

Departament d'Ecologia  
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**CLIMATE and ATMOSPHERIC CO<sub>2</sub> EFFECTS on IBERIAN PINE  
FORESTS assessed by TREE-RING CHRONOLOGIES and their  
potential for CLIMATIC RECONSTRUCTIONS**

**Efectes del clima i del CO<sub>2</sub> atmosfèric en pinedes ibèriques avaluats mitjançant  
cronologies d'anells dels arbres i el seu potencial per reconstruir el clima**

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# **INTRODUCTION**



## OVERVIEW

*“There is an urgent need for greater understanding of nature and causes of the fluctuations in climate that have occurred since the most recent ice age. In a world faced with a growing population and accelerating use of energy, water, and food resources, the demand for estimates of climatic change over the next decades is pressing. This demand cannot be met at present because our knowledge of the course and causes of past climates is too limited. The instrumental records of climate variables such as temperature, pressure, and rainfall are sparse over much of the globe before the beginning of the 20<sup>th</sup> century. In order to gain some understanding of climatic variations on timescales of up to a few decades, climate records for much of the globe over several centuries are needed”* (Hughes *et al.* 1982). Nowadays, after 25 years of climate research, despite many attempts have been done to reconstruct past climate (e.g. Jones *et al.* 1998; Mann *et al.* 1999; Briffa 2000; Esper *et al.* 2002; Guiot *et al.* 2005; Moberg *et al.* 2005; Wilson *et al.* 2005), the results vary very much, particularly concerning long-term changes (Briffa and Osborn 2002; Esper *et al.* 2004; D’Arrigo *et al.* 2006). Consequently, the scientific community is still investing sources and time to determine to what extent current global changes are unusual, as well as to improve long-term predictive models. Last year, the European Union founded the Millennium project (“European climate of the last millennium”, IP 017008, <http://ralph.swan.ac.uk/millennium/>) addresses to answer the key issue: “*whether the magnitude and rate of 20<sup>th</sup> century climate change exceeds the natural variability of European climate over the last millennium*”. This is only an example that illustrates that the need of understanding past, present and future climate is still an important demand of our society.

Climatic variations can occur as a result of natural changes in the forcing of the climate system, for example variations in the strength of the incoming solar radiation or changes in the aerosols concentrations arising from volcanic eruptions. However, the important role that human activity is playing in current global climate change is currently well accepted (Jacoby and D’Arrigo 1997; Mann *et al.* 1998; Crowley 2000; Stott *et al.* 2000; IPCC 2001a, 2007a). For instance, increases in the atmospheric concentrations of greenhouse gases and aerosols since pre-industrial time period, due primarily to fossil fuel use and in a lesser degree to land use change, are considered forcing agents causing

climate change. Since industrial era, the atmospheric concentration of CO<sub>2</sub> has increased from 280 ppm in 1750 to 379 ppm in 2005, exceeding CO<sub>2</sub> concentration in 2005 by far the natural range over the last 650000 years (180 to 300 ppm) as determined from ice cores (IPCC 2007a). The *Third Assessment Report* published that the global average surface temperature increased by  $0.6 \pm 0.2$  °C for 1901 to 2000 (IPCC 2001a). However, the up-dated 100-year linear trend (1906 to 2005) of 0.74 °C (0.56°C to 0.92°C) is therefore larger. Moreover, the linear warming trend over the last 50 years (0.13°C per decade) is nearly twice that for the last 100 years, ranking eleven of the last twelve years (1995-2006) among the 12 warmest years in the instrumental record since 1850 (IPCC 2007a). Although any human-induced changes in climate will be embedded in a background of natural variations, most of the observed increase in global average temperature since the mid-20<sup>th</sup> century is very likely due to the reported increase in anthropogenic greenhouse gas concentrations (IPCC 2007a). On the other hand, numerous long-term changes in climate at continental, regional and ocean scales have been observed and compiled in the IPCC (2007a). These include widespread changes in precipitation amounts and extreme weather events. Although precipitation is highly variable spatially and temporally, long-term trends from 1900 to 2005 have been detected over many large regions. More intense and longer droughts have been observed over wider areas since the 1970s; heavy precipitation has increased its frequency over most land areas; and widespread changes in extreme temperatures have been observed during the last 50 years (cold days, cold nights and frosts have become less frequent, while hot days, hot nights and heat waves have become more frequent).

