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Comparative analyses of multiple tree-ring parameters of beech along latitudinal gradients in different climatic regimes

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Tesi di dottorato di::

Dott. Negar Rezaei

Coordinatore del corso

Prof. Bartolomeo Schirone

Firma 

Tutore

Dott. Alfredo Di Filippo

Firma 

Co-tutori

Dott. Marco Lauteri

Firma 

Dott. Giorgio Matteucci



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1- Introduction

1-1- European beech (*Fagus sylvatica* L.) and climate change

One third of Europe's land surface is covered by forests, with important economic and social value. They constitute the most natural ecosystems of the continent. The dynamic behaviour of forest trees and stands is being changed constantly by interactions between cover, biotic and abiotic conditions, and especially climatic and microclimatic conditions.

European beech (*Fagus sylvatica* L.) represents one of the most important European forest tree species (Tarp et al. 2000; Brus et al. 2012), hence possible adverse factors affecting productivity and physiology of this species can have strong ecological and economic impacts in Europe (Peuke et al. 2002; Geßler et al. 2007). Beech were used extensively in tree ring study (Skomarkova et al. 2006; van der Werf et al. 2007; Čufar et al. 2008), taking advantage of the widespread distribution, sensitivity to climate and longevity of beech. European beech approaches the southern edge of its geographic range in Italy, where its altitudinal range extends more than 1500 m, from about 300–400 to 2000–2100 m in central and southern Italy, and from 200–300 to 1500–1600 m in the Alps (Di Filippo et al. 2007).

Global climate change is affecting the frequency and intensity of extreme events. Extremes of both warm and cool temperature are important indicators as they can have strong impacts on natural systems. For instance, a temperature that is normal for one region may be extreme for another region that has not regularly experienced this temperature in the past. However, precipitation changes across Europe show more spatial and temporal variability than temperature. Since the mid-20th century, annual precipitation has been generally decreasing in parts of southern Europe but increasing across most of northern Europe, most notably in Winter. On the other hand, climate change is expected to have major impacts on forest ecosystems. Rising atmospheric CO₂ concentration, higher temperatures and changes in precipitation are likely to have significant effects on the vegetation period, growth, health and distribution of trees as well as on forest ecosystems, and thus on the goods and services provided by forests. As an example various extreme climatic events like the 2003 drought in large parts of Europe and severe windstorms already had strong negative impacts on forests (Ciais et al. 2005; Usbeck et al. 2010).

In the 21st century, the Mediterranean Basin could be most sensitive to climate change due to global warming among the European regions (Schroter et al. 2005, EEA Report No 12/2012, FAO 2013). Since the 1970s, extreme climatic events as drought periods have been observed more frequently, (McCabe & Palecki 2006), and natural forest productivity in the northern part of the region seems to decline (Boisvenue & Running 2006). In beech stands, productivity decline related to climate was observed in northeast Spain (Jump et al. 2006) and in the central Apennines (Italy) (Piovesan et

al. 2008). Most of the current climate projections for Central Europe predict increased temperatures that are expected to cause an increase in the frequency and duration of intense summer droughts (Lindner et al. 2010). Owing to its sensitivity towards low water availability (Ellenberg 1996) and longer drought periods (Fotelli et al. 2002), physiological performance, growth and competitive ability of European beech may be adversely affected by such changing environmental conditions (Peuke et al. 2002). The situation may be aggravated by the fact that the area of distribution of beech includes many sites with shallow limestone-derived soils of low water storage capacity (Geßler et al. 2005).

Species that occupy large geographic ranges respond to contrasting environmental conditions by genotypic variation and phenotypic plasticity (Peuke & Rennenberg 2004). Several case studies have investigated the drought sensitivity of beech provenances along a gradient of decreasing precipitation from northern to southern Central Europe and to the Mediterranean region (Peuke et al. 2006; García-Plazaola & Becerril 2000).

1-2- Effect of forest management on tree physiology and different types of silvicultural systems

Forest cover modifies the climatic parameters and creates a microclimate whose characteristics depend on the general climate itself and the physical characteristics defining the nature and structure of the cover (Aussenac 2000). In fact, trees and vegetation in which it consists, adapts to new microclimatic conditions by modifying its specific architectural and functional components. Thus, it is really an interactive and even a feedback and feedforward system: any change in one of the components results in an adjustment of the others, and so on (Figure 1). In reality, for a forest tree it is the overall ecophysiological behaviour which is affected by these interaction phenomena, as much in terms of photosynthetic processes, transpiration, translocation, transport and storage of assimilates, as growth, flowering or fruiting phenomena.

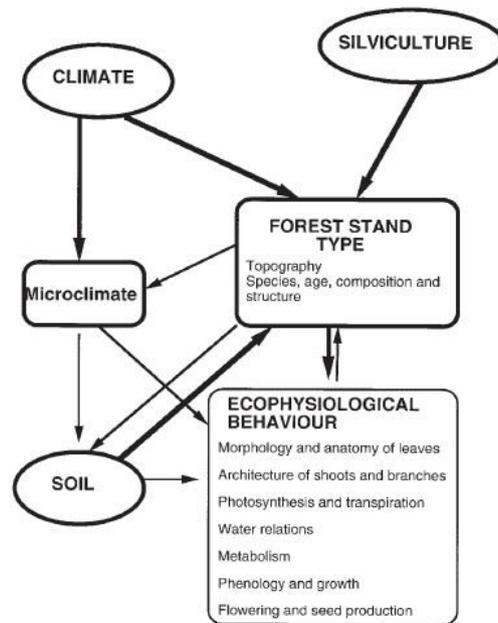


Figure 1. Interactions between climate and forest stand (Aussenac 2000).

Usually, the direct effect of cutting increases light intensity and may provide higher photosynthesis rate, provided water is enough. In previously dense and, after management, heavily thinned stands effects of temperature stress or photo-inhibition phenomena are possible (Aussenac, 2000).

Under silvicultural treatments, stem circumference growth reacts to described phenomena and it is influenced by the:

- Increase of light in the lower parts of the tree crown, which increase photosynthesis
- Increase of soil water availability and reduction of water consumption.-Increase of nitrogen compounds released through faster humus mineralization as a consequence of improved light under the cover and an increase in the surface soil temperature.

Shelterwood cutting refers to a progression of forest cuttings leading to the establishment of a new generation of seedlings of a particular species or group of species without planting. This silvicultural system is normally implemented in forests that are considered mature, often after several thinning interventions. The desired species are usually long-lived and their seedlings would naturally tend to start under partial shade. Once the desired species is established, subsequent cuttings give gradually the new seedlings more light and the growing space is finally fully passed to the new generation.

What differentiates shelterwood cuttings from other regeneration systems, such as clearcutting or seed tree, is that the new seedlings are established before the mature trees are fully (or mostly) removed. This gives the forester more control over the species that are regenerated, and is more

flexible if the first regeneration effort fails. At the end, all mature trees may be removed, creating a young even-aged forest, or a considerable number of reserves may be kept. Further to more or less regular thinnings, shelterwood systems (Figure 2) may include preparatory cuttings, establishment cuttings, and one or more overstory removal cuttings:

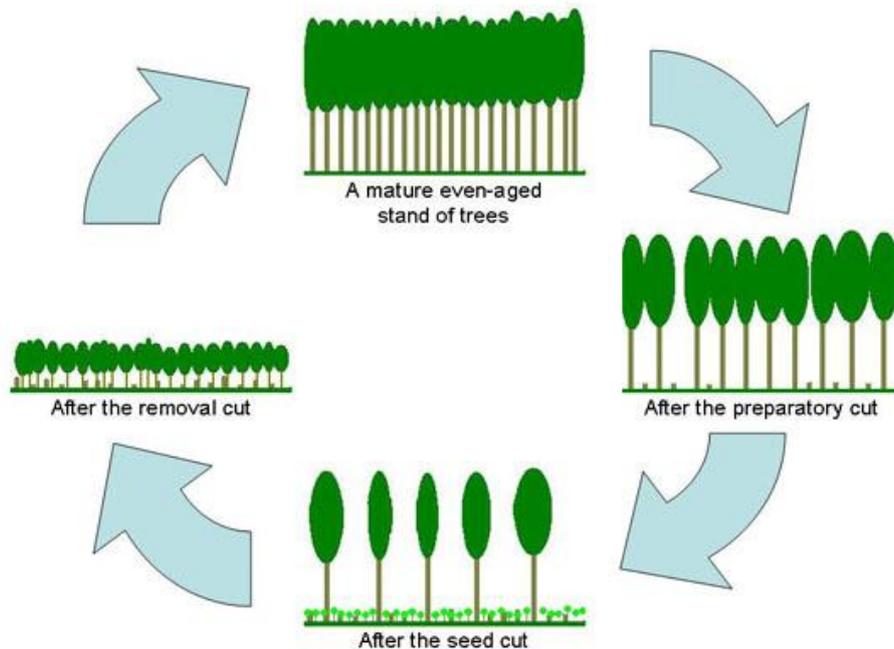


Figure 2. Uniform Shelterwood system

Preparatory cuttings are sometimes, but not always, the first step in a shelterwood regeneration system. The purpose of this cut is to remove species that are not desired so that they do not contribute seeds to the establishment cut and enlarge the trees crowns to stimulate fructification.

