.g. Bellot *et al.*, 2004; Hentschel *et al.*, 2013). In this regard, assessing the seasonal-course of tree-to-tree interactions might help to explain how pines and evergreen oaks often co-exist in long-term equilibrium in areas with limited water resources (Zavala & Zea, 2004).

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Figures and Tables

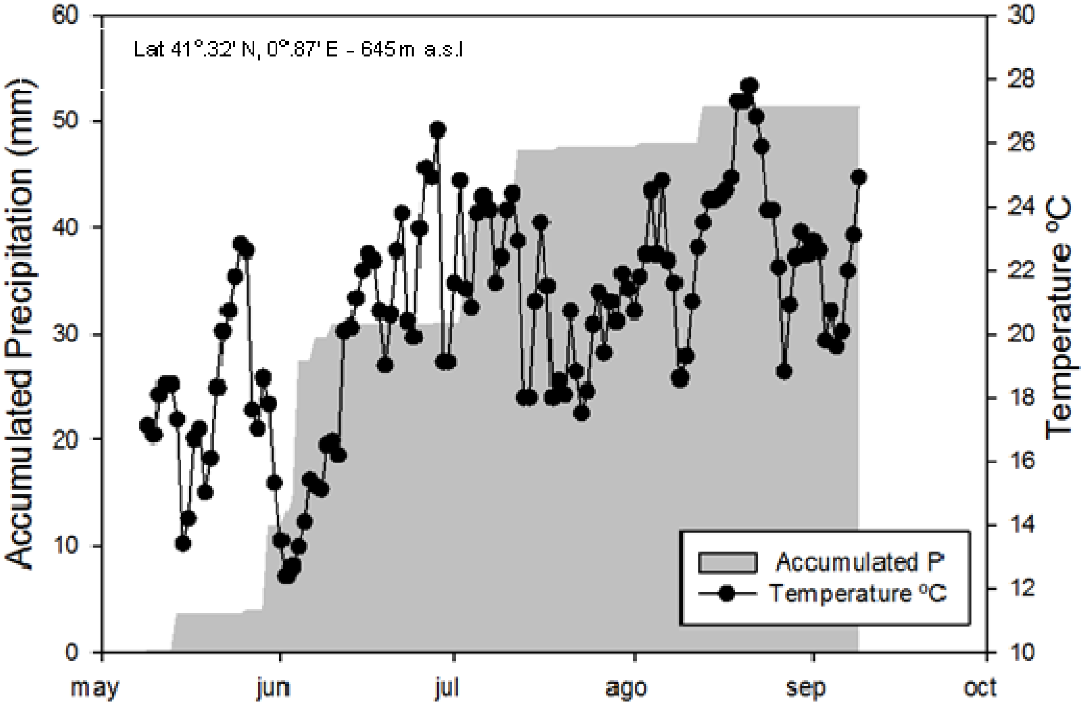

Fig. 1. Environmental context. Meteorological data of the four months before sampling, showing mean temperature and accumulated precipitation. The data correspond to the automatic meteorological station of Ulldemolins, located at 3.5 km from the samplig site.

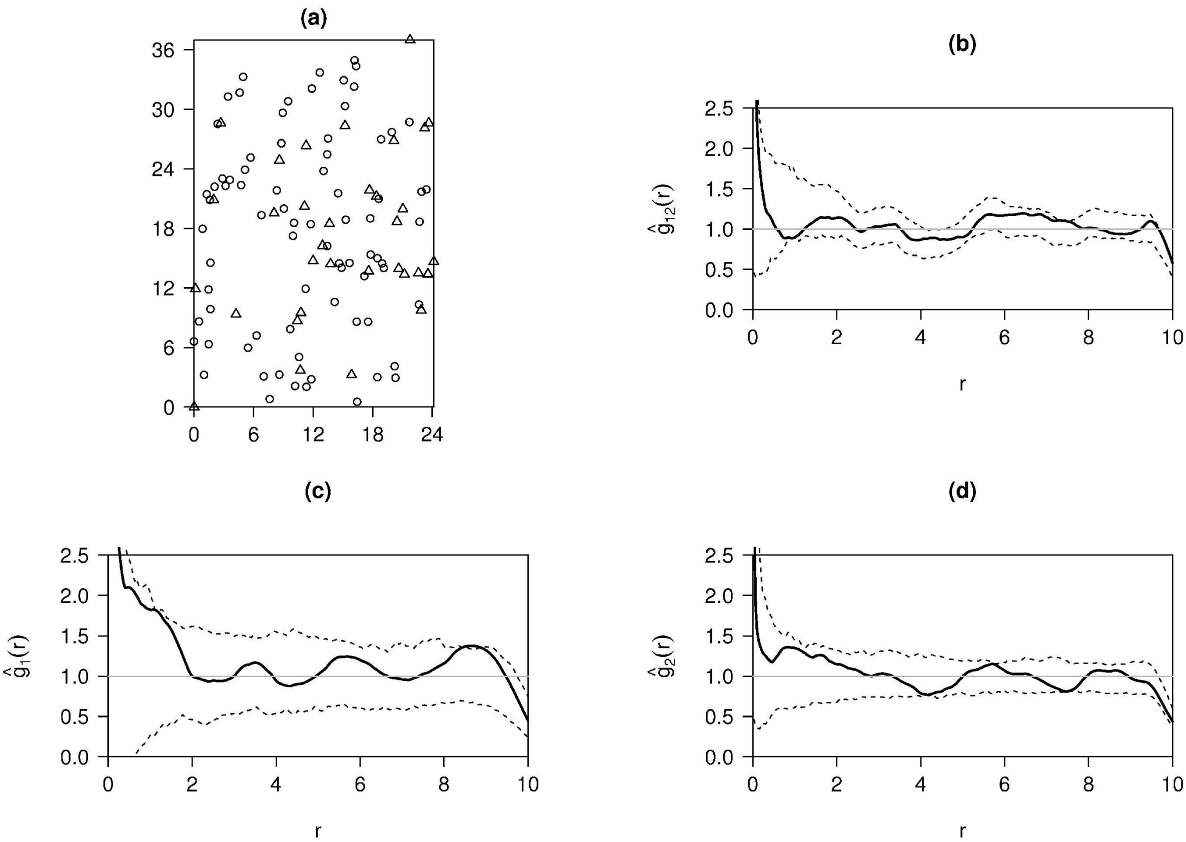

Fig. 2. Bivariate point pattern. (a) Bivariate point pattern of *Q. ilex* (triangle) and *P. halepensis* (circle) in a forest stand in Central Catalonia (Spain) together with (b) the resulting cross-pair correlation function (Eq. 2), assuming random labelling, and empirical pair correlation functions (Eq. 1) for (c) *Q. ilex* and (d) *P. halepensis* and their fifth-largest and smallest envelope values (dashed lines) based on 199 random simulations according to these two null hypotheses (random labeling and Poisson point randomizations); inter-tree distance (r) is given in metres.

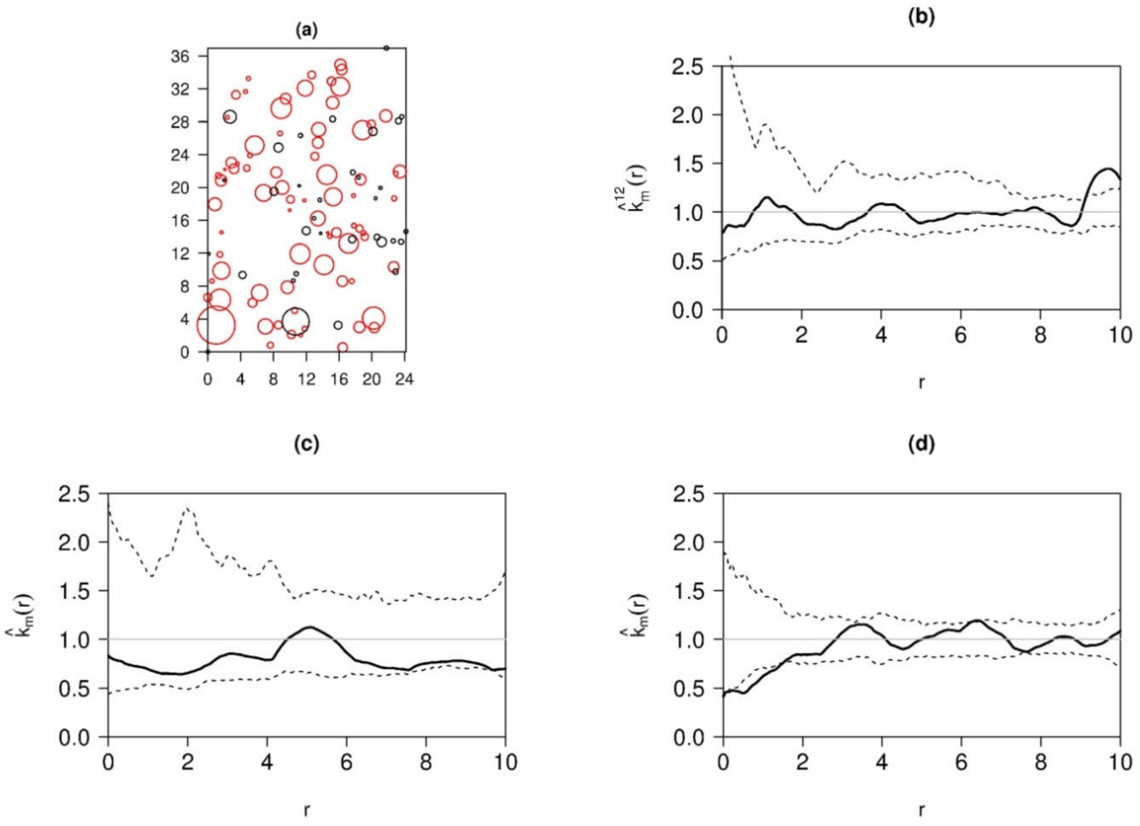

Fig. 3. Mark correlation function (basal area). (a) Bivariate marked point pattern for basal area, involving two tree species, *Q. ilex* (black circles) and *P. halepensis* (red circles), together with (b) the estimated cross-mark correlation function (Eq. 4), and resulting mark correlation functions (Eq. 3) for (c) *Q. ilex*, (d) *P. halepensis*. The fifth-largest and smallest envelope values (dashed lines) are based on 199 random labelling and marking (b), and 199 random marking (c and d) over fixed point positions. Circle plot radius is proportional to each mark and inter-tree distance r is given in metres.

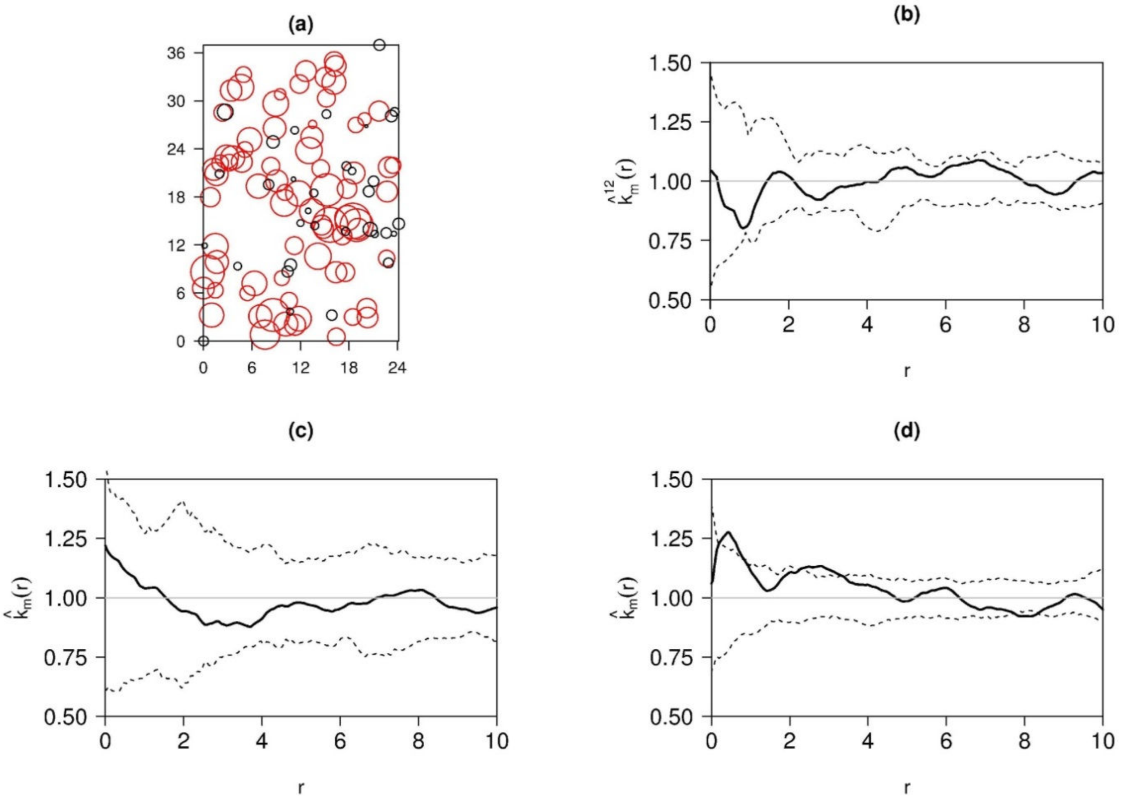

Fig. 4. Mark correlation function (oxygen-18). (a) Bivariate marked point pattern for oxygen isotope composition ($\delta^{18}O$) residuals, involving two tree species, *Q. ilex* (black circles) and *P. halepensis* (red circles), together with (b) the corresponding cross-mark correlation function (Eq. 4), and the resulting mark correlation function (Eq. 3) for (c) *Q. ilex*, (d) *P. halepensis*. The fifth-largest and smallest envelope values (dashed lines) are based on 199 random labelling and marking (b), and 199 random marking (c and d) over fixed point positions. Circle plot radius is proportional to each mark and inter-tree distance r is given in metres.

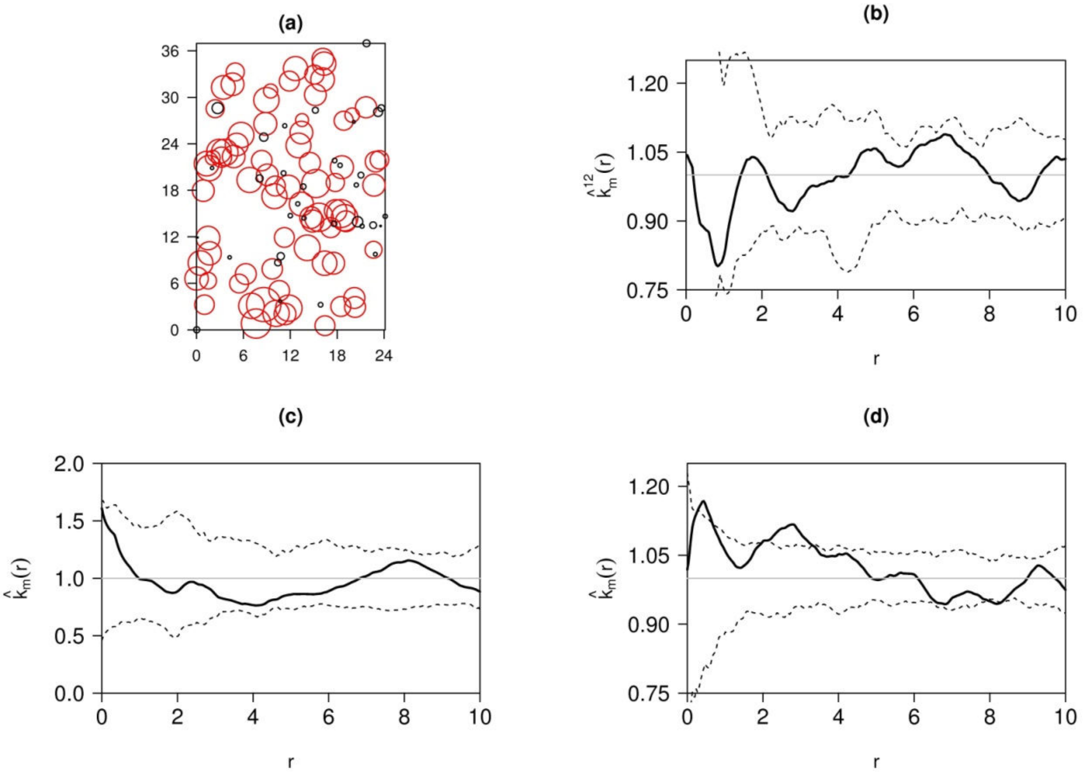

Fig. 5. Mark correlation function (hydrogen-2). (a) Bivariate marked point pattern for hydrogen isotope composition (δ^2H) residuals, involving two tree species, *Q. ilex* (black circles) and *P. halepensis* (red circles), together with (b) the corresponding cross-mark correlation function (Eq. 4), and the resulting mark correlation function (Eq. 3) for (c) *Q. ilex*, (d) *P. halepensis*. The fifth-largest and smallest envelope values (dashed lines) are based on 199 random labelling and marking (b), and 199 random marking (c and d) over fixed point positions. Circle plot radius is proportional to each mark and inter-tree distance r is given in metres.