Overall, observational data of key global parameters compared to the IPCC 2001a projections have not exaggerated, but may in some respects even have underestimated the change (Rahmstorf *et al.* 2007). A global assessment of data since 1970 has shown how anthropogenic warming is likely to have had a discernible influence on many physical and biological systems. The 89% of 29000 observational data series from 75 studies, that show significant changes in physical and biological systems, are consistent with the direction of change expected as response to warming (IPCC 2007b). Many changes in phenology, distribution area, ecological amplitude, biodiversity, community composition and dynamics were observed at the end of the 20<sup>th</sup> century (Menzel and Fabian 1999; Barber *et al.* 2000; Chapin *et al.* 2000; Walther *et al.* 2002). Significant links between these phenomena and the current climatic change were established

(Parmesan and Yohe 2003; Root *et al.* 2003; Parmesan 2006). Moreover, several apparently gradual biological changes were linked to responses to extreme weather and climate events (Easterling *et al.* 2000). This increase in climatic variability could have important effects on ecosystems because, although living beings have an enormous adaptive capacity, many species could be not able to adapt as a consequence of the speed of the changes.

Climatic change is very complex, and not homogenous around the planet, varying depending on regions and ecosystems. In southern Europe, climate change is projected to worse conditions (high temperature and drought) in a region already vulnerable to climatic variability, being reduced water availability, hydropower potential and crop productivity, as well as increased heat waves and wildfires frequency (IPCC 2007b).

In the Iberian Peninsula, exceptionally high temperatures and a great inter-annual climatic variability have characterized climate during the second half of the 20<sup>th</sup> century (Font Tullot 1988; Romero *et al.* 1998; De Luis *et al.* 2000; IPCC 2001b; Giorgi *et al.* 2004). Some models that assess impacts of global change on species distribution, pointed out Spain as one of the regions with the most dramatic predictions in relation to species losses (Bakkenes *et al.* 2002). Growth model simulations for Mediterranean regions, if water is available, predict an increase in leaf area index, a reduction in mean leaf life span (in evergreen species) and higher production in response to the increase of atmospheric CO<sub>2</sub>; however, temperature and rainfall may constrain growth if rainfall is scarce (Sabaté *et al.* 2002). Several studies reported how climatic change is affecting or could affect Iberian terrestrial ecosystems. The atmospheric CO<sub>2</sub> concentration rise may produce an increase in plant growth (Peñuelas *et al.* 1995) or in photosynthetic rate (López *et al.* 1997). On the other hand, warming can produce an enlargement of the growing period (Peñuelas and Filella 2001) and/or shifts in the plant species and biomes distributions (Peñuelas and Boada 2003). However, drought stress linked to higher evapotranspiration (due to temperature increase and/or precipitation decrease) can enhance its influence as limiting factor of plant biomass growth or photosynthetic rate (López *et al.* 1997; Ogaya *et al.* 2003; Peñuelas *et al.* 2004), produce damage in woody flora (Peñuelas *et al.* 2001; Lloret *et al.* 2004) and even sometimes induce mortality (Martínez-Vilalta and Piñol 2002).

Two main purposes divided this Thesis into two blocks, taking into account that the need of understanding current climatic fluctuations is still an important request of population living in the 21<sup>th</sup> century and the enormous wide range of responses observed to these changes depending on regions, ecosystems and/or species. The first main objective is to assess how climate and atmospheric CO<sub>2</sub> concentration changes are affecting Iberian pine forests. The second aim is to extract the climatic signal registered by the studied stands with the aim of reconstructing past climate. Therefore, we will use the relationship between trees and climate in both directions: assessing the effects of climate on the forests, and afterwards, using these results to estimate climatic conditions before the existence of instrumental records. The methodology proposed is based on the study of width and  $\delta^{13}\text{C}$  tree-ring chronologies established at different sites along the north and east of the Iberian Peninsula.

## **PALAEOCLIMATIC RECONSTRUCTIONS**

As mentioned in the overview, a better understanding of past climate changes would be very valuable in determining to what extent current global changes are unusual, as well as to improve the reliability of climate prediction models. Moreover, reconstruction of past climate could contribute to explain certain changes in the history of humanity or natural processes as species evolution or their current phytogeographical range of distribution (Ferrio 2005).