Establishment cutting aims to establish the regeneration, and is done in a year when the seed production is good. The intention is to provide a certain amount of light that is necessary for new seedlings to start, but not necessarily grow freely. In many cases the mineral soil is intentionally exposed, encouraging germination by providing a moister seedbed than the leaves and needles that normally cover the forest floor. The severity of the cut depends on the species being targeted: fewer trees are removed for species that tolerate more shade, more trees removed for species that require more light.

Overstory removal or **partial overstory removal** is performed to give more light to the established seedlings, allowing them to grow freely. This is the most important part of the shelterwood system because the site resources are deliberately transferred from one generation of

trees to the next. Without this cutting, seedlings will stagnate or even die as the crowns of the older trees grow. At the end, all the mature trees may be removed, or some may be left as reserves. These will continue to grow and may be harvested several decades later, or may be left to die naturally and contribute ecological values to the site (deadwood).

When this series of treatments is performed across an entire stand, it is called a uniform or regular shelterwood. Irregular shelterwoods take a variety of options, and are becoming increasingly popular on small woodlots and public land. One variation is the **Femelschlag** (Figure 3), a German version meaning "**expanding group shelterwood with reserves**". The **group** is located or established by cutting, and the mature trees surrounding this patch are cut, with the exception of several high-quality reserve trees. Regeneration then establishes around the edges of this new gap in response to the increased light. When this regeneration has established, the gap is expanded by cutting the trees around the edge of the gap, again leaving some reserves. The forest is regenerated in concentric circles that eventually meet, providing a diverse structure and steady income from repeated cuttings.

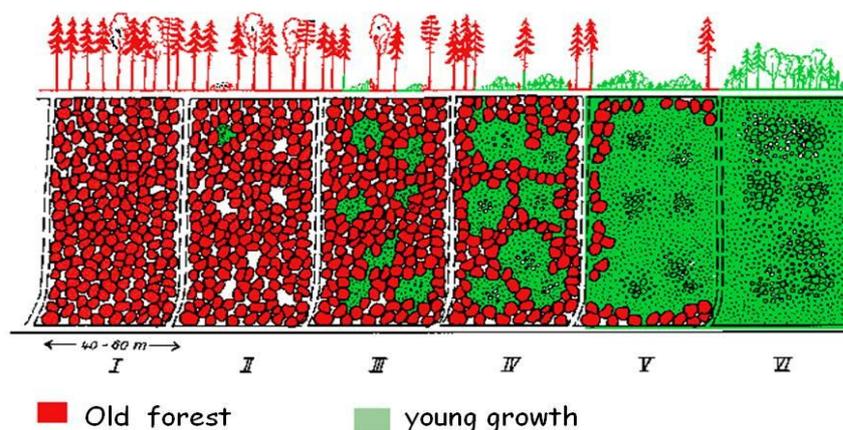


Figure 3. Expanding group shelterwood system (Smith et al. 1997)

In the upper mountain belt of the Apennine range, where beech coppices with standards are mainly located, depopulation and changes in the socio economical conditions over the last 60 years led to a pronounced drop in local demand for small size timber, firewood and charcoal. As a consequence, many coppice stands have been almost completely abandoned due to lack of active management and, lately, no monitoring of their natural dynamics. Over the same period, mountain forests were considered increasingly important as a defense against natural hazards, for biodiversity conservation, for the development of recreational and tourist activities, for the protection of water resources and so on. These processes are common to other European Mediterranean countries as well (Ciancio et al. 2006).

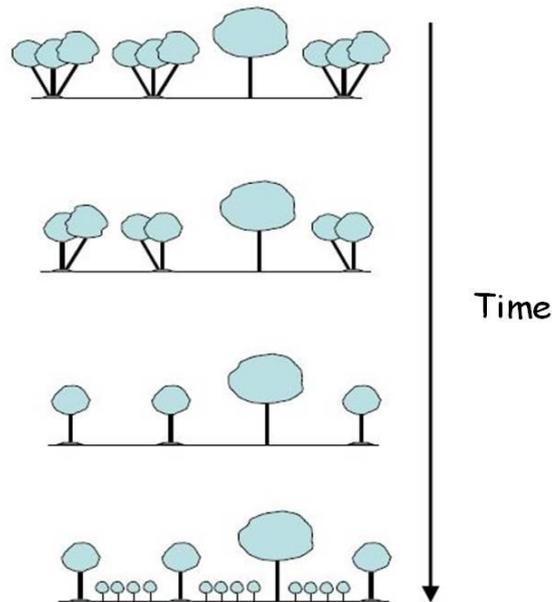


Figure 4. The conversion of coppices into high forests

The conversion of coppices with standards into high forests (Figure 4) is considered one of the preferred management alternatives both for the public good and as an improvement for the owners future revenue by the yield of larger size trees. Such a management option can be framed as a process from a silvicultural system based on clearcutting (the beech coppice with standards) towards a silvicultural system based on continuous cover (the beech high stand to be managed by shelterwood, group shelterwood or selection systems at the end of the conversion).

In beech coppices with standards, conversion is usually carried out by gradually **thinning the stand** in order to prepare, in the shortest possible time, suitable microsite conditions for regeneration from seeds. The best sprouts in terms of vigour, size and shape are favoured by early thinning, which cull the weaker trees. Later interventions can also target the dominant layer. The new high stand starts growing under cover of the released sprouts or after the regeneration fellings, which can be carried out by shelterwood, group shelterwood or selection cutting according to the desired forest structure. Overall, conversion of beech coppices in mountainous areas is quite a long process. The final transformation from a coppice stand at the rotation age into an adult high forest can last up to 150 years, depending on site fertility (Ciancio & Nocentini 2004).

1-3- Tree ring archives

Tree rings are uniquely widespread relative to all comparable natural archives of climate signals. Accordingly, a significant amount of dendroclimatology research has been focused on the extraction and validation of climatic variability from tree-ring data series (Fritts 1966; Fritts et al. 1979)(Briffa et al. 2001; Cook 2002; Lloyd-Hughes & Saunders 2002; Briffa et al. 2004; Gray et al. 2004;

Watson & Luckman 2004; Salzer & Kipfmüller 2005).

Reliably annual rings of woody plants are formed wherever the local climate imposes a single dormant season and a single growing season each and every year. Such conditions are widespread in the boreal, temperate, and sub-temperate regions, and in some parts of the tropics. At middle and high latitudes or elevations, this pattern of one dormant and one growing season per year in the formation of wood is imposed by annual day length and temperature cycles.

The layer of new xylem or wood laid down each year by the cambium of a tree (the annual ring) is a natural archive of growth history of that year. The environmental conditions influencing that growth may leave an imprint on the properties of the ring. Thus the size, structure, and composition of the ring may contain information on those conditions. So the most common way to extract climatic signals from tree rings is to measure the width of the rings (Fritts 1966). Where growth is limited by some environmental factor, such as drought or summer temperature, variability in the ring width is likely to reflect variability in that limiting factor, providing climatic signal. However, there are a number of limitations that restrict the potential of tree ring width for extracting climatic signals. A significant problem in using ring widths to reconstruct climate is that the correlation between ring widths and climate may not be always very strong. Trees use their resources in many ways, such as producing leaves, stools, roots, flowers and seeds, and adding girth is unlikely to be the most pressing necessity. Variations in ring width are best seen as a general reflection of changes in net photosynthesis rather than as a direct response to a particular climate parameter. The effect is that individual trees in a stand are likely to respond in different ways to the climate of a given year, so that there is considerable variability in the relative widths of the rings formed. The ring widths of trees at different sites may also differ because local edaphic and microclimate conditions affect their response to the regional climate that is of interest (Schweingruber & Briffa 1996).

1-4- Isotope fractionation in trees and Stable Carbon isotope theory

The main constituents of wood are carbon, oxygen and hydrogen and variability in the isotope composition of each of these elements could potentially provide an environmental signal (McCarroll & Loader 2004). The water isotopes enter the tree through the roots and move up through the xylem to the leaves, where evaporation leads to preferential loss of the lighter isotopes. The source of carbon is atmospheric CO₂, which enters the leaves through the stomata. In the leaves, photosynthesis combines the three elements (Carbon, Hydrogen and Oxygen) to produce sugars, some of which are transported down the phloem and used to build wood cells around the stem, forming the tree rings.

The first source of isotope variation in tree rings is differences in the isotope composition of the

source water and of atmospheric carbon dioxide. Within the leaf there is fractionation of carbon prior to photosynthesis, mediated by the action of the stomata. As carbon dioxide diffuses through the stomata into the intercellular spaces there is a fractionation against ^{13}C . This ‘fractionation due to diffusion’ remains at about -4.4% , irrespective of changes in temperature and vapour pressure and is even insensitive to changes in the size of the stomatal opening. The difference in diffusion of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ is simply due to the fact that as the molecules bounce around, the lighter ones bounce furthest, and so are more likely to pass through the stomatal opening. Only when the stomatal apertures are very small, fractionation increase markedly, because interactions with the guard cells, as well as the other gas molecules, start to become important.