Table 1. Mixed model analysis of variance (ANOVA) for the three variables studied. Covariates accounting for variation along the X and Y axes of the two-dimensional space (alone and interacting with the species factor, i.e. testing for separate slopes) are included in the models. In parentheses, standard errors of variance components.

Source of variation ($\delta^{18}O$)				
Fixed effects	Num DF	Den DF	Variance ratio	Pr > F
species	1	105	264.7	<.0001
X Coordinate	1	105	21.71	<.0001
Y Coordinate	1	105	1.44	0.2323
X × species	1	105	10.05	0.0020
Y × species	1	105	1.62	0.2055
Random effects			Variance component	
Residual (*Q. ilex*)			0.68 (0.177)	
Residual (*P. halepensis*)			2.40 (0.391)	

Source of variation ($\delta^{2}H$)				
Fixed effects	Num DF	Den DF	Variance ratio	Pr > F
species	1	105	115,5	<.0001
X Coordinate	1	105	20.9	<.0001
Y Coordinate	1	105	0.1	0.9284
X × species	1	105	4.4	0.0384
Y × species	1	105	2.3	0.1353
Random effects			Variance component	
Residual (*Q. ilex*)			12.43 (3.209)	
Residual (*P. halepensis*)			45.85 (7.487)	

Source of variation (individual basal area)				
Fixed effects	Num DF	Den DF	Variance ratio	Pr > F
species	1	105	18.54	<.0001
X Coordinate	1	105	0.03	0.8524
Y Coordinate	1	105	0.85	0.3578
X × species	1	105	0.17	0.6776
Y × species	1	105	0.09	0.7685
Random effects			Variance component	
Residual (*Q. ilex*)			22600 (5835.4)	
Residual (*P. halepensis*)			45780 (7475.9)	

Chapter IV

“Survivors aren't always the strongest; sometimes they're the smartest, but more often simply the luckiest.” Carrie Ryan, *The Dark and Hollow Places*

Competition for water in a mixed oak-pine Mediterranean forest: a stable isotope approach

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Abstract

Water is the most important limiting factor for plant growth in Mediterranean ecosystems. In this context, we aimed at detecting biotic interactions for water uptake among two typical Mediterranean tree species (*Quercus ilex* and *Pinus halepensis*) coexisting in a mixed forest on a sloping site with shallow soils. We used xylem water stable isotopes (δ^{18}O and δ^{2}H) for all trees found in the studied stand (*ca*. 900 m^2) at six different dates covering an extreme summer drought that occurred in 2011. We applied point-process statistics and other methods that integrate stand density information to evaluate possible tree to tree interactions for water use. Our results suggest the existence of inter- and intra-specific competition for water. We also found that there is clear niche segregation between the two species in periods of maximum drought, in which *Q. ilex* used groundwater to maintain a higher physiological activity, typical of its anysohydric behavior. On the other hand, *P. halepensis* seemed to remain inactive during the summer drought illustrating a drought avoidance-isohydric performance. Future drier conditions may affect the coexistence of both species by decreasing opportunities for complementarity, leading to reduced stand density and, ultimately, threatening the persistence of this widespread forest structure.

Keyword index: point patterns, stable isotopes, water uptake, *Pinus halepensis*, *Quercus ilex*, extreme drought, water-use strategies

Introduction

The actual composition and dynamics of forest systems in the Mediterranean basin is likely to undergo important modifications due to the predicted increase in the frequency of drought and heatwaves for the next decades (IPCC 2013). In this region water is usually the most import limiting factor for plant functioning, and subtle changes in the water available to plants can have profound effects at the ecosystem level (e.g. Granda et al. 2014; Linares and Camarero 2012). The resilience of Mediterranean forests is related to differences in functional traits among trees, allowing co-occurring individuals to have differential water-use strategies both at the inter-specific (e.g. Filella & Peñuelas 2003) and intra-specific levels (e.g. Voltas et al. 2015).

A typical situation in coastal ranges along the western Mediterranean basin is the formation of pine-oak mixed forests (Gil *et al.* 1996). Pines, as water-saving, drought-avoiding species share space and resources with more drought-tolerant, less conservative species such as evergreen oaks (Lookingbill & Zavala 2000; Zavala et al. 2000; Zavala et al. 2011). Traditionally, pine-oak mixed forests were considered as transient states in ecological succession towards an oak-dominated climax vegetation (Rivas-Martínez 1987). However, increasing evidence points towards niche segregation as one of the mechanisms determining the long-term persistence of these communities (Comas et al. 2015; Zavala *et al.* 2000; Zavala and Zea 2004). Whether facilitation or competition mechanisms are enhanced under such resource-limited coexistence is still a matter of debate (Díaz-Sierra *et al.* 2010; Maestre *et al.* 2005; Zavala and Bravo de la Parra 2005).

Competition for water is mainly driven by resource availability, which benefits species that are best adapted to drought (Craine & Dybzinski 2013). In this context, water scarcity leads to an increase in competition for water in mixed-species stands (Grossiord et al. 2014). Jucker et al. (2014) found in Iberian oak-pine mixed forests that species benefit less from mixing under dry conditions, suggesting that drought decreases opportunities for complementarity between pines and oaks. While oaks were more resistant to drought and less sensitive to environmental changes, experiencing little changes in aboveground growth, pines benefited under abundance of resources by growing faster, but suffered more in periods of resource scarcity. Alternatively, other studies suggest that pines use less water than oaks while having similar carbon gains throughout the year (i.e. higher water-use efficiency, WUE) (Klein et al. 2013a), which may give them competitive advantage.

A representative Mediterranean tree such as the evergreen *Quercus ilex* has shown a great sensitivity to xylem embolism in extreme summer droughts due to groundwater depletion (Barbeta et al. 2015), otherwise allowing it to remain physiologically active during summer (i.e. anysohydric behavior). Contrary to that, *Pinus halepensis*, which typically forms mixed forest with *Quercus ilex*, strongly reduces stomatal conductance under drought, which may lead to mortality after periods exceeding 90 days without access to water pools (i.e. isohydric behavior) (Klein et al. 2014a). The different strategies of these complementary species are related to contrasting physiological and anatomical traits such as stomatal sensitivity and root system distribution (Ferrio et al. 2003; Klein 2014).

Understanding inter- and intra-specific belowground interactions and competition is problematic because of the lack of appropriate technologies for accessing and monitoring root distribution in trees (Maeght et al. 2013; Rewald & Leuschner 2009). Rewald & Leuschner (2009) showed that the fine root systems of four species broadly overlap within the upper 20 cm of soil in a mixed-stand temperate forest, with lateral roots being up to 10 times larger than tree crowns, being such observation in accordance with several root inventories in forest. This suggests a high interaction and competition for soil resources between individuals. However, the vertical root distribution of Mediterranean woody species is often deep and able to find its way through fractured bedrocks, or even able to penetrate directly through the bedrock if growing over calcareous terrain, which allows trees to remain physiologically active during drought episodes (Canadell et al. 1996). This is particularly the case of species from the *Quercus* genus. Conversely, Mediterranean pines appear to have shallower root systems with higher densities on the top soils, exhausting water relatively fast in an opportunistic manner (Bellot et al. 2004; Klein et al. 2014b) (but see Voltas et al. 2015). But not only the different root distribution between oaks and pines may allow niche partitioning and stable coexistence between these species. Also the lower water potentials that oaks can reach in order to uptake soil water (Klein et al. 2013a; Klein 2014) may allow them to access tightly-bound water during dry periods (Brooks et al. 2009).

In the Mediterranean region forest trees usually grow under relatively shallow soils and their root systems may not reach the phreatic water, but they may still thrive by using water stored in the weathered bedrock (Barbeta et al. 2015; Maeght et al. 2013). A recent study (Comas et al. 2015) has suggested niche segregation for Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) during a period of intense drought through the analysis of the isotopic composition of xylem water. Based on point process statistics, the authors reported intra-specific competition for water among pine individuals, but neither among oak individuals nor between oaks and pines (Comas et al. 2015). In this work, we aim to test whether this niche segregation is dynamic, hence associated with the scarcity of resources (e.g. due to different ability to uptake water from the soil), or static, related to constitutive differences among the two species (e.g. due to contrasting root distribution). For this purpose, we have characterized the temporal evolution of spatial interactions for water uptake during one complete year (from spring 2011 to spring 2012) in a mixed pine-oak stand by integrating information from remote-sensing and other physiological indicators, which complement the seasonal patterns observed through xylem water stable isotopes. This experiment took place during an extraordinarily dry April to September period, the driest since 1975 in the study region (Barbeta et al. 2015).

Material and Methods

Site description

The study area is a mixed forest of holm oak and Aleppo pine located in the Montsant range (41^{o} 19' 47.3" N, 0º 50' 2.6" E, 750 m a.s.l), in the northeast of the Iberian Peninsula. The site is close to the optimal distribution range for both species (topo-climatic suitability index > 0.8; Ninyerola et al. (2010)). The climate in the region is Mediterranean temperate with continental tendency, with a mean annual precipitation of 517 mm and mean annual temperature of 12.3 °C. It is characterized by a dry and a relatively warm summer (mean summer precipitation of 89.5 mm, mean average temperature of 20.9 °C) and wet spring and autumn seasons. The forest stand originated from natural regeneration of pine and oak in a former agricultural terrace which, according to the age of the oldest individuals, was abandoned more than 100 years ago. Understory vegetation is relatively scarce and current regeneration from both species is rare. According to USDA soil taxonomy (Soil Survey Staff 2010), soil is a loamy-skeletal, carbonatic, termic, active calcic pachic haploxeroll, with soil depths ranging from *ca.* 20 cm in the upper part of the plot to *ca.* 50 cm in its lower part. This topographic characteristic, together with a sloppy terrain (see next section), makes this site to be relatively xeric.

Sample collection and tree mapping

Sampling took place in a rectangular plot area (24 x 37 m) with a strong slope (15-22%) facing west (X-axis), and a gentle slope (3-7%) facing south (Y-axis). Within this plot, stand density (dbh>10 cm) was 874 stems ha^{-1} for pine and 560 stems ha^{-1} for oak (in terms of basal area -BA-, 30.5 m^2 ha^{-1} and 7.2 m^2 ha^{-1}, respectively). Oaks stems originating from the same stool were considered as a single individual; hence the individual density for the oak was of 370 stools ha^{-1}. Tree position was determined using a high resolution GPS technology (GeoExplorer 6000 Series Handheld, Trimble Navigation Limited, California, USA). The distribution of individuals was found to be totally at random for both species (Comas et al. 2015).

We performed six extensive field sampling campaigns (i.e. including all individuals: 33 oaks and 78 pines) during one seasonal cycle, from spring 2011 to spring 2012. Sampling dates were May 26th, July 20th, September 9th, October 19th, November 18th (2011) and March 27th (2012) (Fig. 1). For each sampling time, we collected xylem samples from sun-exposed twigs of the upper third of the crown. Soil samples were also collected from 6 points in the plot at two depths: topsoil (2-10 cm, A horizon) and subsoil (B horizon, 5 cm above the maximum soil depth, from 10-55 cm). Xylem was separated from the bark and, together with soil samples, immediately frozen with dry ice in air-tight tubes for subsequent water extraction by cryogenic distillation (Dawson et al. 1993). In order to discard evaporative enrichment in the branch as the cause of a potential uncoupling between soil and xylem water (Palacio et al. 2014; Evaristo et al. 2015) we performed an additional sampling campaign in summer 2013. For this purpose, we selected 10 individuals to be representative of the range of isotopic variation and spatial distribution of the trees in the plot. In this case, samples were collected simultaneously from main trunk and twigs, and midday water potential was determined with a pressure chamber.

Water isotope analysis

Water isotope ratios of hydrogen and oxygen of the xylem and soil water were determined using a Picarro L2120i analyzer coupled to a high-precision A0211 vaporizer (Picarro Inc., Santa Clara, California), and expressed in delta (δ) notation (‰) relative to V-SMOW (i.e. isotopic composition of oxygen, $\delta^{18}O$, and hydrogen, $\delta^{2}H$). The estimated precision, based on the repeated analysis of four reference water samples, was 0.10‰ and 0.40‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. The potential presence of organic contaminants was checked using the post-processing software Picarro Chem-Correct 1.2.0, giving in almost all cases negative results. Nevertheless, for consistency we applied to all samples the post-processing correction described in Martín-Gómez et al. (2014). In agreement with the low level of contamination, we found a very strong correlation between corrected and uncorrected values (r^2=0.995 for $\delta^{18}O$; r^2=0.979 for δ^2H, *N*=680), with 92% of the samples showing differences lower than 0.4‰ for $\delta^{18}O$ and 4‰ for δ^2H. As a reference for the range of local precipitation, we used historical data (2000-2009) from Tortosa, a nearby station (*ca.* 60 km) of the Spanish Network of Isotopes in Precipitation (REVIP; Capilla et al. 2011).

Evaluation of evaporation processes in water

In order to evaluate potential evaporation processes in xylem and soil water we looked at changes in δ^2H with respect to $\delta^{18}O$ by plotting them together and against the global and local meteorological water lines, which state an average relationship between the H and O isotopes ratios in natural terrestrial waters (Craig 1961). Evaporation processes lead to greater changes in O than H isotopes ratios and, thus, to a displacement from the global and local meteoric water line. Therefore, any displacement of the water isotopic values from the meteoric line may indicate evaporative processes and should be considered in the interpretation of water sources for plants.

Analysis of covariance

Xylem isotopic records were subjected to analysis of covariance (ANCOVA) considering the species effect (pine, oak) and the linear variation along the X and Y axes of the two-dimensional space (covariates), allowing for heterogeneity of regression slopes at the species level. This was done to check for (possible) differential systematic variation in the response variables ($\delta^{18}O$ and δ^2H) following X and Y directions, i.e. spatial anisotropic effects. We also allowed for heterogeneity of residual variances at the species level, which was checked by means of log likelihood ratio tests. For the difference between two nested models (homocedastic and heterocedastic), minus two times the log likelihood ratio is distributed asymptotically as χ^2 with degrees of freedom equal to the difference in the number of parameters (Verbeke & Molenberghs 2000).