Since instrumental climate records prior to the 20<sup>th</sup> century are scarce and sparse, estimates of global climate variability during past centuries are based on indirect “*proxy*” indicators. A proxy indicator is a natural or documentary record prior to instrumental climatic data that goes back in time and contains some kind of climatic or environmental information. Palaeoenvironmental reconstructions of climate variations or environmental conditions occurring in the past can be performed using this kind of proxies. Careful calibration and cross-validation procedures are necessary to establish a reliable relationship between a proxy indicator and the climatic variable/s that it is assumed to represent, providing a “transfer” function through which past climate conditions can be estimated (Ferrio 2005).

There are many natural archives of palaeoclimatic information as ice cores, pollen records, ocean and lake sediments, coral reefs or borehole measurements. However, as was pointed out by McCarroll and Loader (2004), none of them provide the two great advantages of tree rings. The first advantage is the achievement of a perfect annual resolution, as the dendrochronological techniques allows the exact dating of each ring. The second advantage is the possibility to define confidence limits calculating variability of each year, as tree-ring chronologies are built over-lapping several series of individual trees. Besides, the fact that trees are widespread introduce the possibility to examine past climate in a wide range of different geographical locations, which could be very useful for predicting the consequences of future climate change in combination with current estimates of global or hemispherical conditions.

## **TREE-RINGS AND CLIMATE**

The statement that climate influences tree growth is generally accepted (Schweingruber 1996). At sites where trees form annual growth layers, the characteristics of those layers or tree-rings reflect the conditions under which they were formed. Differences in tree-rings may be synchronic in many trees within a region indicating that growth is influenced by some common set of external factors. When similarities in growth variation are strong and spatially extensive, it is assumed that climate is related to the external agents that are forcing the observed pattern of common variability among trees. There are no other environmental factors likely to act on the same range in the space, time and frequency domains. Dendroclimatology is based on the assumption that is possible to extract a record of the climate variables recorded in tree-rings of wood formed in the past (Hughes *et al.* 1982).

Since the early works of Douglass in 1914 (Fritts 1976), tree-rings have been extensively used as palaeoclimatic proxies. Dendroclimatology, the science of reconstruction of the past climate by the use of tree rings, is a well developed subdiscipline of dendrochronology. The prefix *dendro* is from the Greek word for tree, *dendron*, and the word *chronology* is the name of the science that deals with time and the assignment of dates to particular events (Fritts 1976). Dendrochronology is a science that allows the identification of the exact year in which each tree ring was formed and to assign specific calendar dates to the rings with an absolute precision, comparing



patterns of wide and narrow rings or other anatomical characteristics (e.g. density fluctuations) among several tree-ring samples. The same sort of comparison can be made among living trees already dated and wood fragments of unknown dating from old buildings, fallen logs, stumps, submerged trunks and other kind of sub-fossil material in order to establish the date when the fragment was part of a living, growing tree. The procedure of matching ring patterns among trees or wood fragments in a given area is called *cross-dating* (Fritts 1976).

Tree growth is not only determined by climate, being also affected by other non-climatic factors as the age-size related trend, forest stand dynamics, exogenous disturbances or particularities of each tree. For dendroclimatic studies, this non-climatic variability or noise should be removed through standardization, remaining only the common information among trees or signal. Standardization transforms the ring widths into tree-ring indices that have a defined mean of 1 and a relatively constant variance (stationary series), by fitting a curve to the tree-ring series and then dividing each ring width value (observed) by the corresponding curve value (predicted). Negative exponential curves, cubic smoothing splines of different year length (Cook and Peters 1981) or low- and high-pass filters are used depending on the objective of the study and the characteristics of the series. Additional noise reduction techniques such as robust mean estimation and autoregressive modelling could be also applied. The common signal is a statistical quantity representing the common variability present in all the tree-ring series at a particular site (Cook and Briffa 1990). The variance of any series contains this common forcing signal, but each series also have specific tree or sample variability (statistical noise). As this noise is uncorrelated from sample to sample, it can be cancelled out depending on the number of series being averaged. Therefore, standardization procedure is two fold: (1) to remove non-climatic signals related to age trends or other sources from the ring-width series; and (2) to allow the resultant standardized values of individual samples to be averaged (Cook and Briffa 1990). However, the risk of removing low-frequency climatic variability when using these statistical treatments must be taken into account (Cook *et al.* 1995). The result of standardization is the *mean chronology* that represents the common growth pattern in a region, being an estimation of the common signal shared by the analyzed trees that usually can be related to climate.