Photosynthesis combines leaf water and intercellular CO_2 to produce sugars, and this step also involves fractionation of carbon and the water isotopes (Francey & Farquhar 1982; Farquhar et al. 1982; Ehleringer & Monson 1993). The fractionation of carbon is about -27% , so that the ratios in leaf sugars are depleted relative to the intercellular CO_2 . However, if photosynthesis uses CO_2 faster than it can be replenished by stomatal conductance, then the intercellular concentration of CO_2 falls and that CO_2 becomes enriched in ^{13}C . Although the fractionation remains near constant at about -27% , the amount of ^{13}C in leaf sugars will increase as the ratio in the source (intercellular CO_2) increases. The stable carbon isotope ratios of leaf sugars are therefore controlled principally by changes in the isotope ratios of the CO_2 within the leaf which, if the ratios in ambient air do not change, is in turn controlled by the internal concentration of CO_2 . Low internal concentrations lead to higher concentrations of ^{13}C and therefore higher (less negative) ^{13}C values in leaf sugars.

Samples with greater amounts of ^{13}C have less negative $\delta^{13}\text{C}$ values and are referred to as isotopically enriched. Samples with lower ^{13}C contents are more negative and are said to be isotopically depleted.

The isotope ratios of atmospheric CO_2 have changed since industrialization, due to release of isotopically lighter CO_2 from fossil fuel combustion and cement manufacture (McCarroll & Loader 2006). Sugars produced in the leaf may move down the phloem of the stem and be used to produce wood cells, or they may be stored as starch and used later. Where sugars are used directly to produce wood, further fractionations of each of the three elements occurs. The main components of tree ring cells are cellulose and lignin, and these have different ^{13}C values.

It should be clear that fractionation occurs at several steps in the path from CO_2 to wood (Figure 5) and that there is no single environmental factor that completely controls the stable isotope ratios of carbon, oxygen or hydrogen (McCarroll & Loader 2004). Carbon isotopic ratios in tree rings reflect the concentration of CO_2 within the leaves during the growing season and thus the balance between the rate at which CO_2 enters, by stomatal conductance, and the rate at which it is removed by

photosynthesis.

Discrimination (Δ) of carbon isotopes by trees, and other C3 plants (Farquhar et al. 1982), is described by:

$$\Delta \approx a + (b - a)c_i/c_a$$

The constants represent the theoretical value assigned to the isotopic fractionation due to diffusion of CO₂ through stomata to the site of carboxylation ($a \approx 4.4\%$) and the isotopic fractionation when CO₂ is used by the photosynthetic enzyme ribulose-1,5-bisphosphate (Rubisco) ($b \approx 27 - 28\%$) and C_i and C_a are intercellular and ambient CO₂ concentrations. The isotopic ratio of the photosynthetic products is given by:

$$\delta^{13}\text{C}_{\text{plant}} = \delta^{13}\text{C}_{\text{air}} - \Delta$$

Since the fractionation constants (a and b) do not vary in response to climate and the atmospheric concentration of carbon dioxide (C_a) is known, the carbon isotope measurements from tree rings are essentially a record of changes in the internal concentration of CO₂ (C_i). The amount of CO₂ in leaves is regulated by two processes: stomatal conductance and photosynthetic assimilation rate. These processes vary in response to environmental controls such as relative humidity, temperature, and soil moisture deficit such that stable carbon isotope series can capture a range of climatic variables depending upon which factors limit these elements.

Stable carbon isotope measurements from tree rings are thus a proxy for the internal concentration of CO₂ within the leaf space as regulated by those two processes mentioned above. Because the climatic signal is usually dominated by the response of either stomatal conductance or photosynthetic assimilation rate, the same $\delta^{13}\text{C}$ value can arise from different climatic forcing, in different locations (Saurer & Siegwolf 2007).

For example, a sunny growing season, at a cool, moist site, may force an increase in photosynthetic assimilation rate, resulting in a drop in C_i and a larger proportion of ¹³C moving through the pathway to be stored in that year's carbohydrate (Gagen et al. 2011). In such a situation the carbon isotope signal would capture sunny summers as high $\delta^{13}\text{C}$ values. Alternatively, a particularly dry summer, at a more arid site, might force a reduction in stomatal conductance with the same effect on C_i and $\delta^{13}\text{C}$. In this case, high $\delta^{13}\text{C}$ values would record low relative humidity/antecedent precipitation. In both cases, owing to the close association typically observed between these meteorological variables, it is likely that $\delta^{13}\text{C}$ would also correlate with summer temperature.

Variation in intrinsic water use efficiency (iWUE) estimated as the ratio between photosynthesis and stomatal conductance is recorded in the variation of the carbon isotope discrimination $\Delta^{13}\text{C}$ of

the annual growth rings that are laid down during each growing season e.g. (Duquesnay et al. 1998). Long-term changes in the gas exchange metabolism of established trees will be recorded in the variation of this carbon isotope discrimination ($\Delta^{13}\text{C}$), which will give insight into how naturally growing trees respond, or have responded, to increasing atmospheric CO_2 concentrations. Experimental results (Morison 1993; Picon et al. 1996) indicate that plants are able to increase their iWUE as CO_2 levels rise. However, short term experimental results from studies of plants in growth chambers, may not be reliable to predict the behaviour of mature trees growing in their natural environment, as trees are able to adjust their physiological response over time to gradually increasing CO_2 concentrations.

Several studies of this nature on growing trees have revealed that trees vary in their responses to increasing atmospheric CO_2 concentrations (A. Duquesnay et al. 1998; Waterhouse et al. 2004). The apparent reason is that the carbon isotope discrimination ($\Delta^{13}\text{C}$) in tree rings also responds strongly to many silvicultural and environmental variables.

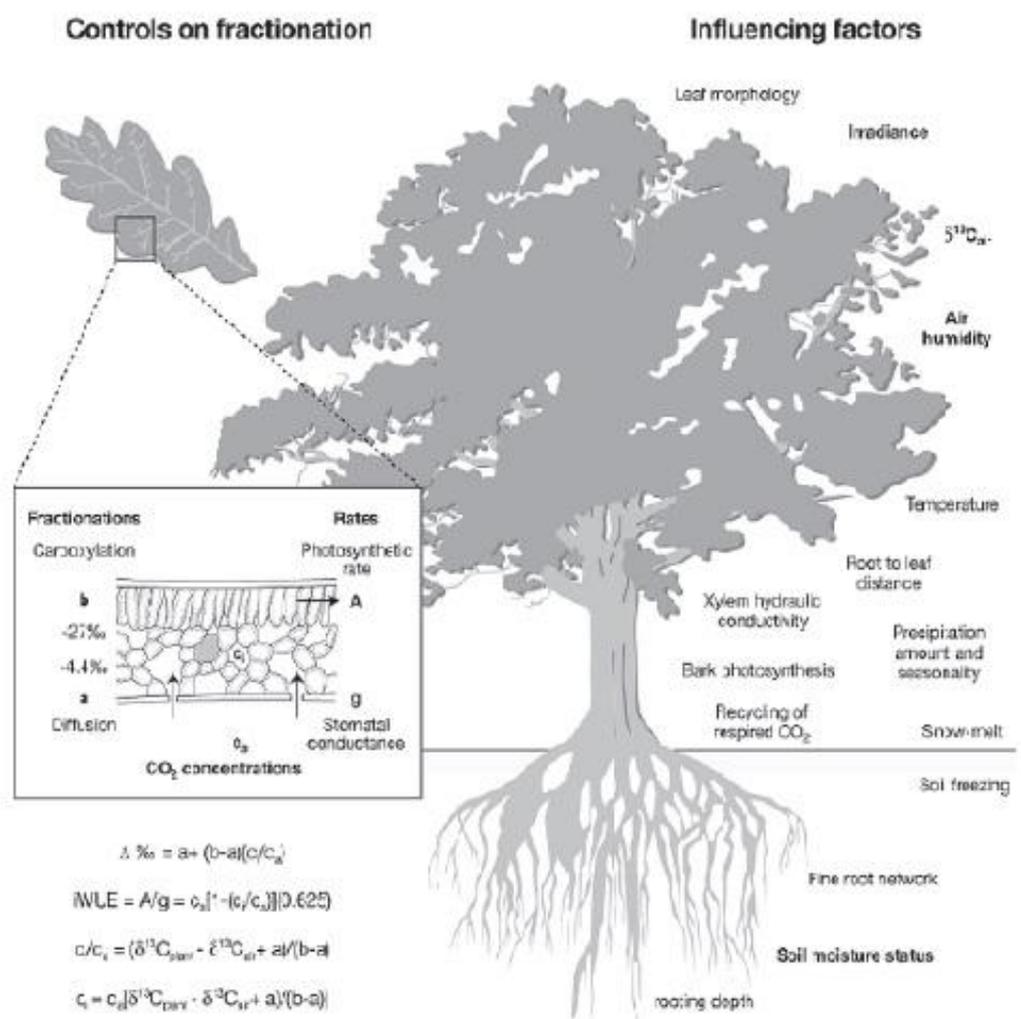


Figure 5: Diagram of broad-leaved tree showing the main controls on fractionation of carbon isotopes and environmental factors that influence them. The equations are described in the text (McCarroll & Loader 2004).