Spatial statistics

To analyse the marked point ($\delta^{18}O$ or $\delta^{2}H$) patterns of oaks and pines associated to each tree location we used the mark correlation function $k_m(r)$ (Stoyan and Stoyan 1994) for each sampling time. This function describes the spatial structure of tree marks and an estimator of this function can be written via

$$\hat{k}_m(r) = \frac{1}{2\pi\hat{\lambda}^2|A|}\sum^{\neq}_{(x_1,m_1),(x_2,m_2)\in\varphi_m}\frac{m_1m_2\kappa(\|x_1-x_2\|-r)}{e(x_1,\|x_1-x_2\|)\hat{g}(r)\mu^2} \qquad (1)$$

for a forest stand A with area $|A|$, where φ_m is a marked point pattern and m_1 is the mark value for tree 1 (say) and x_1 its spatial location, $\hat{\lambda}$ is an estimator of the point intensity, $\kappa(\cdot)$ is the Epanechnikov kernel function, $\sum^{\neq}$ stands for the summation over all pairs such that $x_1 \neq x_2$ and $e(\cdot)$ is the Ripley's factor (Ripley, 1976) to correct for edge effects, for a given inter-distance r between points (trees). This function denotes independence between marks when $k_m(r)=1$, $k_m(r)>1$ indicates positive mark correlation, whilst $k_m(r)<1$ implies mark inhibition for all $r>0$. Following the outcome of the ANCOVA, we corrected for mark anisotropic effects by considering the residuals of the marks after assuming a deterministic, species-dependent linear trend through the X-axis. These residuals were subsequently scaled to avoid negative values. The resulting marked point pattern of residuals is not affected by the linear trend and therefore ensures isotropy.

To study the correlation between species (qualitative mark) with regard to a particular tree quantitative characteristic (i.e. the xylem water isotopic compositions) we adopted a derivation of the mark correlation function initially proposed by Penttinen et al. (1992). Here the point pattern consists of two distinct tree species together with a mark associated to each tree position. Penttinen et al. (1992) defined an estimator of this cross-mark correlation function through:

$$\hat{k}^{12}_m(r) = \frac{1}{2\pi\hat{\lambda}_1\,\hat{\lambda}_2|A|}\sum_{(x_1,m_1)\in\varphi_{m_1},(x_2,m_2)\in\varphi_{m_2}}\frac{m_1m_2\kappa(\|x_1-x_2\|-r)}{e(x_1,\|x_1-x_2\|)\hat{g}_{12}(r)\hat{\mu}^2_{12}} \qquad (2)$$

where φ_{m_1} is the marked point pattern for class 1 (say), $\hat{\lambda}_1$ its point intensity, $\hat{\mu}_{12}$ is an estimator of μ_{12} (i.e. the expectation of m_1m_2, marks from classes 1 and 2) and $\hat{g}_{12}(r)$ stands for an estimator of the cross-pair correlation functions. The interpretation of $\hat{k}^{12}_m(r)$ is similar to that of $k_m(r)$. It indicates spatial positive correlations for the spatial pattern when $\hat{k}_m^{12}(r) > 1$, $\hat{k}_m^{12}(r) = 1$ is the Poisson case, whilst $\hat{k}_m^{12}(r) < 1$ implies negative dependencies. For the Epanechnikov kernel function, we chose the bandwidth to be equal to $c/\sqrt{\lambda}$, where typically $c = 0.1 - 0.2$, as suggested by Stoyan and Stoyan (1994) (here $c = 0.2$).

Each kind of spatial correlation function was tested for spatial independence following a Monte Carlo approach based on the random simulation of (marked) point patterns from the null hypothesis (Poisson). We simulated 199 (marked) point patterns under the null hypothesis of spatial independence, and for each one, an estimator of one of the correlation functions defined above was obtained. We rejected the null hypothesis (spatial independence) if the resulting (empirical) estimator of this correlation function lay outside the fifth largest and/or smallest envelope values obtained from the set of simulated functions with a significant level of $\alpha = 2 \times 5/(199+1) = 0.05$. Testing for spatial independence of marked point patterns of oaks and pines, respectively, was based on the *random marking* approach (Illian et al. 2008). For the point pattern consisting of both tree species together with a mark associated to each tree position we assumed *random labelling* of tree species over the fixed point positions, and then over this new bivariate point pattern, we considered a random marking approach to generate independent bivariate marked point configurations (see Penttinen et al. 1992).

For the statistical analysis of point patterns, we considered the computational implementation in the statistical package Spatstat for the R statistical environment (R Development Core Team, 2007).

Examining density-related effects

Mark-correlation analyses are powerful tools for the study of direct inter-individual interactions, but are based on the comparison of pairs of trees against plot averages. Hence, they do not take into account potential additive effects caused by several individuals (e.g. for the correlation function a single tree in a radius of 1 m from a given individual is equally considered as if they were 4 trees within the same radius). As complementary approach we also examined the effect of local variations in the stand density (as stem ha^{-1}) and stand BA (m^2 ha^{-1}) of oaks and pines on the isotopic composition of each individual. For this purpose, we determined the number of individuals and accumulated BA in a 5 m-radius around each individual. Relationships between target variables and density estimates around each individual were tested using pair-wise Pearson correlations. As an ad-hoc, conservative solution to correct for border effects in the correlations, each sample was weighted as a function of its distance to the border. Considering a minimum inter-tree distance of about 0.5 m, we applied a weight from 0 to 1 and proportional to the distance to the border of the plot for those trees located at less than 4.5 m from it. All trees located at 4.5 m or more from the border were assigned a weight of 1. We also tested other radius (4 and 6 m), as well as more restrictive corrections for border effects (e.g. removing the samples at 3, 4 or 5 m from the border), resulting in comparable results (not shown).

Remote sensing data

A pixel (250 m x 250 m) containing the spectral variation (biweekly) of the Normalized Difference Vegetation Index (NDVI) for the study period in two nearby pine-dominated and oak-dominated stands was obtained from the MODIS Land Subsetted Products at the MODIS web service (2014). Seasonal changes in soil water content for the same stands were derived from data acquired from the ESA´s Soil Moisture and Ocean Salinity (SMOS) mission, available at http://www.smos-bec.icm.csic.es; the data is a high resolution soil moisture delayed product, a data set of soil moisture maps of the Iberian Peninsula with 1 km spatial resolution, which contains two maps per day, corresponding to SMOS ascending (6 A.M.) and descending (6 P.M.) passes. Such maps are obtained using the downscaling algorithm, which combines the brightness temperature measurements from ESA-SMOS with Land Surface Temperature and NDVI data from Terra/Aqua MODIS.

Results

Evolution of climatic and biophysical site conditions during the 2011-2012 season

Precipitation was abundant during spring and early summer of 2011 (May and July respectively). However, high May-June temperatures lowered the soil water content (SWC) in summer as compared to spring (Figure 1). During the transition from spring to summer the NDVI of oaks and pines started to drift apart, with pines showing lower NDVI values (Figure 1). As drought progressed due to lack of precipitation and high mean temperatures in late summer and September 2011, NDVI values of oaks declined down to values similar to those of pines. Furthermore, the drought conditions exacerbated during October 2011 as precipitation was very scarce, leading to both a clear reduction in SWC and a strong decline in NDVI for both species. At this point, both species showed a similar minimum annual NDVI. Later, NDVI values recovered to an annual maximum with the arrival of autumn-winter precipitation (November 2011), which also led to annual maximum SWC values. From this time on, a long period of drought during winter to early spring took place which forced SWC as well as NDVI values down to summer records, showing only a partial recovery after the first rains in April 2012.

Seasonal changes in the isotopic composition of soil and xylem water

The isotopic composition of water from xylem and soil showed, in most sampling times, a clear deviation from the Global and Local Meteorological Water Line (Figure 2), which was symptomatic of evaporative enrichment of both water types. Furthermore, topsoil was more enriched than subsoil in four out of the six sampling times (Figure 2), suggesting an evaporative enrichment defining the isotopic gradient within the soil vertical profile. Particularly, we found a high correlation between soil depth and either $\delta^{18}O$ or $\delta^{2}H$ in May and September 2011, as well as in March 2012, but not in October and November 2011. Due to missing records, this association could not be properly assessed for July 2011. Groundwater values from a nearby fountain were -7.9 ‰ for $\delta^{18}O$ and -49.1 ‰ for $\delta^{2}H$, similar to the values found during soil recharge periods and at the deepest soil layer.

We also observed distinct seasonal patterns in isotopic signatures at the species level. During the wet spring and early summer (May and July 2011), the isotopic values of xylem water overlapped in both species, falling mostly within the range of subsoil water, which was less enriched than the topsoil water (Figure 2a, 2b). However, as the dry season progressed, reaching the period of maximum drought (September-October 2011), we found a clear distinction in isotopic signatures between species together with notable changes in soil values, despite the lack of relevant precipitation events (Figure 2c, d). During this period, xylem water in pines fell outside the range of contemporary soil values and became isotopically enriched, lying along the evaporation line of soil and xylem values characteristic of the end of the wet season (July 20th) (Figure 2b). On the other hand, oaks shifted to the new range of subsoil water values in September 9th. In October 19th, however, the water in oaks fell outside the range of soil values, following a distinct evaporation line similar to that of September 9th. During drought recovery (November 18th), the isotopic signature of pines and oaks were still more enriched than the soil water, although they were in line with the soil records (Figure 2e). Conversely, most trees from both species showed more depleted values as compared with soil records after the next important rain event occurring in March 2012, but again the xylem isotopic values were placed along the soil evaporation line.

We also found significant correlations between the individual isotopic values of xylem water across different sampling times, although stronger and more consistent for oaks than for pines. For oaks, the $\delta^{18}O$ and $\delta^{2}H$ values of xylem water in July 20th were significantly correlated with September 9th and October 19th values (r= 0.75 and r= 0.70 for $\delta^{18}O$; r= 0.76 and r= 0.70 for $\delta^{2}H$; $P< 0.05$). For pines we only found a weak correlation for $\delta^{2}H$ between July 20th and September 9th (r= 0.27, P=0.017). As drought progressed, correlations involving September 9th and October 19th records were also stronger for oaks (r= 0.75, P<0.001, for both $\delta^{18}O$ and $\delta^{2}H$) than for pines (r= 0.34, P=0.003 for $\delta^{18}O$; r= 0.47, P<0.001 for $\delta^{2}H$). Notably, the signal of such spatial configuration persisted during drought recovery (November 18th), as seen in the strong correlation between xylem values in October and November for both oaks (r= 0.71 and r= 0.82 for $\delta^{18}O$ and $\delta^{2}H$, P<0.001) and pines (r= 0.40 and r= 0.41 for $\delta^{18}O$ and $\delta^{2}H$, P<0.001).

Seasonal changes in inter and intra-specific spatial dependences

The mark univariate and bivariate correlation functions overpassed their envelopes in many occasions at different sampling times and distances, but often showing inconsistent patterns (Figure 3 and 4). The most consistent trends in both isotopes were observed in September 9th for pines and November 18th for oaks. Both showed intraspecific competition at distances of 0 to 4 meters (Figure 3c, 4c and 3e, 4e), as indicated by the higher mark values of $\delta^{18}O$ and $\delta^{2}H$ in short distances (i.e. belonging to more surface water within the soil vertical profile). However, although weakly significant or even not significant (i.e. the mark correlation line was close to the upper envelope limit), we observed similar spatial trends at different times: In the case of pines, there was a positive significant interaction at distances that ranged from zero to five meters in all sampling times, except for July 20th (Figures 3 and 4), suggesting a consistent intra-specific competition pattern. In the case of oaks, the October 19th pattern (end of dry period), although not significant, resembled that of November 18th (Figures 3 and 4). Surprisingly, no consistent patterns in terms of spatial dependencies were found at the inter-specific level in most sampling times for the bivariate cross-mark correlation function, indicating lack of relevant competition for water uptake between oaks and pines, at least at the tree-to-tree level. Only during drought recovery (November 18th), we observed a positive, nearly significant interaction at around four meter distance, resembling the intra-specific competition patterns observed in both species (Figure 3e and 4e).

Contrasting with the results of point-process analysis, although we observed a positive trend of $\delta^{18}O$ and $\delta^{2}H$ in response to con-specific density for both pines and oaks, this trend was only significant for the oaks (Figure 5). Conversely, we observed a positive response of isotope values in pines in relation to the local density of oaks, although only significant in September 9th (Figure 5a), whereas the isotopic signature of xylem water of oaks was negatively correlated with the local density of pines (Figure 5b). This indicates a contrasting rooting pattern in response to inter-specific competition for the two species.

Isotopic signals of twig and trunk and relationships with water potential

In the additional sampling campaign of summer 2013 we found that the isotopic compositions of twig xylem water were more enriched than that of trunk in both species, but they were more depleted than topsoil and subsoil records (Figure 6). In addition, the isotopic signatures of twig water fell along the line of soil water, but this was not the case of trunk water (Figure 6). In the case of oaks, there was no significant correlation at the individual level between twig and trunk isotopic water values ($r = 0$ and $r = -0.25$ for $\delta^{18}O$ and δ^2H, respectively; $n = 10$), but the associations were significant for pines ($r = 0.85$ and $r = 0.59$ for $\delta^{18}O$ and δ^2H, respectively; $n = 10$).

Midday water potentials were correlated to neither twig-water nor trunk-water isotopic values in oaks ($r = -0.36$ and $r = 0.11$ for $\delta^{18}O$ and δ^2H in twigs; $r = 0.14$ and $r = -0.19$ for $\delta^{18}O$ and δ^2H in trunk, $n = 10$); however, they were strongly correlated in pines ($r = 0.80$ and $r = 0.90$ for $\delta^{18}O$ and δ^2H in twigs; $r = 0.83$ and $r = 0.62$ for $\delta^{18}O$ and δ^2H in trunk; $n = 10$). We also found that the measured water potentials in pines were significantly correlated to twig $\delta^{18}O$ and δ^2H from the driest sampling times of the seasonal study: September 9th ($r = 0.88$ and $r = 0.93$, respectively, $n = 10$) and October 19th ($r = 0.78$ and $r = 0.87$, respectively, $n = 10$). No significant correlations were found in the case of oaks (results not shown).

Discussion

Inter-specific morphophysiological differences allow coexistence during drought

Pinus halepensis is a shallow rooted species able to exhaust rapidly (<15 days) the available water after precipitation events, particularly at 0–10 cm soil depth (Bellot et al. 2004; Klein et al. 2014b), and then switching to deeper soil layers where there is moisture availability (Klein et al. 2014b; Voltas et al. 2015). Contrary to that, *Q. ilex* develops less root biomass in the upper 10 cm of the soil than in the deeper soil horizons (Moreno et al. 2005), possibly because of its inability to tolerate high soil temperatures in summer in Mediterranean ecosystems (Barbeta et al. 2015), or as a consequence of the competition with early successional species, such as *P. halepensis*.

We found several lines of evidence supporting a distinct rooting pattern as major functional trait allowing niche segregation of coexisting *Q. ilex* and *P. halepensis*. Differences between oaks and pines in the isotope compositions of xylem water can be attributed, at least partially, to a different root system distribution (Fernández et al. 2008; Eggemeyer et al. 2008; Filella & Peñuelas 2003). Although both species appeared to use similar sources of water during the wet season, they shifted to a distinct water source during the drought period, returning back to a similar source in the next wet season (cf. Figure 2). These seasonal dynamics agree with other studies concluding that *Q. ilex* uses a higher amount of groundwater during the summer drought than during other periods of the year (Barbeta et al. 2015). David et al. (2007) also pointed out that the deep root system of *Q. ilex* (up to 13 m) allows this species to access groundwater, therefore remaining physiologically active during the summer drought. On the other hand, while the isotopic signatures of xylem water in *P. halepensis* seemed to fall well within the evaporative line of the July sampling, at the beginning of the drought period, they were above the range of contemporary soil values during the intense, out of season September drought (cf. Figure 2). This suggests that pines may have extremely reduced its physiological activity due to rapid soil water consumption and depletion as a drought-avoidance mechanism. In line with our findings, Klein et al. (2013a) reported a higher water use along the year by the oaks than the pines in mixed stands of *P. halepensis* and *Quercus calliprinos* (an evergreen oak). Bellot et al. (2004) obtained similar results on the physiological performance of *Quercus coccifera* (a deep-rooted shrub), which was unaffected by the presence of *P. halepensis*, unlike other shallow-rooted shrubs that suffered from increasing water competition as stand density and summer drought augmented.