## RING-WIDTH AND DENSITY

Past climate can be reconstructed from the year-to-year changes in annual ring width and ring density (e.g. Fritts 1976). Although density records have the advantage that the common signal between trees is generally stronger than among ring width series (e.g. Wilson and Luckman 2003), up to now, fewer density chronologies has been established as this proceeding is much more expensive than measuring tree rings. Tree-ring width and maximum late wood density variations have most successfully been used for climate reconstructions at sites where tree growth is limited by one dominant factor. For example, temperature is the main limiting factor in cold regions as for example in Fennoscandia (Briffa *et al.* 1990, 1992), European Alps (Büntgen *et al.* 2006), Mongolia (D'Arrigo *et al.* 2000) or the northern hemisphere (Esper *et al.* 2002; Briffa *et al.* 2004); while precipitation is the most limiting factor in arid or semi-arid regions as for example Mexico (Díaz *et al.* 2001; Cleaveland *et al.* 2003), Turkey (Touchan *et al.* 2003) or Morocco (Till and Guiot 1990).

Trees located at the edge of their natural range (altitudinal or latitudinal) are particularly sensitive to climate variations (Fritts 1976), as usually a factor limiting physiological processes can be easily identifiable. For instance, different growth responses were reported along altitudinal transects (Tardif *et al.* 2003; Adams and Kolb 2004). However, not always old living trees are located at the most suitable sites. For example, in temperate regions like Europe, where lots of species are living in the optimal range of their distribution area, sometimes climate growth relations cannot be found. Little useful climate information can be extracted from these series due to the complexity of tree-ring responses to climate. Isotope ratios in tree rings have the added advantage that physiological controls on their variation are reasonably well understood and relatively simple in comparison to the numerous factors controlling annual growth increment (McCarroll and Loader 2004). In this respect, the use of stable isotopes in tree-rings may be particularly promising as the rapid technical development during recent years has given the opportunity to establish millennial tree-ring isotope chronologies (Helle and Schleser 2004). By using stable isotopes, it may be possible to take the advantage of the tree-ring chronologies, avoiding some problems associated with the characteristics of ring width and density series that make compulsory the use of standardization methods.

## CARBON STABLE ISOTOPES IN PLANT SCIENCE

Carbon, oxygen and hydrogen are the three main elements in wood, having all of them more than one stable isotope. These isotopes have almost identical chemical properties but the difference in mass allows physical, chemical and biological processes to discriminate against one of them, thereby imparting an environmental signal (McCarroll and Loader 2004). This section is just a little introduction to carbon stable isotopes in plant science since this Thesis is focused in the study of tree-ring chronologies in which these stable isotopes were analyzed.

The atomic number is the number of protons in the nucleus and defines each element. However, the nuclei of a given element may have varying numbers of neutrons. The atoms of the same element that differ in the numbers of neutrons are called isotopes. The different number of neutrons is responsible that the various isotopes of an element have different masses. The mass number or atomic weight, left superscripted to an element, is the sum of the number of protons and neutrons of an isotope. For example, all isotopes of carbon have 6 protons combined with 6, 7 or 8 neutrons, resulting in carbon atoms with mass numbers of 12, 13 or 14 denoted by  $^{12}\text{C}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ , respectively.  $^{14}\text{C}$  is a radioactive or unstable isotope as it disintegrates spontaneously over time, characteristic that is used for dating materials. On the other hand,  $^{12}\text{C}$  and  $^{13}\text{C}$ , are non-radioactive or stable isotopes that remain constant along geological time scales. Almost the 98.89% of atmospheric  $\text{CO}_2$  is formed by the lighter carbon ( $^{12}\text{C}$ ), while a little portion, 1.11%, is represented by the heavier carbon ( $^{13}\text{C}$ ) (Ehleringer and Rundel 1988).

By convention, the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  is expressed in delta ( $\delta$ ) notation with reference to a standard material for which the isotope ratio is known. The carbon isotope ratio ( $\delta^{13}\text{C}$ ) is expressed in parts per thousand (‰) as:

$$\delta^{13}\text{C}_{\text{sample}} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

R denotes the ratio between the heavy and the light isotope ( $^{13}\text{C}/^{12}\text{C}$ ), while  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the ratios in the sample and standard, respectively. The chosen standard was a subfossil belemnite from the Pee Dee formation of South California, which was exhausted and replaced by Vienna Peedee belemnite or VPDB (Coplen 1995).