2- Objectives

In this context the objectives are to: (1) assess the effect of climatic parameters on growth and $\Delta^{13}\text{C}$, (2) evaluate the effect of different type of forestry management on tree physiology and growth, (3) assess the response to recent CO₂ concentration increment of trees growing in different sites

Hence, we selected four beech forest along a latitudinal gradient: Central and southern Apennines (Pian di Limina, CAL1 and Collelongo ABR1, Italy), pre-Alps (Pian del Cansiglio VEN1, Italy), and middle European lowlands (Zoolithenhöhle ZOO, Germany) (Figure 6).

3- Material and methods

3-1- Sites description

3-1-1. German site

This forest stand is located in the center of Germany (49° 46' 45"N, 11° 16' 58" E) at 450 m a.s.l.. It is a mixed beech, fir and spruce forest, and as many lowland German forest are essentially man made, following the large scale reforestation throughout the 19th century. The bedrock is limestone and the soil is classified a cambisol with mild pH values.

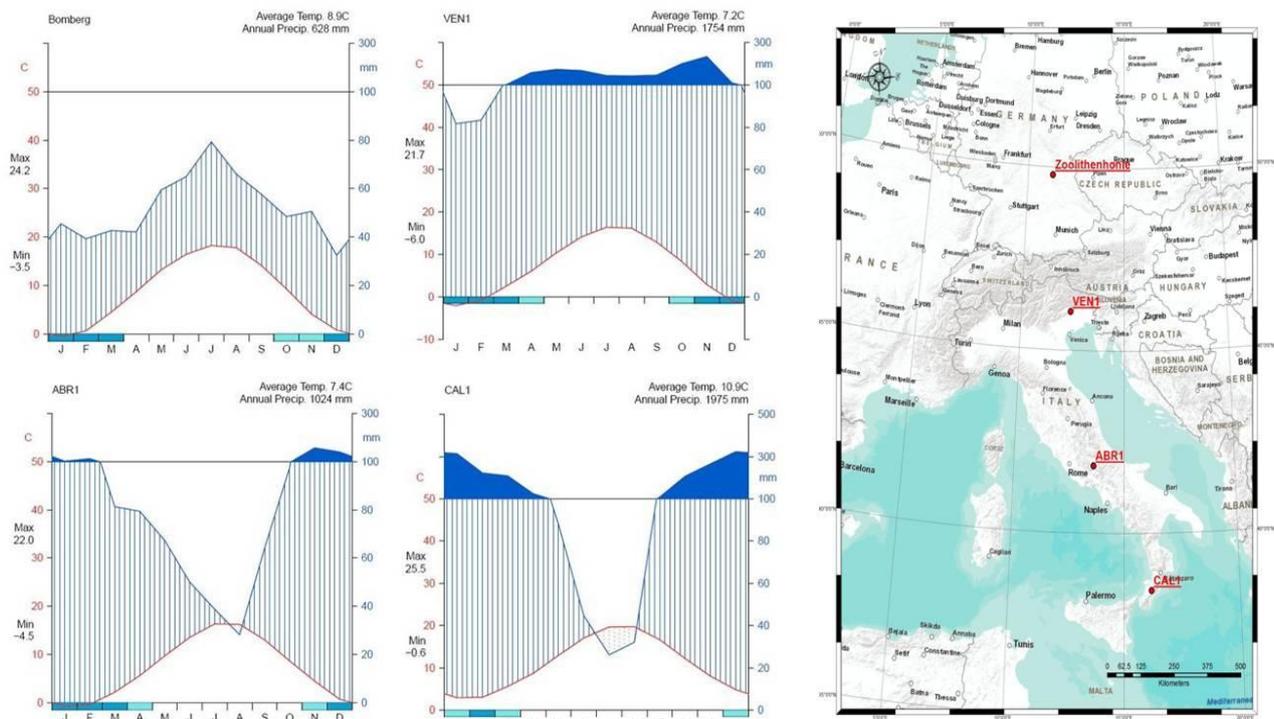


Figure 6. Geographical locations of the study sites in Germany and Italy (right), and Walter climate diagram of each region (left).

3-1-2. Italian Sites

Pian Del Cansiglio (VEN1) is located in the Veneto Region, in Province of Belluno (at the border with the Province of Treviso) (46° 03' 26" N — 12° 01' 56" E) at 1100-1200 m a.s.l . The management is directly carried on by the National Forest Service of Italy. It is included in the Natural Biogenetic Reserve Pian Parrocchia-Campo di Mezzo (established in 1977). The total area is 667 ha and the dominant plant species are *Fagus sylvatica* L. (tree layer) - *Stellaria nemorum* L. - *Athyrium filix-foemina* (L.) Roth (understory layer). The main management type is high forest treated with shelter-wood cuttings(Figure 2). Soil type is classified according to the IUSS working group WBR (2006), as Haplic Luvisols (ACID) on sedimentary rocks (calcareous).

The forest is included in Special Protection Zones (ZPS, 79/409/CEE) and in Sites of Community Importance (SIC, 92/43/CEE). Since 1996, the forest is also included in the Italian network of the forest ecosystem monitoring (CONECOFOR), part of the of the UN/ECE International Cooperative Programme of Forests (ICP Forests, <http://www.icpforest.org>) that, in 2009-2010, was monitored under LIFE+ FutMon (<http://www.futmon.org>).



Figure 7. Studying sites. In the left column, Zoolithenhöhle (up), ABR1 (down) and right column, VEN1 (up), CAL1 (down)

Table 1. Location and main characteristics of the sites and samples as well as mean annual temperature, and total annual precipitation .

Location	Pian di Limina	Collelongo	Pian del Cansiglio	Zoolithenhöhle
Elevation	1100 m	1500m	1100 m	450 m
Slope and Aspect	20° NE	5-10° S	5° W	0°
Soil type	Haplic Podzols	Humic Acrisols	Haplic Luvisols	Cambisol
Mean annual precipitation	1900 mm	1000 mm	1700mm	650mm
Mean annual temprature	11 °C	7 °C	7 °C	8°C
Bioclimatic area	Mediterranean	Mediterranean	Middle Europe	Middle Europe
Forest type	High stand beech forest	High stand beech forest	High stand beech forest	High stand mixed beech forest

Collelongo (ABR1) is located in Collelongo (AQ, central Italy; coordinates 41°50'58"N—13°35'17"E, 1560 m above sea level). In the last inventory (2012), stand density was 755 trees ha⁻¹, basal area was 38.5 m² ha⁻¹ with a mean diameter of 25 cm and a mean height of 21.9 m, the maximum age in 2014 was 120 years. Soil depth exhibits high spatial variability ranging from 40 to 100 cm and is classified, according to the IUSS working group WBR (2006) as a Humic Alisol (Chiti et al. 2010) on Sedimentary Rocks (Calcareous). Dominant plant species in this site are include, *Fagus sylvatica* L. (tree layer) - *Galium odoratum* L. - *Rubus idaeus* L. (understory layer) . The site is equipped with an eddy-covariance tower measuring the exchange of CO₂, H₂O and energy fluxes since 1993 (Valentini et al. 1996) and is part of the CARBOEUROPE, FLUXNET and ILTER international networks.

Pian di Limina (CAL1) is located in the Calabria Region, Province of Reggio Calabria (south Italy; coordinates 38° 25' 38" N —16° 10' 47" E, 1100 m a.s.l above sea level). The management is directly carried on by the National Forest Service of Italy (CFS).

It is an high forest originated from regeneration following the final cutting by the shelter-wood system or clear-cut with reserves, performed at mid 19th century close the end of 2nd World War. Soil type is classified as Haplic Podzols (ACID) on Volcanic rocks (granites). The dominant plant species are *Fagus sylvatica* L. (high tree layer) - *Ilex aquifolium* L. (low tree layer) - *Ruscus aculeatus* L. - *Cyclamen Hederifolium* Aiton - *Vinca minor* L. and *Galium odoratum* L. (understory layer).

3-2- Tree sampling and sample preparation

At each site, co-dominant trees were sampled and two increment cores at breast height were collected from each tree, using an increment borer (Table 2). Five trees without signs of physical damage were selected at each of the four sites to extract cores for the analyses of stable carbon isotopes. Cores were placed into groove boards, wrapped with string to prevent twisting and left to air dry. Furthermore, in the Italian sites we measured the tree height and the crown insertion height. Then we calculated crown depth as the difference between tree height and insertion height. Once

the cores were dry, they were prepared for tree ring analysis using standard dendroecological methods (Stokes & Smiley 1996). After surfacing, tree-ring width was measured at 0.01 mm resolution using a LINTAB (Frank Rinn, Heidelberg, Germany) table fitted with a Leica MS5 stereoscope (Leica Microsystems, Germany) and the TSAP-Win software. Crossdating of all the tree-ring data was verified using the Program COFECHA, which assesses the quality of crossdating and measurement accuracy of tree-ring series using the segmented time-series correlation technique (Holmes 1983).

Table 2: Characteristics of the sampled trees, N_chro number of trees used in site chronology, Range_chr max and minimum sampled trees diameter, N_iso trees used for isotopic analysis, Range_iso max and minimum sampled trees diameter used for isotopic analysis

Site	species	N_chro	Range_chr (cm)	N_iso	Range_iso (cm)
ZOO	<i>F. sylvatica</i>	10	38-100	5	40-46
VEN1	<i>F. sylvatica</i>	18	37-56	5	41-56
ABR1	<i>F. sylvatica</i>	20	25-54	5	40-53
CAL1	<i>F. sylvatica</i>	15	38-57	5	44-52

We only used the successfully cross-dated cores, e.g. those having a significant Gleichläufigkeit (GLK) and TVBP. This is a statistical measure of the year-to-year agreement between the interval trends of the series, based on the sign of agreement. We also used the Student's t test, which determines the degree of correlation between curves (Kaennel & Schweingruber 1995).