A further evidence of the physiologically active condition of *Q. ilex* during the prolonged drought of summer-winter 2011 is provided by the high NDVI values observed during most of the dry period in nearby mono-specific oak stands as compared to pine stands (cf. Figure 1), which indicates a better water status. In contrast, *P. halepensis* experienced a fast NDVI reduction coincident with the arrival of the drought period. Del Castillo et al. (2014) found that Aleppo pine NDVI drops are associated to drought periods, and attributed this to a decrease of either leaf area index or photosynthesis (Klein et al. 2014a). Drought dormancy and/or drought deciduousness to avoid water stress is a characteristic of shallow-rooted species employing a profligate/opportunistic strategy (Moreno-Gutiérrez et al. 2012). In-situ observations from nearby road-cuts also provided empirical evidence of distinct rooting patterns between the two species. On the one hand, *Q. ilex* presented a long tap root able to penetrate through small rock cracks and with far fewer lateral roots in the upper soil layer than *P. halepensis*. Conversely, *P. halepensis* showed large superficial lateral roots that extended several times the tree crown.

Inter and intra-specific interactions for water during drought

The positive values of the mark-correlation function indicate that the closer two individuals are, the more positive their xylem water values will be. Our findings suggest that water uptake is shallower at closer inter-tree distances for *P. halepensis*. Moreno-Gutiérrez et al. (2015) reported similar effects between *P. halepensis* and understory shrubs, with shrubs becoming more enriched in their xylem water values as they were located closer to pines. These authors suggested that strong belowground competition forced the shrubs to rely on more superficial soil water. In our study, intraspecific competition among pines may have led individuals at short distances to develop shallower roots as a mean to maximize their ability to capture short precipitation pulses, suggesting that pines were not able to switch successfully to deeper soil layers during drought to avoid competition for water. In this context, inter-specific competition with *Q. ilex* may play an important role, as this species has a greater ability to compete for belowground resources (Jucker et al. 2014).

It would be tempting to hypothesize that oaks do not compete for water because they have access to an unlimited groundwater supply. However, silvicultural studies support the claim that holm oak intensively compete for water with neighbors, as stand thinning enhances secondary growth (Ducrey & Toth 1992), tree water status and physiological performance (Moreno & Cubera 2008). Indeed, although we did not find clear inter-specific interactions on a tree-to-tree level (i.e. based on point-process statistics), we observed a consistent negative relationship between oak isotope values and pine density, particularly strong under severe drought and subsequent recovery (Figure 5). This suggests that a single pine may not be enough to impact on the root distribution of oaks, but a dense pine cluster may prevent the oaks from colonizing the upper soil layers. Hence, *Q. ilex* may have been forced to further develop its deep-root system as a consequence of competition between species. Similarly, Sardans et al. (2004) reported that seedlings of *Q. ilex* increase the allocation of biomass in roots if this species has *P. halepensis* as neighbor, but not in the presence of conspecific seedlings. Other studies suggest that the presence of shrubs with much shallower root systems than *Q. ilex* can compete for water with this species during dryer than usual periods (Rolo & Moreno 2011).

Inter-specific responses during drought recovery

Right before the end of the drought period (November), the water status of *Q. ilex* might have been hampered by a prolonged depletion of groundwater, as suggested by the decrease in *Q. ilex* NDVI values. This agrees with a similar study in a nearby area (Barbeta et al. 2015) demonstrating that *Q. ilex* exhausted groundwater reserves during the extremely dry summer-autumn of 2011, being unable to meet a very high evaporative demand, which led to tree mortality. This event may explain why the isotopic signatures of xylem water of *Q. ilex* in November 18th resembled that of the two previous field samplings during the summer drought, despite the considerable amount of accumulated rainfall in the previous 30 days (161 mm). In this regard, the amount of water infiltrating to lower soil layers could have been limited by a fast uptake by pines (Klein et al. 2014b).

The observed intra- and inter-specific competition (see e.g. Figure 3e) suggests that both species are competing for similar water reservoirs, and that the recovery after drought (November 2011) is limited in those individuals most affected by competition and, hence, potentially weakened by the extreme drought event. At this moment, it is very likely that both species were using a mixture of both sources (soil water and groundwater) as the isotopic values were closer to soil and groundwater values than in October (Figure 2e). Such water uptake pattern, right after the first autumn rainfall events, has been documented for *P. halepensis* (Voltas et al. 2015), *Q. ilex* in a nearby location (Barbeta et al. 2015) and for other tree species like *P. ponderosa* (Eggemeyer et al. 2008).

Density vs. inter-tree interactions: what should we look for?

Interestingly, we observed contrasting patterns when comparing individual tree-to-tree interactions (i.e. through point-process statistics) with density effects on the isotopic values. Particularly, an intra-specific interaction for pines was observed through point-process analysis, but no significant effects of pine density were found; conversely, both inter- and intra-specific responses in oaks were best shown when considering density effects. This points to the need for combining different conceptual approaches to obtain a complete view of spatial interactions for water uptake, and suggests that additive (i.e.

density) and tree-to-tree effects of competition may differ, and thus should be specifically addressed. On the other hand, and contrasting with other studies (e.g. Rodríguez-Robles et al. 2015), we did not find evidence of facilitation through hydraulic uplift from the deep-rooted oak benefiting the shallow root system of the pines. Despite showing contrasting patterns, both intra- and inter-specific interactions pointed towards a negative effect of stand density. So far, our study does not support the prediction of some models stating that facilitation is fostered by increasing resource scarcity (see e.g. Brooker et al. 2008, and references therein), but exemplifies a limiting environment in which none of the dominant species benefits from each other.

Conclusions

Stable isotopes in xylem water indicate that *P. halepensis* and *Q. ilex* have a different rooting distributional pattern and also different functional characteristics such as water uptake capacity or stomatal response, thus allowing coexistence; however, significant interactions for water uptake show that such coexistence may become difficult under future climate. Both species appear to use a similar water source during periods of high soil moisture, but they differ during soil water scarcity. Inter- and intra-specific interactions (particularly competition) are maximized during periods of moderate drought. Theoretically, niche partitioning in mixed forest may bring benefits for both species by favorably influencing inter-specific interactions and by increasing resource-use efficiency (Loreau & Hector 2001). However, this equilibrium may be disrupted under long and intense periods of drought. In this study, the inability of pines to compete for belowground resources may threaten severely this species if drought increases in severity and duration, leading to longer dormancy periods and potentially causing carbon starvation and mortality. In fact, seven pines (8%) died in the stand during the two years following this drought event. Similarly, a high uptake of infiltrated water by *P. halepensis* would increase the sensitivity of *Q. ilex* to drought events, as groundwater levels could become more easily exhausted in the future. Altogether, our results indicate that drought decreases the room for complementarity between species in this mixed Mediterranean forest.

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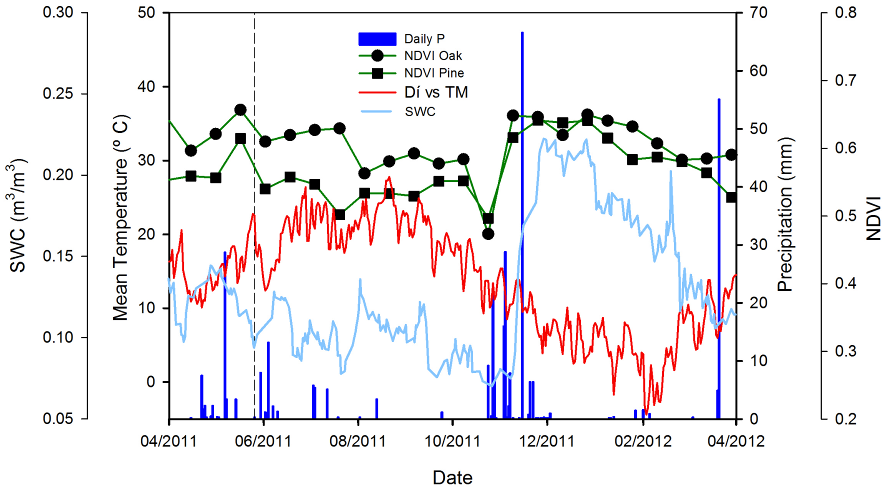


Figure 1. Physiological and environmental variables of the study site including daily temperature (T) and precipitation (P) obtained from the closest (4 km) weather station (Ulldemolins). Soil water content (SWC) from remotely sense moisture (SMOS-BEC Ocean Land Products of the pixel (1 km^2) belonging to the study area . Vegetation index (NDVI) (from MODIS satellite sensor) of the pixel (0.25 km^2) containing the study area and of pixels from other nearby locations in which either of the species were in a non-mixed forest stand.

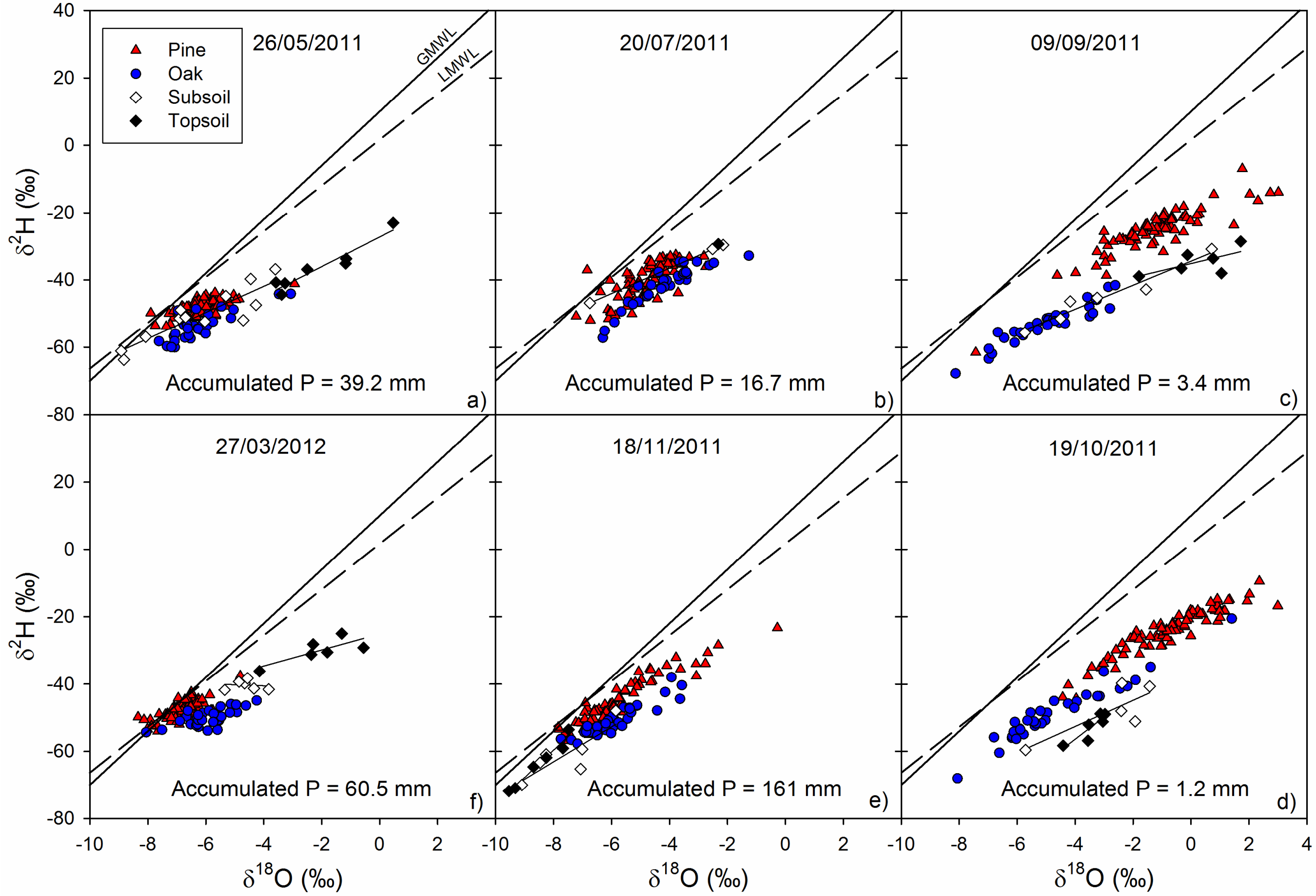


Figure 2. Isotopic signatures of water extracted from twig xylem of pines and oaks, and from the top and subsoil of the studied forest stand.

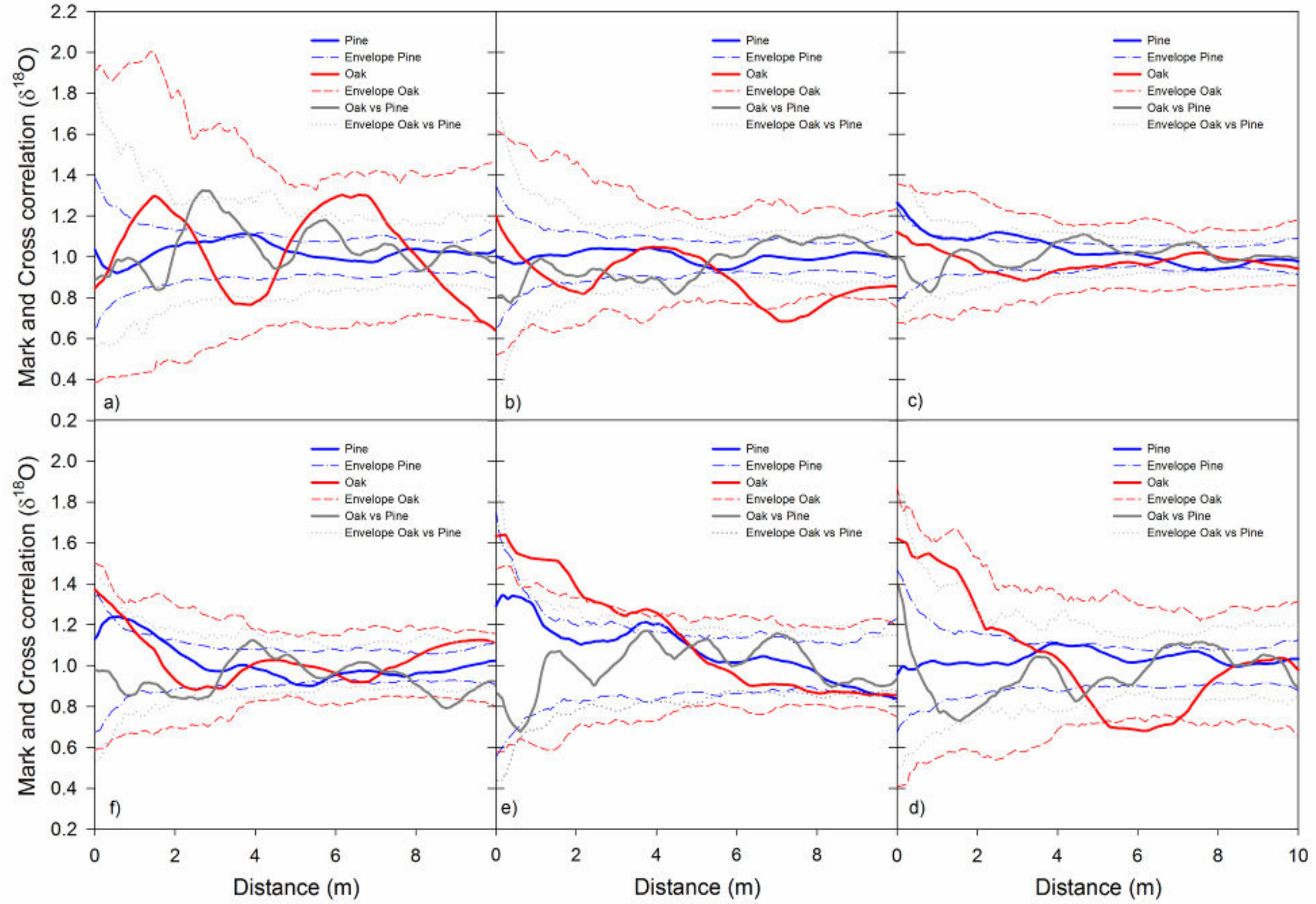


Figure 3. Seasonal (1 to 6 sampling moments) mark and cross-mark correlation functions for δ^{18}O of the twig xylem water from *P. halepensis* and *Q. ilex*. Solid lines indicate actual values of the mark and cross correlation functions and dashed lines indicate the envelopes of random simulations of 100 iterations.