Living beings, equally to all the material of the universe, are composed by atoms that can be isotopes more or less frequent of the same element. Relative proportion between light (lower mass number) and heavy isotopes (high mass number) varies depending on their location. Some substances become enriched due to the incorporation of a higher proportion of heavier isotopes, while others become depleted being the proportion of lighter isotopes higher. This phenomenon is called isotope fractionation. As a result the isotope composition of a given element varies considerable among the different parts of the Earth. Atmospheric elements can show a different proportion of heavy isotopes of the same elements that are found in the lithosphere, hydrosphere or biosphere. Indeed, ratios also vary depending on their specific location in each compartment, kind of life form or within the different parts of an organism.

Current average of the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  is -8‰ but it was -6.69‰ in 1956, becoming this value more negative year by year due to the use of fossils fuels and deforestation (Keeling *et al.* 1979). Terrestrial vegetation incorporates  $\text{CO}_2$  during photosynthesis, discriminating against heavy molecules showing a preference of  $^{12}\text{C}$  in front of  $^{13}\text{C}$ . As a consequence, carbon of terrestrial plants contains less proportion of  $^{13}\text{C}$  than carbon of atmospheric  $\text{CO}_2$ . The leaves and wood of trees present values of -20‰ and -30‰, respectively, demonstrating that trees are depleted in  $^{13}\text{C}$  relative to air (McCarroll and Loader 2004). Discrimination rate depends on levels of  $\text{CO}_2$  in the atmosphere, being higher when  $\text{CO}_2$  concentration is higher.

The seasonal and inter-annual evolution of the isotopic composition of atmospheric  $\text{CO}_2$  ( $\delta^{13}\text{C}$ ) varies between both hemispheres due to their different kind of vegetation cover. Vegetation in Northern hemisphere is concentrated in medium to high latitudes being exposed to seasonal cycle activity, whereas vegetation in Southern hemisphere is dominated by equatorial rainforest where seasons are not present. During the growing period, photosynthesis take place and plants take  $\text{CO}_2$  from the atmosphere preferably poor in  $^{13}\text{C}$ ; as a consequence,  $\text{CO}_2$  that remains in the atmosphere is enriched in this heavier isotope. For this reason, there is higher seasonal variation of atmospheric  $\delta^{13}\text{C}$  in the Northern than the Southern hemisphere.

## PHYSIOLOGICAL BASIS

In many physiological studies, carbon isotope discrimination against  $^{13}\text{C}$  ( $\Delta$ ) is calculated as follows, expressing the difference in isotopic composition between air ( $\delta^{13}\text{C}_{\text{air}}$ ) and plant organic matter ( $\delta^{13}\text{C}_{\text{tree}}$ ) in ‰:

$$\Delta = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{tree}}}{1 + \delta^{13}\text{C}_{\text{tree}}/1000}$$

For C3 plants, the relationship between carbon isotope discrimination and leaf gas exchange has been described by several models, being the following equation described by Farquhar *et al.* (1982, 1989) the most extensively used:

$$\Delta = a + (b - a) \frac{c_i}{c_a}$$

The terms  $c_i$  and  $c_a$  are the needle intercellular spaces and ambient concentrations of  $\text{CO}_2$  ( $\mu\text{mol}\cdot\text{mol}^{-1}$ ), respectively. Isotopic fractionation is based on two main steps: diffusion and carboxylation. The first step, when air diffuses through stomata into the intercellular air spaces towards the carboxylation sites, the  $\text{CO}_2$  that include lighter carbon isotope are able to diffuse more easily. As a result, the internal air is depleted in  $^{13}\text{C}$  relative to ambient air. The fractionation between  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  during diffusion of  $\text{CO}_2$  through the stomata ( $a$ ) is 4.4‰. In the second step,  $\text{CO}_2$  is used by the photosynthetic enzyme or rubisco (RuBP carboxylase). Biological processes tend to use  $^{12}\text{C}$  in preference to  $^{13}\text{C}$ . This discrimination against  $^{13}\text{CO}_2$  carried out by the rubisco ( $b$ ) was estimated to be about ~27‰ by Farquhar and Richards (1984).