To remove age-related growth trends and potential disturbance signals (e.g., due to forest management), the tree ring width (TRW) measurement series were detrended individually using a modified negative exponential method to obtain series of Ring Widths Index (RWI). The "ModNegExp" approach attempts to fit a classic nonlinear model of biological growth of the form $f(t) = a \exp(bt) + k$, where the argument of the function is time (Fritts 2001), with consideration a positive value of slope. Specific site chronologies were produced by averaging the detrended single series using a robust mean (Mosteller & Tukey 1977). Ultimately, standardized tree-ring series were combined to produce a single chronology for each site using program R, dplR package (Bunn 2008).

We used RWI series to analyse the effect of climatic parameters inside each site

3-3- Basal Area Increment (BAI) measurements

In shade tolerant species, growth rates can increase with age after release events (Phipps & Whiton 1988). Hence, the use of Basal Area Increment is especially important because BAI is more correlated to volume increment (Phipps & Whiton 1988).

Tree ring width was converted into tree basal area increment (BAI) according to the following standard formula:

$$BAI = \pi(r_n^2 - r_{(n-1)}^2)$$

where r is the radius of the tree and n is the year of tree-ring formation. To examine the mean growth trend of the trees, BAI for each year was averaged over all individuals.

In shade-tolerant species BAI series of dominant or co-dominant trees in a mature stands typically show a period of early growth suppression (suppression phase) before a rapid increase in annual basal area growth (release phase). BAI may continue to increase in mature healthy mature trees (Duchesne et al. 2002) or stabilize (Foster & LeBlanc 1992).

3-4- Carbon isotope analysis on extracted cellulose

Isotope analysis were made on the 5 trees of every site, selected as previously explained, in the period from 1950 till 2013 according to Boettger et al (. 2007). The procedure to extract cellulose were performed at the Isotope Lab of Erlangen-Nurnberg University.

a. Cutting

For each site, tree-rings were separated using a thin sharp blade into slim shavings of approximately 0.5–0.8 mm and pooled year by year.

b. Cellulose extraction

α -cellulose was extracted to avoid isotope variations due to varying compositions of wood components such as lignin, which show a systematically different isotopic signature.

The wood sample were transferred into a filter funnel. The funnels were loaded with the appropriate sample material, and then plugged into the drainage module. As soon as all openings were closed the module was placed into the water bath, which is then heated up to 60°C. The extraction of cellulose employed acetic acid, sodium chlorite and sodium hydroxide .

The first step consisted of the treatment of wood with a solution of 5% NaOH for 2 h at 60°C, repeated twice, to remove lipids, resins, oil, tannins and hemicelluloses.

In the second step, samples were washed 3 times with hot de-ionized water removing chemical remnants and extracts from each funnels and solution were changed to 7% NaClO₂ and 3–4 mL of acetic acid (pH 4) for a minimum of 36 h that was changed and refilled every 8 hour at 60 °C. Because the solution is only reacting for 10 h. During this stage lignin was digested.

Finally, samples were washed three to four times with boiling distilled water and refilled with NaOH 17% at room temperature for one hour. After that The washing process again simplified 3

times before the samples were flushed with HCL (1%).

c. Homogenization

The resulting α -cellulose samples were transferred from the filter funnels into 2 ml vials and covered with 1 ml de-ionized water. The extracted celluloses, in order to be enough cold during homogenization, were moved to refrigerator for 24 hours and then the samples got homogenized one by one with an ultrasonic device providing a higher sample throughput.

d. Freeze drying

Last step was done After put porous Cap for all eppendorfs in order to not lost the samples and then by put the homogenized samples in freezer till the time that the samples get freeze. This step was taken 3 working day and every time with 3box of samples.

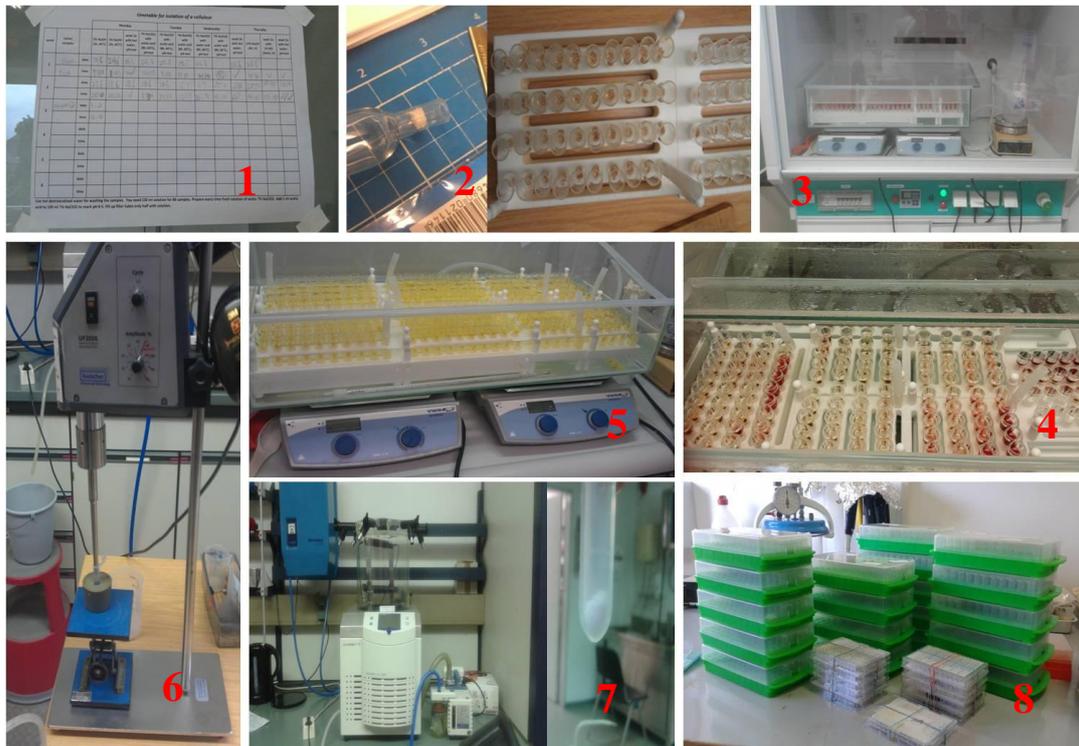


Figure 8. Isotope analysis. In order: cutting (2) , wood treatment with 5% NaOH for 2 h at 60°C (3) , 7% NaClO₂ and 3–4 mL of acetic acid (pH 4) for a minimum of 36 h that was changed and refilled every 8 hour (4) , NaOH 17% room temperature for one hour (5), homogenization (6) , Freeze drying (7) and Weighting (8).

e. Weighting

For $\delta^{13}\text{C}$ analysis, 0.5 to 0.8 mg amount of dry cellulose were weighted and were closed into 3*5 tin capsules.

f. Spectrometer analysis

The carbon dioxide produced from the combustion of organic samples was analysed with a dual inlet mass spectrometer (model SIRA II, VG Isotech, Middlewich, UK) in CNR - IBAF. Organic samples were quantitatively combusted using an elemental analyzer (Model NA 1500, Carlo Erba, Milan, Italy) coupled with the mass spectrometer. Results were expressed as $\delta^{13}\text{C}\%$ corrected by linear regression with international standard.

3-5- $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$ and water use efficiency calculation

Carbon isotope composition of cellulose was calculated according to (Farquhar et al. 1982) as:

$$\delta^{13}\text{C}_{\text{sample}} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{V-PDB}}} - 1 \right] \times 1000 \text{ in per mil}(\text{‰})$$

Which $(^{13}\text{C}/^{12}\text{C})$ are the isotope ratio of samples and of the international standard Vienna-Pee Dee Belemnite (VPDB).