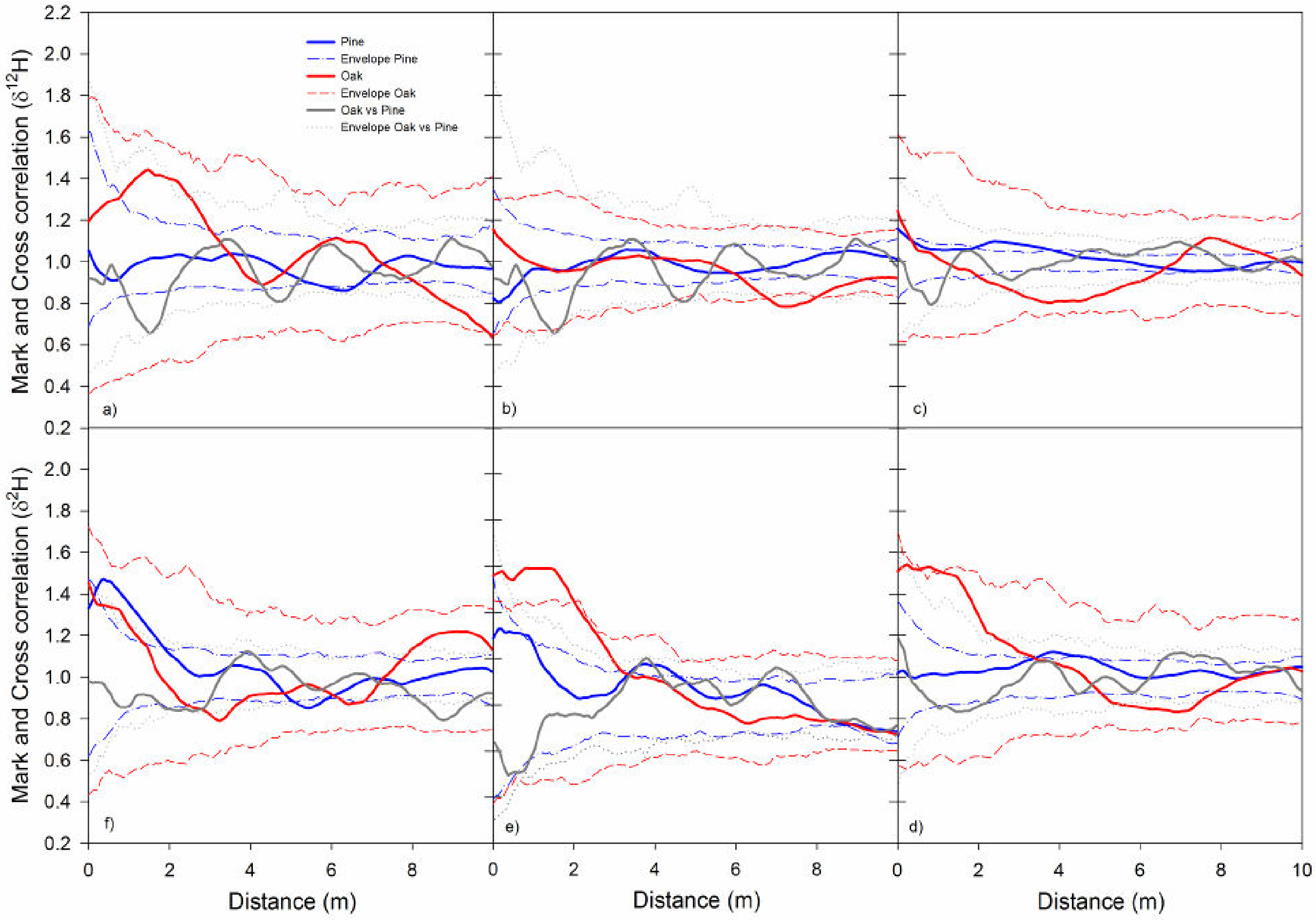


Figure 4. Seasonal (1 to 6 sampling times) mark and cross-mark correlation functions for δ^2H of the twig xylem water from *P. halepensis* and *Q. ilex*. Solid lines indicate actual values of the mark and cross correlation functions and dashed lines indicate the envelopes of random simulations of 100 iterations.

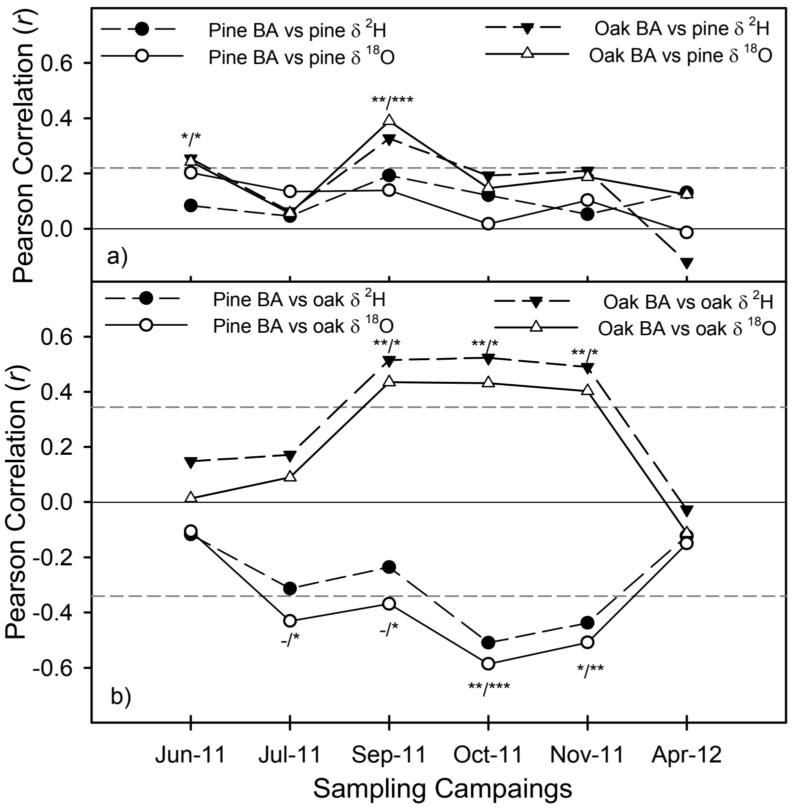


Figure 5. Seasonal changes in pair-wise correlation coefficients between isotopic water values for each individual of either pines (a) or oaks (b) and the density in a 5 m radius. Dashed grey box line indicates the 0.05 significant level (n= 87 for pines, n = 33 for oaks).

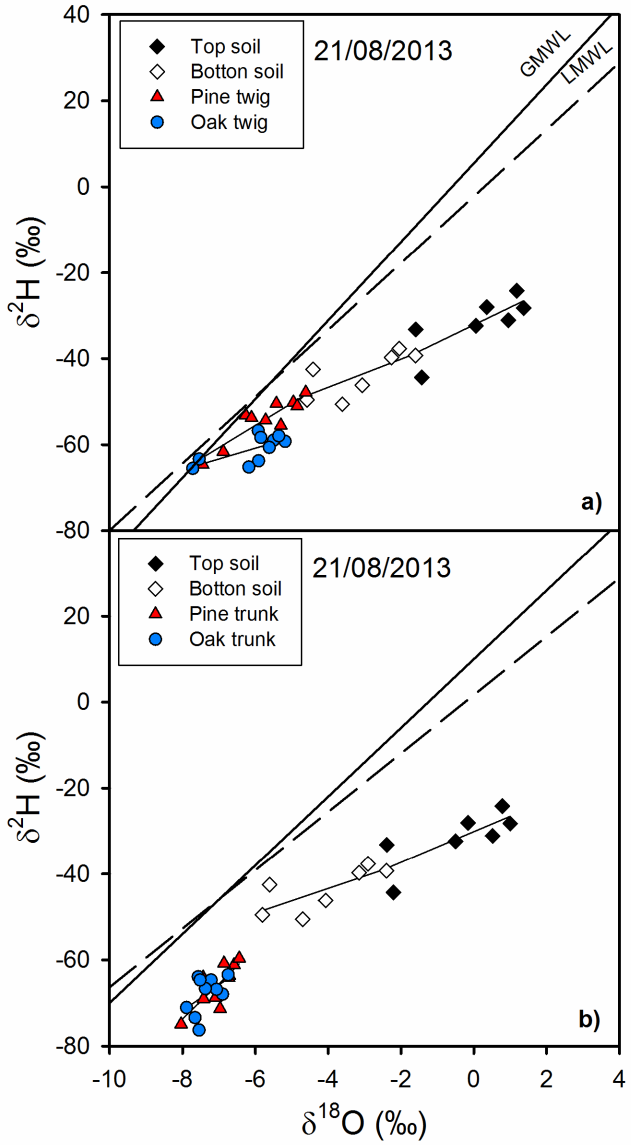


Figure 6. Isotopic signatures of water from the top and subsoil, as well as from the twig xylem (a) and the trunk (b) of 10 representative trees of each species from the forest stand under study.

General Discussion

Simple can be harder than complex: You have to work hard to get your thinking clean to make it simple. But it's worth it in the end because once you get there, you can move mountains. **Steve Jobs**

Complementary water uptake patterns for stand water balance

Earlier studies have proposed that *Quercus ilex* and *Pinus halepensis* may display different physiological responses to seasonal changes in precipitation (Ferrio et al. 2003; Aguilera *et al.*, 2012), most likely as a result of different water utilization strategies; e.g, a differential stomatal sensitivity (Baquedano & Castillo 2007) and a contrasting root distribution (Rodríguez-Robles et al. 2015). Similarly, in Chapter 1 we have shown that the carbon isotope discrimination ($\Delta^{13}C$, a proxy of intrinsic water use efficiency-WUE_i- in dry climates) in the tree-rings of both species show distinct geographic patterns, thus providing complementary paleo-climate information on the annual precipitation of the Northeastern part of the Iberian Peninsula (see Chapter 1). We also found a lower range of variation and absolute values in the $\Delta^{13}C$ of *P. halepensis*, as compared to *Q. ilex.* Although such differences in $\Delta^{13}C$ in response to drought can be attributed to different metabolic pathways in wood formation (Gessler et al. 2014), such differences are likely related to variations in stomatal conductance - references in Ferrio, (2005) - or concomitant changes in leaf area index or leaf chlorophyll content (Chapter 2), WUE_i and total amount of transpired water (Klein et al. 2013). Stomatal conductance responds to VPD and water availability (Mediavilla & Escudero 2004), and in turn relates to leaf water potential in the two species (Baquedano & Castillo 2007; Mediavilla & Escudero 2004), both probably being controlled by xylem anatomy (Miller et al. 2001); with lower average leaf water potential in ring-porous (deciduous oaks) and diffuse-porous (evergreen oaks) than in coniferous (pines) (Klein 2014). This illustrates two contrasting hydraulic behaviors (isohydric and anisohydric) and may imply an ecological advantage for the pine over the oaks in terms of dealing with drought (Ferrio 2005) (Zavala et al. 2000; Klein et al. 2013), as having a more sensitive stomatal conductance can be beneficial to avoid periods of drought and rapidly maximize photosynthesis during periods of water abundance (Baquedano & Castillo 2007). However, oaks generally show less sensitive stomatal regulation than pines, but can work at lower water potentials, being able to exploit particular portions of water within the soil: hence, *Quercus ilex* may have higher effective soil water availability than *Pinus halepensis* at low soil water potentials (Ψs). For instance, while *Pinus halepensis* is unable to extract soil water at $\Psi s < -2.0$ MPa

(Klein et al. 2013), *Quercus ilex* may extract water even at Ψs < -2.5 MPa (Baquedano & Castillo 2007) or -3 MPa (David et al. 2007). In this way, *Quercus ilex* maintains high gas exchange during dry periods in which the pine stops its physiological activity (Baquedano & Castillo 2007), therefore having less limiting photosynthesis through stomatal closure as the pine (see discussion below on niche segregation). This may give oaks a major advantage in the forest interspecies competition, either directly (higher growth) or indirectly (seizure of water resources) (Klein 2014). Furthermore, the dual root system of *Quercus ilex* allows it to exploit water from 2 hydrological niches, the soil and ground/fractures, switching along the seasons as needed (Barbeta et al. 2015). Thus differences in Δ^{13}C in response to drought can be also attributed to this: In fact, further research we conducted shows that oaks presented values of Δ^{13}C associated with significantly lower values of xylem Δ^{18}O than pines during summer i.e. with isotopic values of water at higher depths (data not shown).

In line with this hypothesis, in Chapters 3 and 4, the isotopic composition of xylem water and other ecophysiological indicators revealed that both species show different water use and extraction patterns, which allows them to maximize the available water resources in different hydrological niches (in time and space) (Ehrlinger & Dawson 1992), and to coexist in a relative equilibrium. The equilibrium is relative because small changes in water availability due to variations in climate, edaphic or forest composition may have implications in the long-term coexistence for *Q. ilex* and *P. halepensis* (Zavala & Zea 2004; Barbeta et al. 2013) . The results in chapter 4 agree with the stated above, despite that the continued transpiration of *Quercus ilex* has to do partly with access to groundwater, unreachable to the pine, as suggested by the lowered midday water potential during moderate drought of the pine (Chapter 4). This is also confirmed by the results of chapter one, which provided information on how the WUEi in *Q. ilex* at the spatial level is linked to variations in seasonal precipitation responsible for groundwater recharge (Ferrio et al. 2003); and in chapter 3 and 4, which both showed that the xylem water comes from deeper soil layers in the case of *Q. ilex* than in the case of *P. halepensis*.

In terms of water balance at the stand level, the above ecophysiological differences may translate into different amounts of transpiration, thus in different impacts on the water yield (Klein et al. 2013; Aranda et al. 2012). For instance, Klein et al. (2013)

revealed that in a typical stand density of 300 trees/ha and annual precipitation of 500 mm, the water use of *Pinus halepensis* and *Quercus calliprinus* stands were 357 and 459 mm, leaving 143 and 41mm as water yield, respectively. They also provided an estimate of the maximum carrying capacity for the different stands: 350 and 550 trees/ha for oak and pine, respectively, at the 500 mm iso-precipitation region. However, other study suggest that *P. halepensis* transpire more than *Q. ilex* when water is available, but drastically reduces transpiration during summer drought in which *Q. ilex* can considerably increase transpiration (Baquedano & Castillo 2007). Thus the total annual water budget of each species may end up being similar (Baldocchi et al. 2010); further research is required in this area.

The above studies also suggest that mixed pine-oak forest may have a close to zero or negative water yield some year, perhaps living upon groundwater reserves from other years. Furthermore, the ability to exploit different water sources can maximize water extraction, not only through competition, by forcing species to specialized towards a particular niche, but also through facilitative processes such as water uplift by the oaks that pines may use (Dawson 1996; Rodríguez-Robles et al. 2015; Maeght et al 2013). To understand better the impact of forest dynamics on local and regional water balances, we need to understand the physiological, ecological and environmental factors that influence water loss from trees and forested lands (Dawson 1996). Extreme drought and moderate but persistently drier conditions brought by climate change could make the species in our study region to deplete water reservoirs from groundwater and weathered bedrock (chapter 4), thus increasing mortality and high defoliation to the point the system rebounds towards groundwater recharge (Barbeta et al. 2015). Such scenario could have serious negative consequences for the sustainability of mixed oak and pine forest if drought intensifies, with serious risk of die-back and mortality (Aranda et al. 2012). However, the ecological implications of changes in the stand water balance need to be evaluated under the different and sometimes contradictory theories of coexistence that explain the stand dynamics and composition of forest ecosystem.

Long-term co-existence of pines and oaks: stability or transitional succesion?