## $\delta^{13}\text{C}$ AND CLIMATE

As was described in the previous section, the fractionations that occur due to  $\text{CO}_2$  diffusion into stomata and carboxylation are constant, and consequently insensitive to climate (McCarroll and Pawellek 2001). Therefore, the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in tree rings is controlled dominantly by the ratio between  $c_i$  and  $c_a$ . If  $c_i$  is high relative to  $c_a$ , the amount of  $\text{CO}_2$  available in the stomatal chambers is high and there will be a strong carboxylation discrimination leading low  $\delta^{13}\text{C}$  values. In this case, the stomatal conductance is much higher than the rate of photosynthesis. On the other hand, if the

stomatal conductance is lower than the photosynthetic rate, the internal CO<sub>2</sub> concentration ( $c_i$ ) will drop and there will be less carboxylation discrimination yielding high  $\delta^{13}\text{C}$  values (McCarroll and Loader 2004). Thus,  $\delta^{13}\text{C}$  reflects the internal concentration of CO<sub>2</sub> and therefore the balance between stomatal conductance and photosynthetic rate. In dry environments,  $\delta^{13}\text{C}$  will be dominated by stomatal conductance being related with air relative humidity and antecedent rainfall. In wet environments,  $\delta^{13}\text{C}$  will be controlled by photosynthetic rate being related to photon flux and temperature. This simple model of the influence of climate on the internal concentration of CO<sub>2</sub> in the stomatal chambers and therefore on  $\delta^{13}\text{C}$  values was described by McCarroll and Pawellek (2001).

Drought maps for last centuries in the south-east of USA were developed using  $\delta^{13}\text{C}$  from tree-rings (Leavitt *et al.* 2007), as the  $\delta^{13}\text{C}$  in plants can show drought stress periods. Since plants tend to close their stomata in order to save water during droughts, CO<sub>2</sub> is less available in the stomatal chambers. As a result, plant discrimination is lower yielding to high  $\delta^{13}\text{C}$  values.

A summary of papers that have used tree-ring isotope time-series for palaeoenvironmental research is described in McCarroll and Loader (2004). Up to now, there are few annually resolved millennial long records from tree ring isotopes, and still fewer real reconstructions, as for example a precipitation reconstruction in Pakistan using oxygen isotope records from tree-rings (Treydte *et al.* 2006). Shorter climatic reconstructions using  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  chronologies are scarce as well, but some examples can be given as precipitation in north-eastern Spain for the last 400 years (Planells *et al.* 2006), temperature in northern Finland since AD 1640 (Gagen *et al.* 2007), summer drought since 1880 (Raffalli-Delerce *et al.* 2004) or interannual fluctuations in local summer temperature and water stress for the last four centuries in Western France (Masson-Delmotte *et al.* 2005).



## **OBJECTIVES**





As it was mentioned in the Introduction, this Thesis has established the relationship between trees and climate with two main objectives: (1) to assess climate and atmospheric CO<sub>2</sub> concentration effects on Iberian pine forests; (2) to extract climatic information registered by these forests to reconstruct past climate. Four chapters or papers that develop the topics cited above are included, introducing also more specific questions.

## **1. Climate and atmospheric CO<sub>2</sub> concentration effects**

### **1.1. Climatic effects**

*CHAPTER 1: Climatic effects on regional tree-growth variability in Iberian pine forests*

- To detect the macroclimatic signal shared by the thirty-eight tree ring-width chronologies involved in this study.
- To analyze temporal variability of radial growth and possible climatic drivers.
- To assess growth-climate relationships and their stability throughout time.

### **1.2. Atmospheric CO<sub>2</sub> concentration effects**

*CHAPTER 2: Atmospheric CO<sub>2</sub> concentration effects on five Spanish pine forests*

- To study the responses of five Spanish pine forests to atmospheric CO<sub>2</sub> concentration increase, mainly in terms of their carbon isotope composition and water relations, analyzing the  $\delta^{13}\text{C}$  chronologies performed for each site.

## **2. Reconstructions of climate**

### **2.1. Width and $\delta^{13}\text{C}$ sensitivity to climate**

*CHAPTER 3: Width and  $\delta^{13}\text{C}$  tree-ring sensitivity to climate in Spanish pine forests*

- To assess the nature and strength of the climatic signal registered in the width and  $\delta^{13}\text{C}$  tree-ring chronologies in five Iberian pine forests.

### **2.2. Summer precipitation reconstruction**

*CHAPTER 4: Precipitation reconstruction for the last 400 years in Spain*

- To develop preliminary summer precipitation reconstructions in Spain over the past 400 years based on width and  $\delta^{13}\text{C}$  tree-ring chronologies.

- To compare different statistical treatments of the tree-ring proxies used as predictors in the transfer functions, as well as reconstructions obtained from two different meteorological data sets.
- To conduct a preliminary examination of links between large-scale atmospheric phenomena and our reconstructions.