We thereafter calculated $\Delta^{13}\text{C}$, C_i and $i\text{WUE}$. The $\Delta^{13}\text{C}$ (carbon isotopic discrimination) was calculated as

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}) / (1000 - \delta^{13}\text{C}_{\text{plant}}) * 1000$$

following (Farquhar et al. 1982) and using published values for air $\delta^{13}\text{C}$ from ice core measurements, direct atmospheric measurements and inferred from C4 plants (McCarroll & Loader 2004). Since $\Delta^{13}\text{C}$ is related to C_i (intercellular CO_2 concentration) and C_a (ambient CO_2 concentration) by the following equation:

$$\Delta^{13}\text{C} \text{ ‰} = a + (b - a)(C_i/C_a)$$

where a is the discrimination against $^{13}\text{CO}_2$ during CO_2 diffusion through stomata ($a=4.4\text{‰}$, (Farquhar et al. 1982), b is the discrimination associated with carboxylation ($b=27\text{‰}$, (Farquhar et al. 1982) and given Fick's Law:

$$A = g\text{CO}_2(C_a - C_i)$$

where A is the net photosynthesis, measured as CO_2 uptake, and $g\text{CO}_2$ is the leaf conductance to CO_2 , and given that $g\text{H}_2\text{O}$, the leaf conductance to water vapour is $1.6 g\text{CO}_2$, $\Delta^{13}\text{C}$ can be finally related to the ratio $A/g\text{H}_2\text{O}$ ($i\text{WUE}$)(Osmond et al. 1980) by the following equation:

$$\Delta^{13}\text{C} = a + (b - a)(1 - 1.6A / C_a g\text{H}_2\text{O})$$

we determined the C_i , using the (Francey & Farquhar 1982)'s equation:

$$C_i = C_a (\delta^{13}C_{\text{plant}} - \delta^{13}C_{\text{air}} + a) / (a - b)$$

Finally, we calculated iWUE as,

$$\text{iWUE} = C_a (b - \Delta^{13}C) / (1.6 (b - a))$$

3-6- Meteorological data

Site representative temperature series were constructed starting from the gridded version of the Italian long term climate dataset (Brunetti et al. 2006), which is available from the Institute for Atmospheric Sciences and Climate of the National Research Council of Italy (ISAC/CNR). The grid has 1° resolution, both in latitude and in longitude. It is based on 67 observing stations, interpolated with a Gaussian weighting function constructed to have weight of 0.5 for station distance equal to the mean distance (d) of one grid point to the next. Each grid point record was calculated under one of the following conditions: (i) a minimum of two stations at a distance lower than d or (ii) a minimum of one station at a distance lower than d/2. The grid value computation was then performed considering all stations within a distance of 2d. Grid values were calculated from station anomalies with respect to the 1961–1990 period (Brunetti et al. 2006).

Site-representative precipitation series were constructed with a similar approach and are based on the 111 series of the ISAC/CNR dataset (Brunetti et al. 2006).

Regarding the German site (ZOO) we used data collected by the nearest meteorological station, named Bamberg (49°52'N, 10°54'E), which is at 239 m of elevation. It may be that the study site is a bit cooler and moister (Figure 6).

3-7- Statistical analyses

Regression analyses were used to identify significant relations among the different variables considered in the study, normality was tested using Shapiro - Wilk test and constant variance was tested using Spearman rank correlation.

Correlation were calculated using the Pearson Method and significance was fixed to p-value<0.05.

T-Test and Mann-Whitney Rank Sum Test were used to compare two groups in case of a normal distribution of data and non normal distribution, respectively.

One way ANOVA was used to compare many groups and post hoc Tukey's test to assess differences

among groups.

ANCOVA was used only in case of linear relation between variables to evaluate the different response among the sites. The differences between forest sites were tested by post hoc Tukey test. The relationship of $\Delta^{13}\text{C}$ and the Ring Widths Index (RWI) with climate variables of each site was calculated using bootstrapped correlations, and the statistical significance was fixed at $P \leq 0.05$. Statistical analysis were performed using R 3.0.1 (R Development Core Team 2013) and the packages bootRes (Zang & Biondi 2013) and HH (Heiberger & Holland 2015) and SigmaPlot.

4- Results

4-1-1. Site growth

In ZOO, BAI series typically showed a period of early growth suppression (suppression phase) from 1870 till 1950, when a thinning produced a rapid increase of BAI (release phase). After that growth continued to be stable and this phase represented the maturity phase (till 2013).

Dendrochronological results of VEN1 indicated three phases in forest development (Figure 9.2 A-B); short early growth suppression (suppression phase) from 1870 till 1900 before a rapid increase of growth (release phase) till 1940 and stable maturity phase up to 2013. Since 1970, we observed negative value of the Ring width Index (RWI).

BAI series in Collelongo (Figure 9.3A-B) showed a period of early growth suppression (under Coppice cycle) till 1910 before an increase in growth (release phase) till 1978, effect of the conversion to high stand in 1950's. During maturity phase, BAI continued to be stable but RWI showed a decline.

Pian di Limina is a young beech forest (90 years old) in the south Apennines. The actual forest structure with big trees is the result of the past management (High intensity cutting on wide area).

BAI and RWI series in Pian di Limina forest showed a release phase till 1970 and a not very stable maturity phase up to 2013(Figure 9.4A-B).

Among the samples used for isotopic analysis, we observed a great variability of absolute values of BAI (Figure 10) even though we selected trees with a similar diameter and of the same social class (co-dominant). Furthermore, we calculated the correlation between each tree to assess their accordance. At ZOO all the correlation coefficient were significant and ranging from 0.719 to 0.877 (Pearson method, in all cases p -value <0.01). At VEN1 the correlation coefficient was significant only in 30% of the possible cases with low value ranging from 0.333 to 0.353 (Pearson method, in these cases p -value <0.01). At ABR1 we observed that in 60% of cases the correlation was significant with coefficient ranging from 0.336 to 0.644 (Pearson method, in these cases p -value <0.01). At CAL1 in the 50% of the cases correlation were significant with coefficient ranging from 0.378 to 0.653.

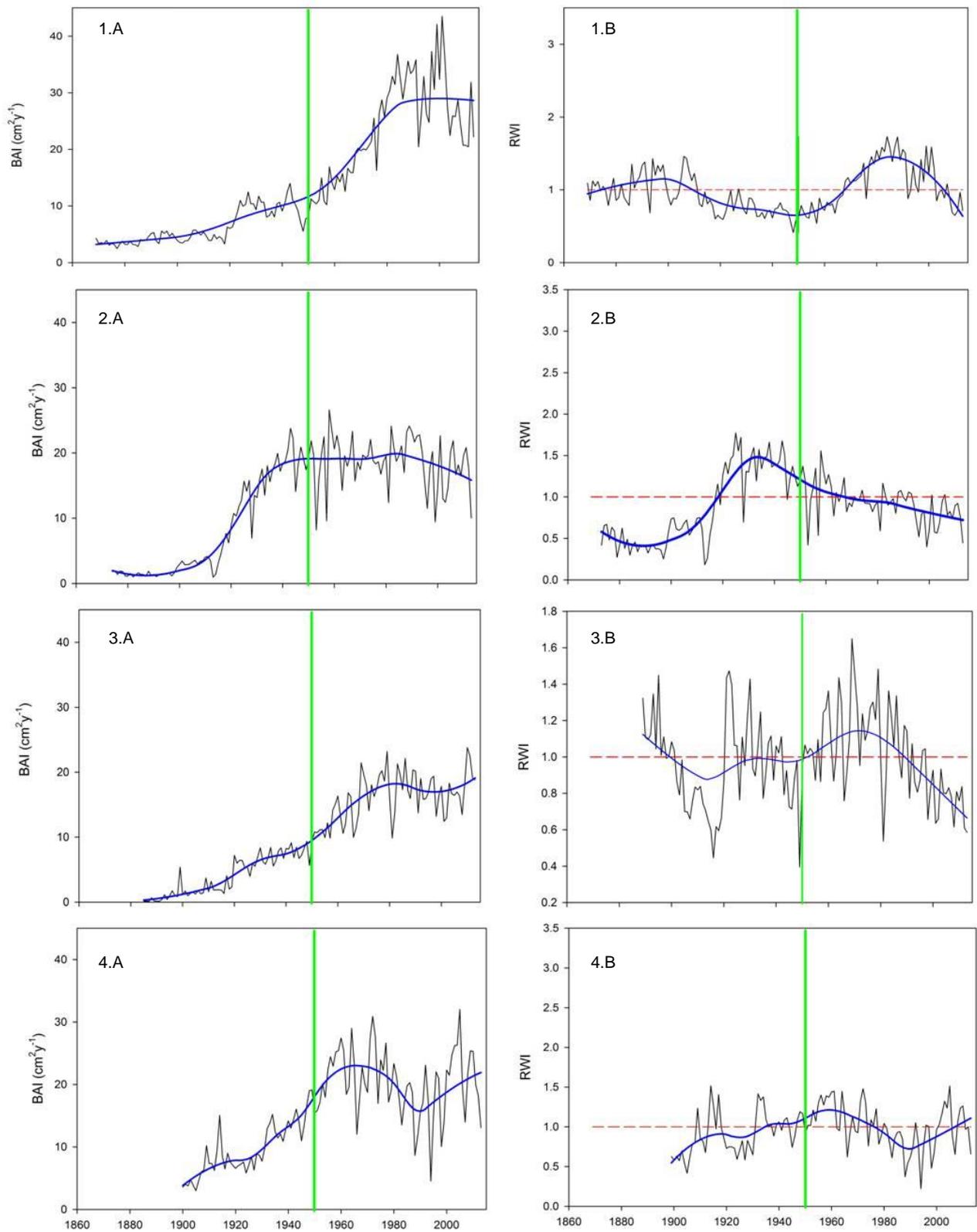


Figure 9: BAI (A) and RWI (B) of Zoolthenhole (1), Pian del Cansiglio (2), Collelongo (3) and Pian della Limina (4). Blue line is 50 years smoothing spline. Green line represent the years in which we started to analyze stable carbon isotope.