So will long-term coexistence of the species be hampered?

This is a difficult question because there are many ecological theories that explain the coexistence of species. At the large spatial scale, distribution of vegetation types has long been associated to climate (Braun-Blanquet 1928). More recently, for a wide range of environments, from drylands to wetlands, it has been suggested that water tolerance (from deficit to excess) is responsible for the segregated distribution of species along gradients (Silvertown et al. 2015). At the local scale, however, community distribution and configuration are the result of overlapping populations interacting with themselves and each other (competition and facilitation), and some deterministic (such as climatic), and random (such as seed dispersal or disturbance regime) processes (Silvertown et al. 2015; Hubbell 2001; Zavala et al. 2000; Lookingbill & Zavala 2000).

Wilson (2011) reviews 12 theories of coexistence, dividing them into equilibrium and stabilizing mechanism, as already described in Chesson (2000). Equilibrium refers to fitness of the species, which can only approximate a similar fitness, but it is never equal, thus only delaying competitive exclusion. However, stabilizing refers to mechanisms that, as it names indicate, will allow indefinite coexistence of the species through an increase-when-rear mechanism (Chesson 2000). Another important theory is the neutral theory of Hubbell (2001), which offers the view that the spatial configuration of the forests respond to purely stochastic processes, in which regional and local scale are connected through dispersal processes, without intervention of local species interaction processes. Wilson (2011) suggested that some of the coexisting theories/mechanisms like: cyclic succession, equal chance (neutrality), initial patch composition, etc. are likely to be irrelevant, or perhaps not even exist. Although, it is beyond the scope of this thesis to do a truly comprehensive review of all of them here, we can look at some of the ecological mechanism reviewed by Willson (2011) and others that operate in the dynamics of pine-oak mixed forests:

Cyclic succession

The mechanisms of ecological succession were first documented by Thoreau (1860) on *The succession of forest trees,* while studying a pine-oak mix forest. Since then, many different types of succession have been described: For instance, cyclic succession has been proposed as a possible mechanism behind the pine-oak forest dynamics. This is despite that recent evidences from large scale recruitment study (Carnicer et al. 2013) agree with mix traditional vegetation models (phytosociological models) (Rivas-Martínez 1987), in that pine-dominated communities are transient states of a secondary succession evolving towards an oak-dominated climax community. The evidences suggest that *Q. ilex* acts as a key driver of large-scale shifts in recruitment in the Iberian Peninsula, negatively affecting most pine species with the advance of forest succession (Carnicer et al. 2013). This is in agreement with our results in chapter 4, in which the high water competition among the dense pine population (Moreno-Gutiérrez et al. 2014) may not support new competitors like the *Q. ilex*, able to switch its water extraction at different depths (Barbeta et al. 2015). The exclusion rates of *P. halepensis* by *Q. ilex* is a function of water and light availability at the local site (Carnicer et al. 2013; Zavala & Zea 2004). As a result, pines and oaks would conform to a regional compositional gradient associated with the precipitation regime, time since last disturbance and history of the forest. However, the proportion of *P. halepensis* increases toward the driest border while the oak becomes the dominant species in areas with higher precipitation (Carnicer et al. 2013; Zavala et al. 2000; Urbieta et al. 2011). For instance, 440 mm of annual rainfall are required for these *Q. ilex* forests to persist (Terradas & Savé 1992), whereas *P. halepensis* can tolerate drier conditions (289 mm) (Klein et al. 2012; Klein et al. 2014). This totally agree with differential responses of each species in terms of leaf-level physiology, i.e water use efficiency, as well as their distributional patterns that were reported in chapter 1. Furthermore, only major anthropogenic or natural disturbances like forest fires, land use change, e.g such as land abandonment as in the case of our sampling site (see Chapter 4), will reset things towards the primary successional stage in which seeders like *P. halepensis* will rapidly colonize (Lookingbill & Zavala 2000).

However, the above might be an overly simplistic view of successional dynamic (Mendez et al. 2008), as forests are dynamic systems reaching only relative equilibriums. For instance, the long-term persistence in a climax stage of mono-specific *Q. ilex* mature forest in the absence of disturbances has been questioned (Zavala et al. 2000). This is because small changes (either allogenic or autogenic mechanisms) in light and water availability may lead *Q. ilex* population to oscillate with another species population, usually a gymnosperm (Ducrey, 1992; Zavala et al. 2000). Our results in chapter 4 suggest that during prolong drought, depletion of the alfa-niche of oaks (groundwater) may end up being a problem for *Q. ilex*, as it relies frequently on groundwater sources. For instance up to 70% of the transpired water during summer drought can come from groundwater sources (David et al. 2007), and high mortality rates were documented in a nearby location to our study site due to groundwater depletion (Barbeta et al. 2015). Furthermore, the presence of the pine, an opportunistic competitor which rapidly uses available water (chapter 4) (Moreno-Gutiérrez et al. 2014), may further impact groundwater recharge, thus negatively affecting (Barbeta et al. 2013) and even outcompeting oak population.

If such droughts are to become more frequent due to climate change, oak recruitment, which is normally favored by the understory shade of pines (Urbieta et al. 2011; Carnicer et al. 2013), can result particularly difficult due to high competition for water resources with the pines (Zavala et al. 2000). Stand composition may then be reverted towards a pine-dominated equilibrium with autosuccessional dynamics, typical of arid, disturbed regions, to which the highly drought tolerant *P. halepensis* is better adapted (Zavala et al. 2000; Zavala & Zea 2004; Urbieta et al. 2011). Although the dendrochronology of our forest stand agrees with the traditional successional-climax model, where *Q. ilex* is displacing the decaying pine population suffering from strong intra-specific and inter-specific competition for the water resources, slow changes in climate or forest dynamics and composition could reverse this situation (for instance, chapter 4 showed dead pine trees, $n=7$, after extreme drought, were significantly ($p=0.05$) smaller than average, and had more neighbors of pines ($p=0.05$) or pines+quercus ($p=0.10$) than average, as well as significantly different extraction water pattern than the rest). Therefore, the dominance of either species in a transition zone, with suitable habitats for both species (like is the case in our study area), may fluctuate with long-term climatic variability affecting ecological interactions, such as competition for water and light as a function of canopy closure (figure 1). For instance, the aforementioned large-scale study at the Iberian Peninsula level on recruitment, described a scenario of global change dominated by the widespread expansion of *Q. ilex*, with increased suppression of *P. halepensis* recruitment (and growth) by *Q. ilex* only at sites no warmer than (MAT > 15 °C), with low stand basal areas and low rainfall (< 600 mm) (Carnicer et al. 2013). Therefore, the above advocates that cyclic succession, and even classical stable climax vegetation might not be as important as other stabilizing mechanisms such as environmental fluctuations (Wilson 2011), with important mechanism such as niche segregation and the storage effect being at play here (see further disscusion below).

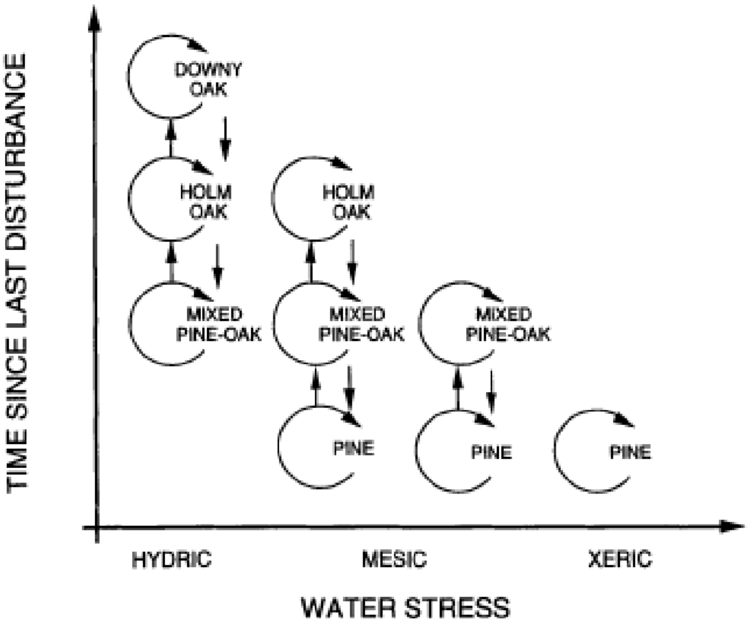


Figure 1. Idealized model of transition and dynamics observed in mixed holm oak-Aleppo pine forests. The X axis illustrates water balance measured as a drought index. The Y axis represents average time since last disturbance (that is, decreasing levels of light reaching the ground). Circular arrows indicate the possibility of autosuccession (from Zavala et al 2000).

Neutral theory

The neutral theory (Hubbell 2001), developed from the dispersal-assembly theory, states that the presence or absent of species at intermediate scales is dictated by random dispersal and stochastic local extinction and speciation (Hubbell 2001). Our results suggest that our species were randomly distributed (Chapter 3), possibly due to the random dispersal and colonization by pines (Lookingbill & Zavala 2000); however, mortality (chapter 4, high competition) and recruitment of the oaks (shade-tolerant species) under the understory of the pines (Lookingbill & Zavala 2000) followed deterministic processes defining their spatial distribution. This is very likely to shape the future spatial configuration of the forest, and the entire landscape, by responding to species-specific requirements. Such deterministic processes thus question the neutral theory as a coexistence mechanism. The neutral theory is an equalizing mechanism that argues that coexisting species have an equal chance of survival due to equal fitness. However, and despite that measuring fitness is a complex task (Chesson 2000), our results suggest that differences in plant fitness, for instance in physiological and decay rates (dead pines), are responsible for the spatial configuration of the forest through an screening process of natural selection (see below). Furthermore, our results may disagree with the neutral theory in that niche partitioning (a stabilizing mechanism) is unnecessary to plant coexistence (Hubbell 2001), as we found strong evidences of it (Chapter 3 and 4). However, it is also possible having both mechanisms operating at different scales: for instance niche-based mechanisms operating at the local scale (Lookingbill & Zavala 2000) may explain the segregation of species along a successional gradient, while species-specific habitat requirements could explain segregation along altitudinal and rainfall gradient (Galindo-Jaimes 2012; Hubbell 2001), with a balance between neutrality and niche structuring mechanisms (Purves and Pacala 2005).

Niche segregation

For Wilson (2011), niche segregation, together with other stabilizing mechanism such as allogenic disturbance, environmental fluctuation (relative non-linearity and/or the storage effect) and pest pressure are the most important in plant coexistance. Our results suggest that the fate of both species in terms of presence, absence, abundance and spatial distribution may respond to species different functional roles and niche partitioning, the classical Niche-Assambley theory. In our case, either oak and pines could be observed as an equilibrium of forces, exploiting separate hydrological niches within the soil-ground profile or along the seasons through stomatal regulation (see below) (Filella & Peñuelas 2003), despite that they also compete for water resources during some parts of the year, as seen in chapter 4. CLEMENTS et al. (1929: 316–317) wrote: "When the immediate supply of a single necessary factor falls below the combined demands of the plants, competition begins". Our results also suggest a typical phenomenon of the increase-when-rare process, which refers to when a species is rare, the resource that it particularly exploits will be present in greater abundance (Wilson 2011). This agrees with the rare *Q. ilex* becoming established in the dominant pine population of our study site by exploiting groundwater, and perhaps by not encountering strong competition from pines due to pines lower functioning during drought (chapter 4). Our results also agree with 43 studies on hydrological niches reviewed by Silvertown et al. (2015), which also found hydrological niche segregation (HNS) in coexisting species. These studies suggest that soil moisture gradients (chapters 3 and 4) from highly local to large geographic scales appear to control plant community composition, as in chapter 1 and 2. However, the precise mechanisms and consequences of HNS are still not totally clear (Silvertown et al. 2015).

As aforementioned, Chesson (2000) described an interesting ecological theory of equilibrium and balancing mechanisms as drivers of coexistence. It is interesting the refinement of this model by MacDougall et al. (2009) (Figure 2), which illustrates how successful invader establishment depends on either a fitness advantage and niche differences from resident species, but only fitness advantage permits invaders to become dominant. In this regard, different plant functional traits are key to understanding niche segregation and species response to changing environment at the inter-specific but also at the intra-specific level (Laforest-Lapointe et al. 2014).

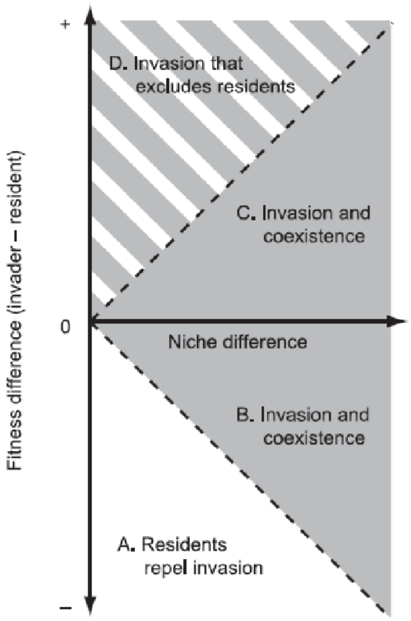
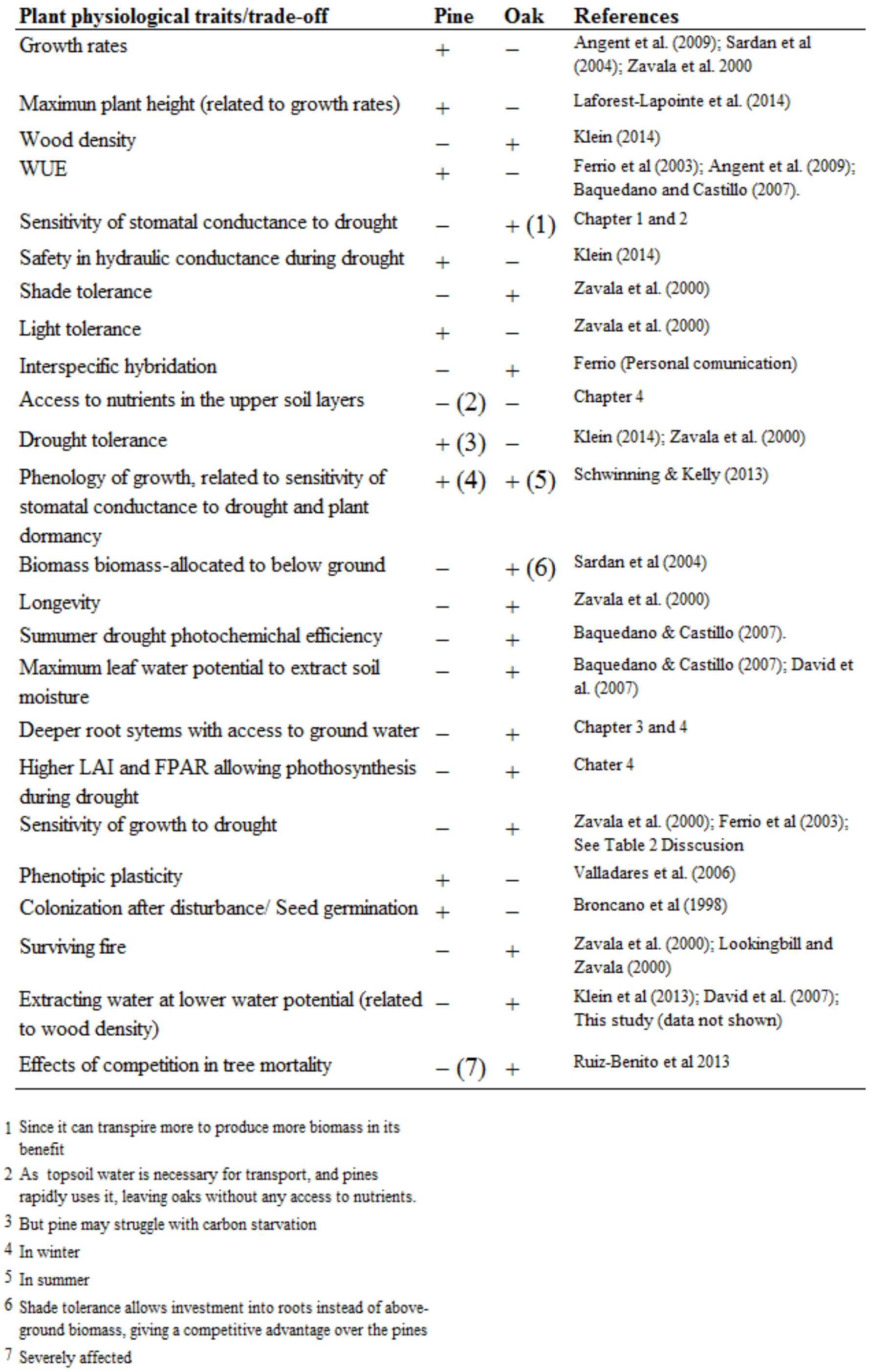


Figure 2. The influence of niche and fitness differences on the outcome of biological invasions following the theory, from MacDougall et al. (2009)

If we consider our study site, with *Q. ilex* as an invader, according to Darwing, the phylogenetic distance with *P. halepensis* (Ferrio 2005) offers the invader an advantage by minimizing overlap in resource use with the resident species, a niche difference (Darwin 1859). However, it is the difference in fitness that will determine dominance (Figure 2). However, advantages of *Q. ilex* over the *P. halepensis* are usually offset by compensating disadvantages, also known as trade-off (Chesson 2000), thus allowing coexistence. Table 1 attempts to summarize the potential effect of these trade-offs and the predicted response of each species in mixed stands. In our case, oaks, with their ability to exploit a deeper water hydrological niche, became established within the forest floor, avoiding part of the fierce competition for water with pines once their roots were deep enough; however, according to the model of Figure 2, it is their better fitness that can allow oaks to exclude pines.