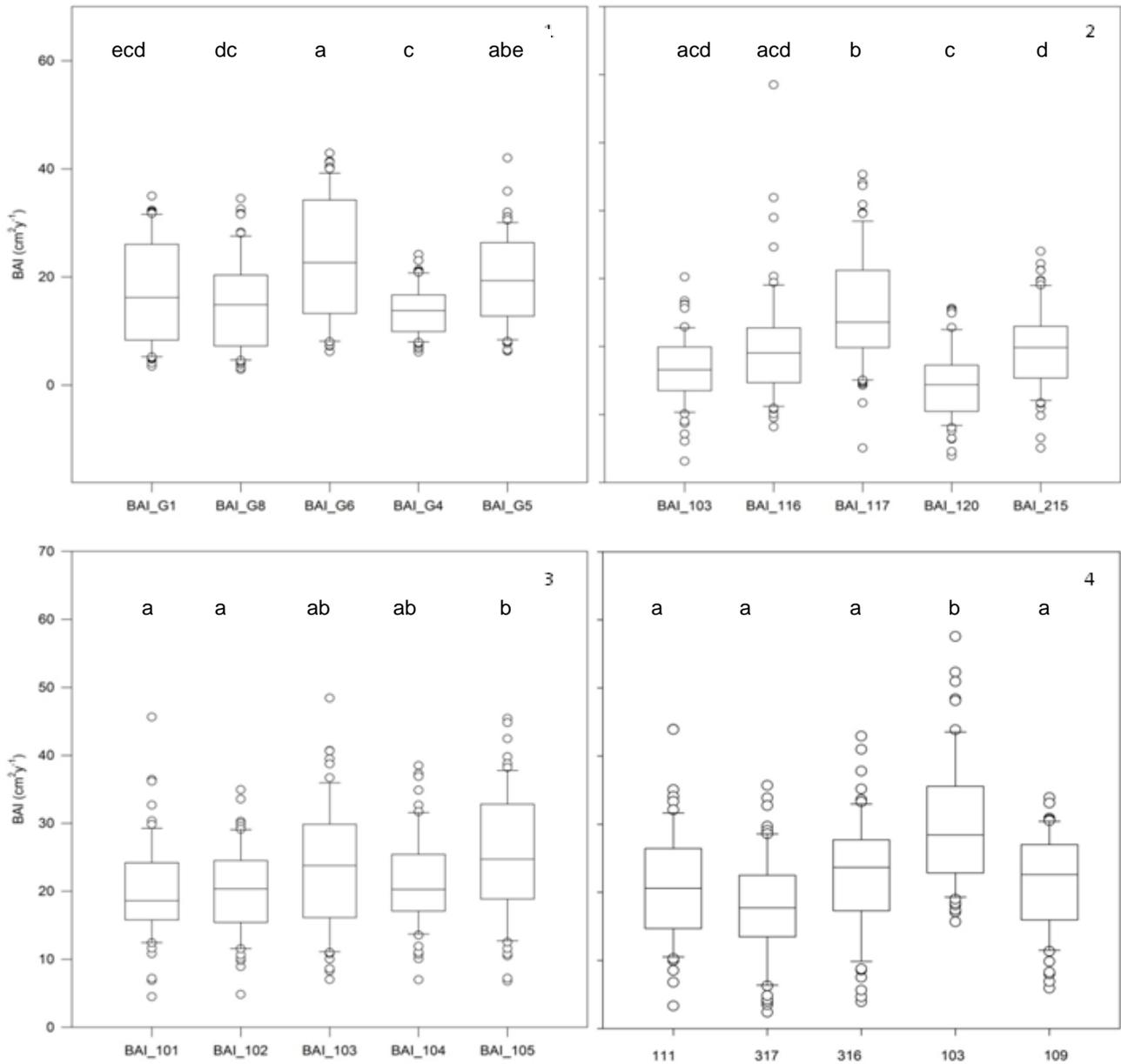


Figure 10: Box plot of BAI of each tree of each site 1: Zoolithenhole, 2: Pian del Consiglio; 3:Collelongo; 4: Pian della Limina. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. Different letters represent differences (ANOVA, Tukey's post hoc test, p-value < 0.05).

4-1-2. Growth and sites climatic conditions

Climatic analysis were performed during the whole study period (1930-2013), two sub- period from 1930-1975 and 1976 - 2013, the last period corresponds also to high frequency of extreme climatic events as drought periods. In the analysis we considered the monthly maximum average temperature and the monthly cumulative precipitations from June of the previous year to September of the current year (Figure 11).

In ZOO, during the whole study period we observed a negative correlation of growth with the temperature of August of the previous year and of April of the current year and a positive

correlation with precipitation of the previous August. Considering the former period (1930-1975), we assessed a positive effect of June precipitation and a negative effect of temperature of the same month. From 1975 the negative correlations with temperature increased and was significant in June and April of the current year and July of the previous year.

In VEN1, correlation between temperature and growth was weak and significant in August of the previous year and March and April of the current year. In the first sub-period (1930-1975) positive correlations of growth with precipitation of the previous winter and with the temperature of June were observed. Since 1975 we observed negative correlations of growth with temperature of April and June and with the precipitation of May.

In ABR1 summer temperatures influenced negatively the growth during the entire period and in the last 37 years. In all the reference period, April temperature was always negatively correlated with the growth. Considering the entire period and the first sub-period, precipitation played a crucial role in the stand growth of the site. We observed a positive correlation of growth with precipitation of current and previous summer and with the precipitation of winter and early spring. More recently, only the precipitations of previous August were positively correlated to growth.

In the southern site (CAL1), we observed negative correlations of growth with summer temperature of current and previous year. Instead a positive effect of precipitation of June was observed during the whole and in the last part of study period.

In the colder sites (ZOO, VEN1 and ABR1), since 1975, we observed negative correlations of growth with the temperature of April and June.

In the whole studying period, the effect of summer temperatures is just the Apennine sites, but in recent times appear similar effect in ZOO and VEN1. In VEN1, during the less warm period growth was positively correlated to June temperature, but in the last period we observed an opposite signal. In addition, CAL1 the southernmost did not show much sensitivity to the summer drought, since it had abundant rainfall.

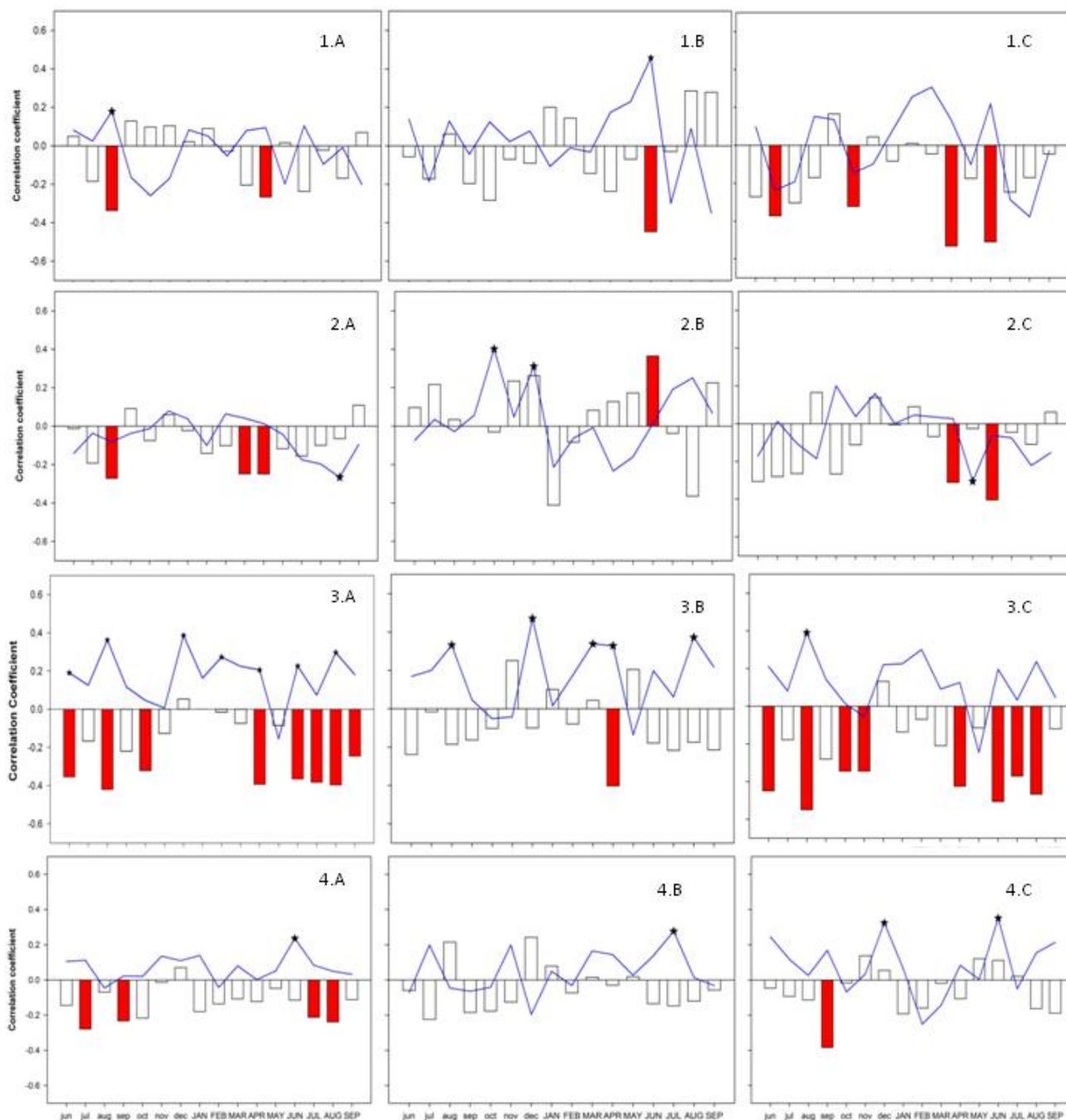


Figure 11: Relation between climatic parameters and RWI. Correlation coefficient were calculated using bootstrapped correlations, and the statistical significance was fixed at $p\text{-value} \leq 0.05$. Numbers represent site:1 = ZOO;2 =VEN1;3 =ABR1:4 =CAL1. Letter are referred to different period: A=1930-2013; B=1930-1975; C=1976-2013. Vertical bars represent correlation coefficients between RWI and monthly max temperature, red ones are significant. Blue line represents correlation coefficients between precipitation and RWI, black stars represents significant coefficients.