Table I. Trade-off mechanisms and predicted specific response in mixed Aleppo pine-Holm oak forest, based on the literature and the results from this Thesis.



Plant physiological traits/trade-off	Pine	Oak	References
Growth rates	+	–	Angent et al. (2009); Sardan et al (2004); Zavala et al. 2000
Maximun plant height (related to growth rates)	+	–	Laforest-Lapointe et al. (2014)
Wood density	–	+	Klein (2014)
WUE	+	–	Ferrio et al (2003); Angent et al. (2009); Baquedano and Castillo (2007).
Sensitivity of stomatal conductance to drought	–	+ (1)	Chapter 1 and 2
Safety in hydraulic conductance during drought	+	–	Klein (2014)
Shade tolerance	–	+	Zavala et al. (2000)
Light tolerance	+	–	Zavala et al. (2000)
Interspecific hybridation	–	+	Ferrio (Personal comunication)
Access to nutrients in the upper soil layers	– (2)	–	Chapter 4
Drought tolerance	+ (3)	–	Klein (2014); Zavala et al. (2000)
Phenology of growth, related to sensitivity of stomatal conductance to drought and plant dormancy	+ (4)	+ (5)	Schwinning & Kelly (2013)
Biomass biomass-allocated to below ground	–	+ (6)	Sardan et al (2004)
Longevity	–	+	Zavala et al. (2000)
Summer drought photochemichal efficiency	–	+	Baquedano & Castillo (2007).
Maximum leaf water potential to extract soil moisture	–	+	Baquedano & Castillo (2007); David et al. (2007)
Deeper root sytems with access to ground water	–	+	Chapter 3 and 4
Higher LAI and FPAR allowing photosynthesis during drought	–	+	Chater 4
Sensitivity of growth to drought	–	+	Zavala et al. (2000); Ferrio et al (2003); See Table 2 Disscusion
Phenotipic plasticity	+	–	Valladares et al. (2006)
Colonization after disturbance/ Seed germination	+	–	Broncano et al (1998)
Surviving fire	–	+	Zavala et al. (2000); Lookingbill and Zavala (2000)
Extracting water at lower water potential (related to wood density)	–	+	Klein et al (2013); David et al. (2007); This study (data not shown)
Effects of competition in tree mortality	– (7)	+	Ruiz-Benito et al 2013

1 Since it can transpire more to produce more biomass in its benefit

2 As topsoil water is necessary for transport, and pines rapidly uses it, leaving oaks without any access to nutrients.

3 But pine may struggle with carbon starvation

4 In winter

5 In summer

6 Shade tolerance allows investment into roots instead of above-ground biomass, giving a competitive advantage over the pines

7 Severely affected

In addition to the above, the differential growth response between pines and oaks to drought reflect the larger plasticity of the former in our sampling site (Table 2). It is important to note that leaf-level mechanisms such as stomatal regulation to ameliorate drought have been included as a form of hydrological niche segregation in dry ecosystems (Silvertown et al. 2015). This agree with results in Chapter 4, where seasonal variation in stomatal sensitivity to drought show that niche segregation in water uptake was not only due to different root disposition, but to leaf functional changes over time. Given the relation between leaf water potentials and stomatal conductance (Klein 2014), this aspect is probably linked to the ability to extract water from the soil with different water potential (Baquedano & Castillo 2007), thus being able to access a portion of water from the soil unreachable to competitors. Therefore, through the study of leaf gas-exchange using $\Delta^{13}C$, we might be able to understand not only ecosystem-scale primary productivity as we have done until now (Diefendorf et al., 2010), but also the temporal constraint of each species linked to their ecological niches. In this sense, the $\Delta^{13}C$ isoscapes from chapter one can be useful to test whether long-term coexistence between species is more or less likely in a given scenario.

In conclusion, although the MacDougall et al. (2009) model may represent coexistence processes in a more realistic way, all the above trade-offs mechanisms can vary spatially and temporally further complicating the picture (Hubbell 2001; Chave 2013; Barbeta et al. 2013). Thus, although it is clear that changes in the dominance of Q. ilex–P. halepensis respond to water and light availability at the local scale (Zavala 2000; Zavala & Bravo de la Parra 2005) and water competition (chapter 3, 4), larger scale studies are required for a proper understanding of forest coexistance. In this context, Wilson (2011) advises of 2 important issues which are central to understanding coexistence: 1) the appropriate spatial scale in community ecology to study coexistence might be impossible to define; 2) since low variability can also be due to low perturbation, how low does the variability to be called stability?

Table 2. Correlations of $\mathrm{TRW}_{\mathrm{index},}$ and precipitation time series.

		SPEI3	**SPEI6**	**P (Lleida)**
Q. ilex	**$\mathrm{TRW}_{\mathrm{index}}$ (1969-2013)**	0.15	0.13	0.01
P. halepensis	**$\mathrm{TRW}_{\mathrm{index}}$ (1969-2013)**	0.52*	0.45*	0.39*

TRW index = Yearly chronologies built for each species using 74 pines and 33 oaks.

SPEI = Standardized Precipitation Evapotranspiration Index (SPEI), space-time scale = 0.5 degrees grid/monthly, SPEI3 = 3 months of drought, SPEI6 = 6 months of drought , the monthly series were integrated into yearly series using the 12 months mean in order to correlate them to TWR index series. "The SPEI is based on precipitation and temperature data, and has the advantage of combining a multi-scalar character with the capacity to include the effects of temperature variability on drought assessment. The procedure to calculate the index is detailed, and involves a climatic water balance, the accumulation of deficit/surplus at different time scales, and adjustment to a Log-logistic probability distribution" (Vicente-Serrano et al 2010).

P = Precipitation (Lleida, 1959-2012) data from the Spanish Meteorological Agency (AEMET).

Spatial configuration and self-organization

Nevertheless, and as mentioned in the introduction, it seems that inter-tree competition, the density and identity of neighbors within a stand must be considered central aspects of tree mortality (Olano et al. 2009; Ruiz-Benito et al. 2013) (Chapter 4), tree regeneration (Comas 2009), growth (Comas et al. 2013; Moreno-Gutiérrez et al. 2014) (Chapter 4), all connected to coexistence of the species and their spatial configuration and dynamics (Fowler 1986). These forests are highly dynamic systems that need to be evaluated at different spatiotemporal scales. The WUEi and plant physiology at large spatial scales as modelled in Chapters 1 and 2 are necessary to understand constrains determining species spatial distribution and the likeliness of forming semi-stable mixed forest (Moreno-Gutiérrez et al. 2014). However, biotic interactions should be considered to understand the complexity of forest ecosystems. It seems that both, random and deterministic factors operating in time, drive succession from a random distribution at primary stages (for instance, because of the arrival through random dispersal of first colonizers after disturbance) to a community complexity and organization (Brulisauer et al., 1996). Here, structures build upon previous structures, unless deleted by disturbance. Thus, the dynamic succession shows a self-organization of the system through a chaotic transient behavior, which can be mathematically characterized by a finite set of parameter, known as the fractal dimension. Changes in the fractal dimension reveal changes in vegetation structure through successional phases (Alados et al. 2003). According to Alados et al 2003, self-organization is in part driven by negative feedbacks from biosynthetic end-products at the individual level, density limitations at the population level, and predation and competition at the community level (Alados et al. 2003). Such processes are operating at different orders of magnitude; at larger scale we could imagine competition taking place not only at the individual level but among different vegetation patches within a landscape mosaics, which are important ecological units to understand ecosystem structures and functioning across spatial scales (Mendez et al. 2008). Point process could be useful to evaluate this. Furthermore, vegetation may incorporate fractality through climate, which time series usually include fractal dimension (a power law relationship), thus defining succession and spatial patterns. A good way to test fractality in successional stages could be through stable isotopes.

In this context, successional changes in community composition through a gradual species replacement mirrors changes in the $\Delta^{13}C$ and $\Delta^{18}O$ due to ontogeny, increasing isotopic values from early- to late- successional species (Resco et al. 2011). In this sense, low $\Delta^{13}C$ in early succession could indicate the need for higher growth to enhance establishment, whereas high $\Delta^{13}C$ later in succession could indicate that these species with higher competition levels applied a more conservative resource (i.e. water) use strategy (Resco et al. 2011). Thus, further exploring $\Delta^{13}C$ from tree-rings with different spatial analysis such as the fractal method could provide interesting results to study species coexistence and spatial distribution. In addition, analysis using $\Delta^{13}C$ as marks in point process could clarify this topic as well as feedback mechanism related to forest canopy closure and root overlapping during succession, which increases competition for resources (Resco et al. 2011).

The $\Delta^{13}C$ considers gas exchange aspects (WUEi) to properly assess the potential competitive ability of a plant in Mediterranean environments (Filella & Peñuelas 2003). However, variations in plant $\Delta^{13}C$ are caused by both changes in light/nutrient and water availability (Cernusak et al. 2009). Improved light and increased nutrient availability, can increase assimilation rates relative to stomatal conductance and thus, decrease $\Delta^{13}C$ (Ehleringer et al. 1986; Cernusak et al. 2009). In fact, changes $\Delta^{13}C$ within a stand were linked to changes in light and not to water availability (Sleen et al.

2013). In this context, dendrochronological and $\Delta^{13}C$ data was recently employed to show how *P. halepensis* highly compete for water, light and nutrients with understory species. For example, the closer shrubs of *R. lyciodies* were to *P, halepensis*, the higher were the values of $\Delta^{13}C$ in the tree-rings, suggesting stomatal closure limiting photosynthesis and growth (Moreno-Gutiérrez et al. 2014). Surprisingly, despite that we found potential evidences for competition using point processes on O^{18} and H^{2} in xylem water (chapter 3 and 4), using $\Delta^{13}C$ from tree rings as marks in point process shows no interaction between species, but at the intra specific level for Q. ilex at short distances (below 2m) (Data not shown).

Reconstructing spatial-temporal patterns in climate and WUE

The results in chapters 1 and 2 show different approaches for spatial and temporal integration across scales of stable isotopes information derived from tree-rings archives: In chapter 1, we reconstructed past spatial patterns of climate and WUE by combining isotope data with GIS technology. In addition, the second chapter illustrated the possibility of developing a high resolution WUE spatial-model at the regional scale by integrating the $\Delta^{13}C$ from tree-rings (a proxy for WUE) with vegetation index (NDVI) derived from satellite remote sensing. Such integrating models of isotopes and non-isotope tracers data provide fundamental tools not only to understanding plant water relations and the metabolism dynamics of entire landscapes and continents (Dawson 1996), but also understanding synoptic climates (Werner et al. 2012).

However, despite the promising results, chapter 2 also showed that the spatial patterns of $\Delta^{13}C$ can vary over time; On the one hand, this is due to large spatial scale climate variations; for instance, changes in the influence of different atmospheric circulation patterns that control *Pinus halepensis* growth, such as North Atlantic oscillation or Mediterranean Oscillation (Pasho et al. 2011), which would also affect $\Delta^{13}C$, as both ($\Delta^{13}C$ and Growth) showed a strong relationship (chapter 2). However, $\Delta^{13}C$ does necessarily covariate always with growth in dry environment, as drought conditions can lead to a lower $\Delta^{13}C$ (higher WUE) without altering growth rates in *Pinus halepensis* (see e.g. Linares et al. 2011). In addition, changes in the climatic forcing over time pose a very strong limitation to the methodology used in chapter 1, in which extrapolating present WUE-climate estimations to the past may not be totally feasible. Furthermore, if we use tree-rings corresponding to an old period of time, validation of this climate models with actual climate data will remain as an important shortcoming (mainly because we lack high density networks of historical climate data).

Besides large-scale changes in the climate forcing, several factors at the site level can affect spatial patterns of $\Delta^{13}C$ over time: ontogeny, site competition or other biological interactions (see the above discussion), responses to other abiotic factors such as temperature (affecting water demand) (Ferrio & Voltas 2005; Warren et al. 2001; Ferrio et al. 2003), changes in soil characteristics and groundwater level affecting water availability (Barbour et al. 2002), different phenotypic plasticity or adaptive processes (Voltas et al. 2008 and et al. 2015); Klein et al. 2012; De Luis et al. 2013), continentality, which affects the effective vegetative period (Shestakova et al. 2014) etc. (see Chapter 2 and references therein). Thus, the environmental and ecophysiological differences between sites generate a mismatch between plant responses (as measured in the $\Delta^{13}C$) that explain why the temporal and spatial variations in $\Delta^{13}C$ are different (Shestakova et al. 2014). In addition, carry over (memory) effects, as seen in chapter 2, e.g. a severe drought affecting the growth or physiological response of trees in subsequent years, also makes the temporal variation in $\Delta^{13}C$ different to the spatial variation. Still, the true effects of what is known as drought legacy (a period of slow growth lasting 3 to 4 years after the drought event) are unknown; this is despite that recent studies at the global scale point to hydraulic failure as a likely cause, with trees and in particular the Pinaceae genera from dry environments experiencing worse recoveries (Anderegg et al. 2015). The results from chapter 2 suggest that changes in leaf area index or chlorophyll content (part of the photosynthetic machinery, probably to adjust WUE to drier conditions may end up affecting growth on the long term (See also figure 3). However, whether such conditions are related to damage at the tree architectural level (e.g. canopy and leafs, roots, xylem etc) instead of pure physiological responses needs further research; not only at the interspecific but also at the intraspecific, i.e. genotypic and phenotypic level (Voltas et al 2008: 2015). This is of paramount importance since the consequences of the drought legacy in terms of carbon storage by forest might be large enough to be included in current global climate models employed for future predictions (Anderegg et al. 2015). Integrating vegetation indexes with tree-ring information will also allow us to see whether such a mismatch is related to a decoupling between secondary growth and leaf physiology with aboveground biomass or chlorophyll concentrations, providing clues about the actual processes controlling the drought legacy (see Figure 3 for slower recovery rates of vegetation indexes relative to radial growth).

On the other hand, as our results in chapter 1 and 2 suggest, and in agreement with global surveys annual precipitation largely controls $\Delta^{13}C$ in plant vegetation matter (including tree-rings) (Diefendorf et al. 2010; Kohn 2010; Korol et al. 1999; Warren et al. 2001; McCarroll & Loader 2004; Stewart et al. 1995; Miller et al. 2001). In addition, $\Delta^{13}C$ is more sensitive to precipitation in drier environments (the ones approaching P/E <1) (Warren et al. 2001; Chapter 2; Introduction). This is due to differential leaf-stomatal responses related to contrasting water strategies at the intra-specific and inter-specific level (Ferrio & Voltas 2005; Klein et al. 2013; Ferrio et al. 2003; Shestakova et al. 2014; Lázaro-Nogal et al. 2013). As mentioned in the introduction, despite that decreases in $\Delta^{13}C$ reflect either a decrease in stomatal conductance (g_s) or increase in assimilation rates (both reflecting an opposite effect on plant performance and lower and higher water availability), our results in chapter 1 and 2 suggest that, in our climatic gradient, $\Delta^{13}C$ consistently increases with precipitation.

Finally, in chapter 2 we also showed that WUE at the landscape level could be inferred through the relationship between $\Delta^{13}C$ and NDVI across sites. Such relationship was better described by a log model, suggesting that the coordination of vegetation greenness and water-use efficiency follows a trend comparable to the response of $\Delta^{13}C$ to precipitation (Fig. 5, chapter 2). Although we found a poor agreement between $\Delta^{13}C$ and NDVI at the temporal scale in the driest sites and suggested it could be the result of a greater relevance of the chlorophyll content signal controlling NDVI in water-limited environments (Chapter 2), other problem related to contaminated signal from other additional species contained within the pixels may have obscured the relationships. In fact, using the trees from the sampling site of chapters 3 and 4 we built chronologies and found a very strong agreement between TRW and annual NDVI derived from MODIS, which has a higher spatial resolution than the AVHRR used in chapter 2 (8 km vs 250 m) (Figure 3). In addition, although for a different time period being compared, we did not find a strong correlation using annual NDVI from AVHRR (Period 1982-2006, $r_{pearson} = 0.29$, $n = 26$). This suggests that $\otimes^{13}C$ may also show stronger correlations with NDVI in time using higher spatial resolution.

In this context, the future holds improved technology which will possibly allow long-term monitoring of individual trees based on high-resolution satellite data. This will eliminate much of the noise related to coarse-resolutions NDVI and would provide accurate physiological tree-responses to supplement tree-ring records for the study of spatial trends in WUE in recent past.

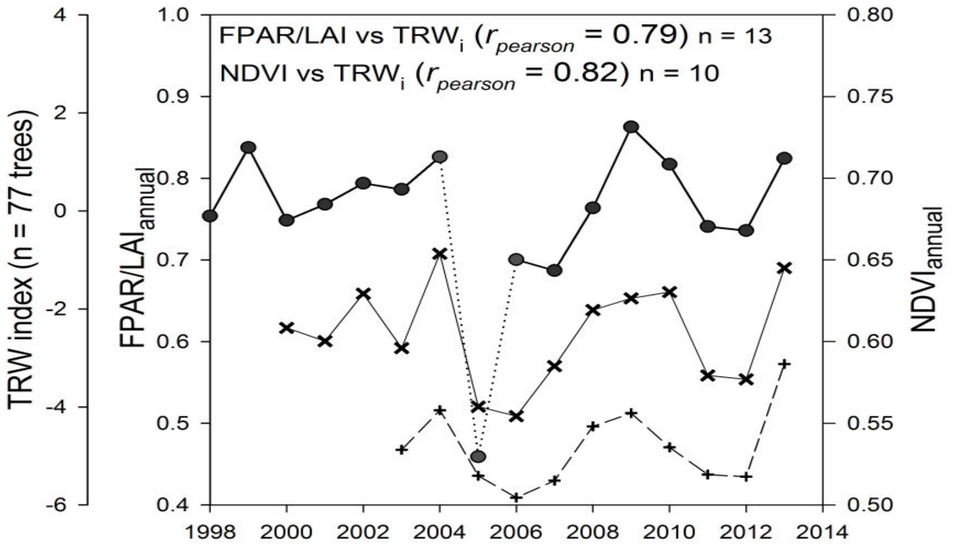


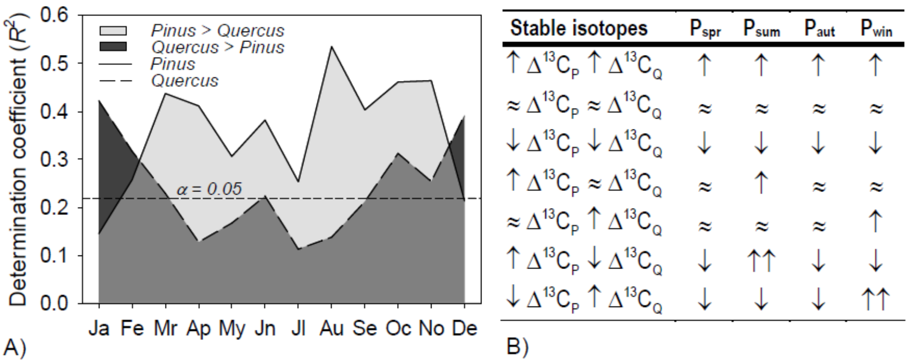
Figure 3. MODIS products (vegetation indexes) vs TRW$_{index}$ from the *Pinus halepensis* for the study site of chapter 3 and 4 (n = 74). MODIS products incudes: NDVI (250 m^2 per pixel) period (2003-2013) and FPAR divided by LAI (2000-2013, time resolution every 15 days (1000 m^2 per pixel), recalculated into annual values (year 2005 was a clear TRW$_{index}$ outlier, value = -5, so opted for not including it in the calculated Pearson correlation). By visual inspection, the forest area adjacent to the studied forest stand were composed mainly by pines, with probably a higher proportion of pines than in the actual sampling plot, which had 78 pines vs 33 oaks.

Modelling future changes in WUE at the large spatial scale will be necessary to understand the water and carbon cycles and therefore the climate of the earth, which is largely controlled by these two cycles. However, local ecological processes such as tree to tree interactions are still key to understand WUE spatio-temporal variability. In particular Mediterranean species, through stomatal conductance adjustment, may increase WUE in response to the rising temperatures and CO2 concentrations of recent decades, but only in sites with competition for water is low. For instance, *Pinus halepensis* can compete for water resources with understory shrubs, leading to severely suppressed shrubs, which are then unable to show such adaptive response (Moreno-Gutiérrez et al. 2014). Less drought-stressed shrubs, as opposed to those under high competition, maintain a constant internal CO2 concentration by closing stomata, thus increasing WUE without increases in assimilation (A) rates (Moreno-Gutiérrez et al. 2014; Battipaglia et al. 2014), On the contrary, suppressed shrubs already operate at low stomatal conductance, thus the margin to increases WUE for this plants might be limited (Moreno-Gutiérrez et al. 2014). In this context, our current work investigating the Δ^{13}C in the tree-rings of our forest stand, as mention earlier, is ideal to unveil this issue. Furthermore, the coordination between WUE and growth found in chapter 2 needs to be further explored in the context of climate change; for instance, the effects on growth of increased WUE due to higher atmospheric CO2 levels is much under debate, with recent studies suggesting no fertilization effect in Iberian conifers (Andreu-Hayles 2011) and the major global forests biomes (Peñuelas et al. 2011). Thus, future work may imply investigating whether WUE-growth coordination driven by precipitation is also altered as a consequence of global change (e.g. changes in atmospheric CO2 concentrations).

Paleoclimate modeling using multi-specific data

Previous work (Ferrio et al. 2003; Aguilera et al. 2012), also suggest that different periods of precipitation are responsible for the Δ^{13}C in the tree rings of *Pinus halepensis* and *Quercus ilex*: The active cambium of *Pinus halepensis* allows it to grow all year around if conditions are favorable i.e. when temperatures and water availability are high, although it may slow down or halt during summer drought and cold winters (De Luis et al. 2013). On the other hand, *Quercus ilex* responds to accumulated precipitation in previous seasons, periods for the recharging of the groundwater in which this species relies. Such mechanisms are further confirmed in chapter 4; here summer drought halts *Pinus halepensis* activity, as seen in NDVI and the probably low circulation of water within the tree (low transpiration) (Chapter 4). In addition, the better water status of the

Quercus ilex during summer drought was due to having access to groundwater, recharged in the autumn/winter time (Barbeta et al. 2015). This agrees with the theory that $\Delta^{13}C$ of tree-rings registered the environmental conditions (including water availability) present during wood formation. So variations in $\Delta^{13}C$ of each species will provide differential tree response information to environmental changes. Ferrio et al (2007) developed a conceptual model which matches the responses we found throughout this thesis (Figure 4). The main difference with this model and the one we used in chapter 1 is that the former was based on not overlapping datasets, whereas the latter was based on a set of sites with co-occurring species. This is positive in that it allows calibration of multiple linear models to define best-fit combination of months, but negative in that it does not cover the whole species distribution, only the overlapping area. Still, the validation across sites of such models with climate data, suggests that also the projected areas through linear fit were well modeled. What is even more interesting, is that this multi-specific climate modeling can be extrapolated to other areas with coexisting species showing differential response; in this case we have used a isohydric and anisohydric species, but a similar approach could be applied e.g. to a deciduous vs evergreen tandem. In this context, a recent work (Shestakova et al. 2014) showed differential sensitivities resulting in a contrasting imprint on stable isotopes (including carbon and oxygen) of the tree-rings. In the future, combining several isotopes we might be able to build isoscapes which can then be converted into seasonal-monthly high resolution climate models. In this way, vegetation will be transformed into the best paleoclimatic archive for spatial reconstruction. Each plant is a weather climate station with the most sophisticated sensors, and as stated by Fritts (1972): we only need to learn how to extract such information thus separating complex ecological and climatological interactions which obscured the tree-ring information.



Stable isotopes	P_{spr}	P_{sum}	P_{aut}	P_{win}
↑ $\Delta^{13}C_P$ ↑ $\Delta^{13}C_Q$	↑	↑	↑	↑
≈ $\Delta^{13}C_P$ ≈ $\Delta^{13}C_Q$	≈	≈	≈	≈
↓ $\Delta^{13}C_P$ ↓ $\Delta^{13}C_Q$	↓	↓	↓	↓
↑ $\Delta^{13}C_P$ ≈ $\Delta^{13}C_Q$	≈	↑	≈	≈
≈ $\Delta^{13}C_P$ ↑ $\Delta^{13}C_Q$	≈	≈	≈	↑
↑ $\Delta^{13}C_P$ ↓ $\Delta^{13}C_Q$	↓	↑↑	↓	↓
↓ $\Delta^{13}C_P$ ↑ $\Delta^{13}C_Q$	↓	↓	↓	↑↑

Fig. 4 A) Determination coefficients (R^2) between $\Delta^{13}C$ in wood and monthly precipitation for Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill.). B) Proposed conceptual model for the interpretation of $\Delta^{13}C$ values in co-occuring oaks and pines, according to their differential response to seasonal precipitation. $\Delta^{13}C_P$, $\Delta^{13}C_Q$, $\Delta^{13}C$ in wood of pines and oaks, respectively; P_{spr}, P_{sum}, P_{aut}, P_{win}; total seasonal precipitation in spring, summer, autumn and winter, respectively; ↑, increasing variable; ≈, steady variable; ↓, decreasing variable. Source: Ferrio *et al.* (2007).

Concluding remarks

The spatial analysis of stable isotopes in trees at different orders of magnitude undertaken in this thesis has contributed to the understanding of a major challenge in ecology, which is to separate abiotic from biotic factors controlling ecosystems functioning, and to integrate them across different scales (Loreau et al. 2001). The ultimate aim of this thesis was to gain a further insight into forest ecophysiology, biogeochemistry (carbon and water cycles) and the potential of tree-ring networks to infer spatial climate variability. The stable isotopes of carbon and water have allowed us to study contrasting root systems (shallow versus deep) and water extraction patterns in two Mediterranean tree species. The results revealed that the less studied belowground processes are of primary importance in understanding forest ecosystem dynamics. In this sense, "looking deeper" into the ground (Maeght et al. 2013) with new exploratory tools to study root systems (e.g., Rodriguez-Robles et al 2015) will go beyond the grain of knowledge provided by this thesis. The future holds a further multidisciplinary integration of science and technology, and it is difficult to forecast which direction forest research will take given the fast technological advances. Surely stable isotopes applications will tend to provide higher temporal resolution in the time series of $\Delta^{13}C$ in tree-rings, analysing every time smaller portions of the wood. Similarly, spatial and temporal analysis will be improved not only because of greater computational power but more importantly by the compilation of large data banks to build complex spatial-temporal networks of information.

The methodologies developed here have also allowed us to model tree responses to changes in water availability, such as stomatal conductance, a major and complex plant physiological process that mediates between carbon fixation and water loss (Aranda et al. 2012). In Mediterranean environments, modeling $\Delta^{13}C$ allows us to understand inter and intra-specific stomatal responses to spatiotemporal variations in environmental conditions (mainly water, which is limiting in these environments). This is vital to understand the future health and water use of the forest ecosystems and to provide information on the ability of the forest to sequester atmospheric CO2; thus $\Delta^{13}C$ isoscapes and spatio-temporal analysis of $\Delta^{13}C$ combined with other information such as growth or NDVI can be decisive information to better estimate the CO2 balance in current global climate models.

Finally, ecological processes like tree to tree interaction and consequent impact on stand water dynamics and successional processes were studied through the analysis of stables isotopes in xylem water with point process statistics. Obtaining other physiological information to be used as marks in point process, like $\Delta^{13}C$ in tree-rings or basal area increments, as our research group is currently doing, will tell us whether the observed tree behavior in water uptake patterns affects long-term physiological performance. To conclude, this thesis constitute a small piece in the complex puzzle of forest ecosystems. Ecosystems belong to complex system theory, and as such, it shares common characteristic with other complex systems (brain, social, traffic, etc). Fluxes, stability, spatial patterns, feedbacks, chaos and order have been described in this thesis and all conform these systems. Often understanding the whole might not be necessary to get a particular information from ecosystems, e.g. climate change impacts on forest, maximizing productivity through forest management, or understanding human impact on the water cycle. It is just a matter of asking the right question without getting lost in the infinite number of possibilities of complex systems which may never provide the so pursuit “ultimate answer” to everything.

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CONCLUSIONS

"A smooth sea never made a skilled mariner". **English proverb**,

Conclusions

i. The combination of multi-specific $\Delta^{13}C$ in tree-rings can provide complementary seasonal information to infer mean annual precipitation (MAP) at the regional level, and the accuracy of MAP models derived from $\Delta^{13}C$ can be comparable to that obtained from direct interpolation of meteorological data.

ii. Geographical information system and in particular the step-wise, multi-regressive modeling provides an opportunity for high resolution spatially-explicit models of $\Delta^{13}C$, which can complement climate data and provide information on changes in plant water status and iWUE at the landscape level.

iii. The $\Delta^{13}C$ spatio-temporal variability of *Pinus halepensis* is mainly governed by changes in precipitation, suggesting a major role of stomatal processes. However, parallel changes in NDVI also indicate a possible change in photosynthesis, either through variations in leaf area index or leaf chlorophyll content, thus finally affecting tree-ring growth. On the other hand, temporal variations in $\Delta^{13}C$ are strongly affected by carry-over effects, particularly evident after severe drought episodes.

iv. As hypothesized, *Pinus halepensis* and *Quercus ilex* use water with different isotopic compositions during severe drought, with stable isotope results indicating that the oaks could extract water at deeper soil levels than the pines, thus supporting niche segregation in root distribution and water uptake.

v. Although inter-specific interactions do not show up in point process methods, density-based competition indices showed some degree of interaction between pines and oaks, pointing to an additive effect of individual interactions. Inter- and intra-specific interactions (particularly competition) among trees are maximized during periods of moderate drought.

vi. Despite the different inter-specific water use strategies, our results suggest that increase drought may decrease the room for complementarity between the two species in mixed forest stands thus compromising the stability and sustainability of the system.