4-1-3. Stable carbon Isotopes and intra-site variability

For ZOO, $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ are shown in Figure 12.1.A-B, with mean values of -24.98‰ and 17.07‰, respectively. Considering the period since 1950 to 1975 (Table 3;

Table 4), we assessed $\delta^{13}\text{C}$ equal to -24.98‰ and $\Delta^{13}\text{C}$ as 17.33‰ . Since 1976, which was an extremely famous drought year, the carbon isotopic composition was -25.13‰ and carbon discrimination was 16.87‰ . Comparing the two periods we didn't find significant differences of $\delta^{13}\text{C}$. At the contrary we found significant differences in carbon discrimination (T-test; $t= 3.142$, $p\text{-value}<0.01$).

Furthermore, there were $\Delta^{13}\text{C}$ differences among the trees in this site (Tukey's post hoc test, $p\text{-value} < 0.05$) (

Figure 13.1).

In VEN1 the mean $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ (Figure 12.2.A-B) were -25.27‰ and 17.39‰ , respectively.

Considering the two different periods (Table 3;

Table 4) we found significant differences between $\delta^{13}\text{C}$ (Mann-Whitney Rank Sum Test, p-value<0.01) but no differences in $\Delta^{13}\text{C}$. Concerning the carbon discrimination of each sampled tree we observed only one different from the other (Figure 13.2).

In ABR1, the mean of $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ (Figure 12.3.A-B) were -24.05‰ and 16.14‰, respectively. As in the case of VEN1, the $\delta^{13}\text{C}$ differed in the two periods (Mann-Whitney Rank Sum Test, p-value<0.01) but the $\Delta^{13}\text{C}$ value before and after 1975 was similar. Among the sampled trees we observed 4 similar $\Delta^{13}\text{C}$ and 1 that was similar only to another one (Figure 13.3).

Variations of tree ring $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ during the study period at CAL1 are shown in Figure 12.4 A-B. The mean of the carbon isotopic composition and discrimination are -25.13‰ and 17.20‰, respectively. As in VEN1 and ABR1 we assessed a difference between $\delta^{13}\text{C}$ of the two period (T-test; t= 6.863, p-value<0.01) but not in $\Delta^{13}\text{C}$ (T-test; t=-0.432, p-value=0.667). $\Delta^{13}\text{C}$ values of each tree were very heterogeneous as shown in Figure 13.4.

Table 3: $\delta^{13}\text{C}$ (‰) in different reference periods in each site

Site	Period	mean	std.dev	Max	Min
ZOO	1950-2013	-24.98	0.76	-22.66	-26.79
VEN1	1950-2013	-25.27	0.62	-24.43	-26.79
ABR1	1950-2013	-24.05	0.68	-22.96	-25.43
CAL1	1950-2013	-25.13	0.61	-24.00	-26.46
ZOO	1950-1975	-24.76	0.42	-24.05	-25.64
VEN1	1950-1975	-24.75	0.23	-24.43	-25.35
ABR1	1950-1975	-23.52	0.41	-22.96	-24.55
CAL1	1950-1975	-24.65	0.39	-24.00	-25.37
ZOO	1976-2013	-25.13	0.90	-22.66	-26.79
VEN1	1976-2013	-25.63	0.54	-24.51	-26.79
ABR1	1976-2013	-24.41	0.60	-23.36	-25.43
CAL1	1976-2013	-25.46	0.50	-24.51	-26.46

Table 4: $\Delta^{13}\text{C}$ (‰) in different reference periods in each site

Site	Period	mean	std.dev	Max	Min
ZOO	1950-2013	17.06	0.63	18.29	15.00
VEN1	1950-2013	17.34	0.30	18.01	16.68
ABR1	1950-2013	16.16	0.38	17.01	15.30
CAL1	1950-2013	17.20	0.35	18.04	16.49
ZOO	1950-1975	17.33	0.49	18.29	16.42
VEN1	1950-1975	17.32	0.28	18.00	16.87
ABR1	1950-1975	16.14	0.40	17.01	15.38
CAL1	1950-1975	17.23	0.41	18.04	16.58
ZOO	1976-2013	16.87	0.64	18.07	15.00
VEN1	1976-2013	17.35	0.31	18.01	16.68
ABR1	1976-2013	16.18	0.36	16.91	15.30
CAL1	1976-2013	17.19	0.31	17.91	16.49

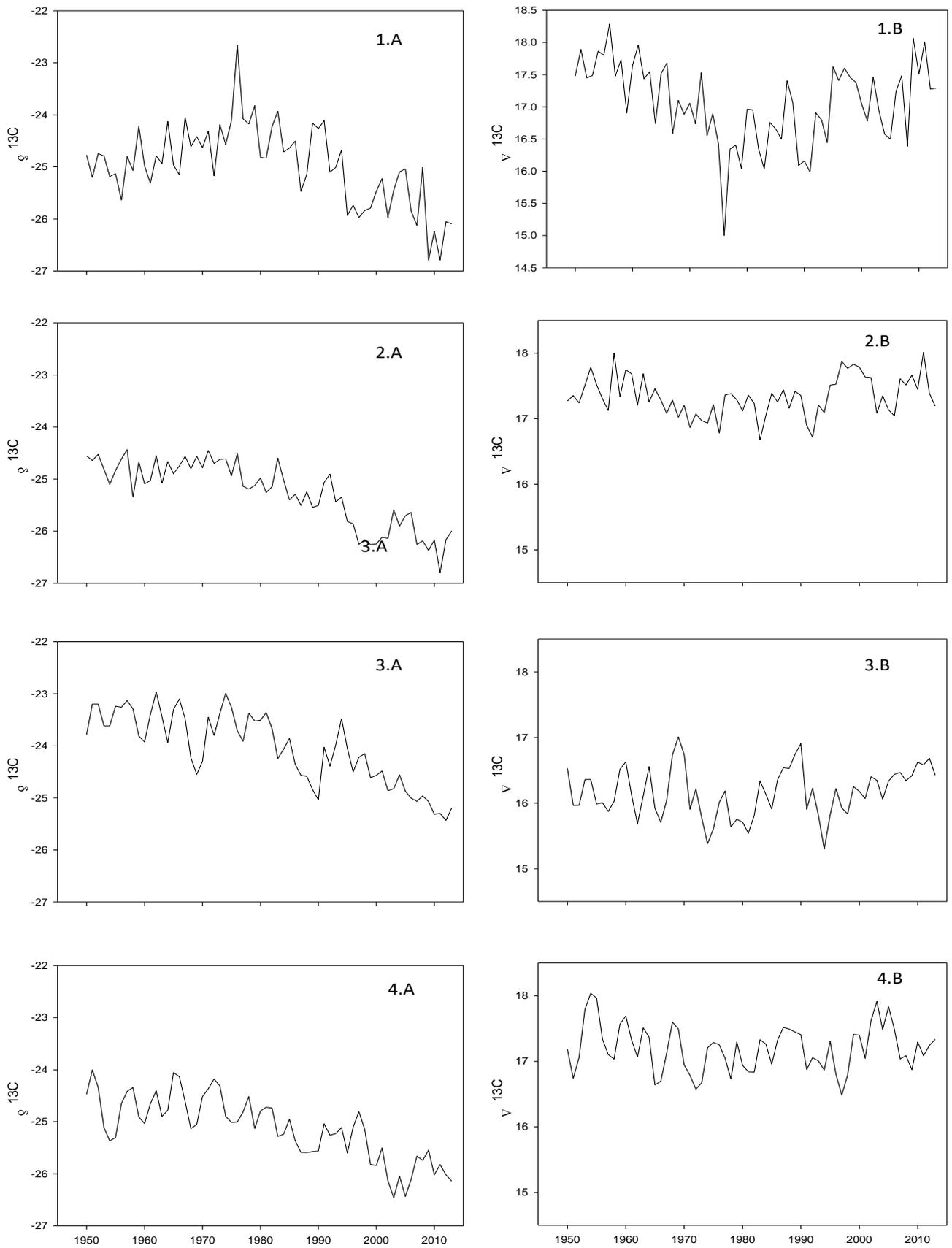


Figure 12: $\delta^{13}\text{C}$ (A) and $\Delta^{13}\text{C}$ (B) in different sites: 1=ZOO; 2=VENI; 3=ABR1; 4= CAL1.

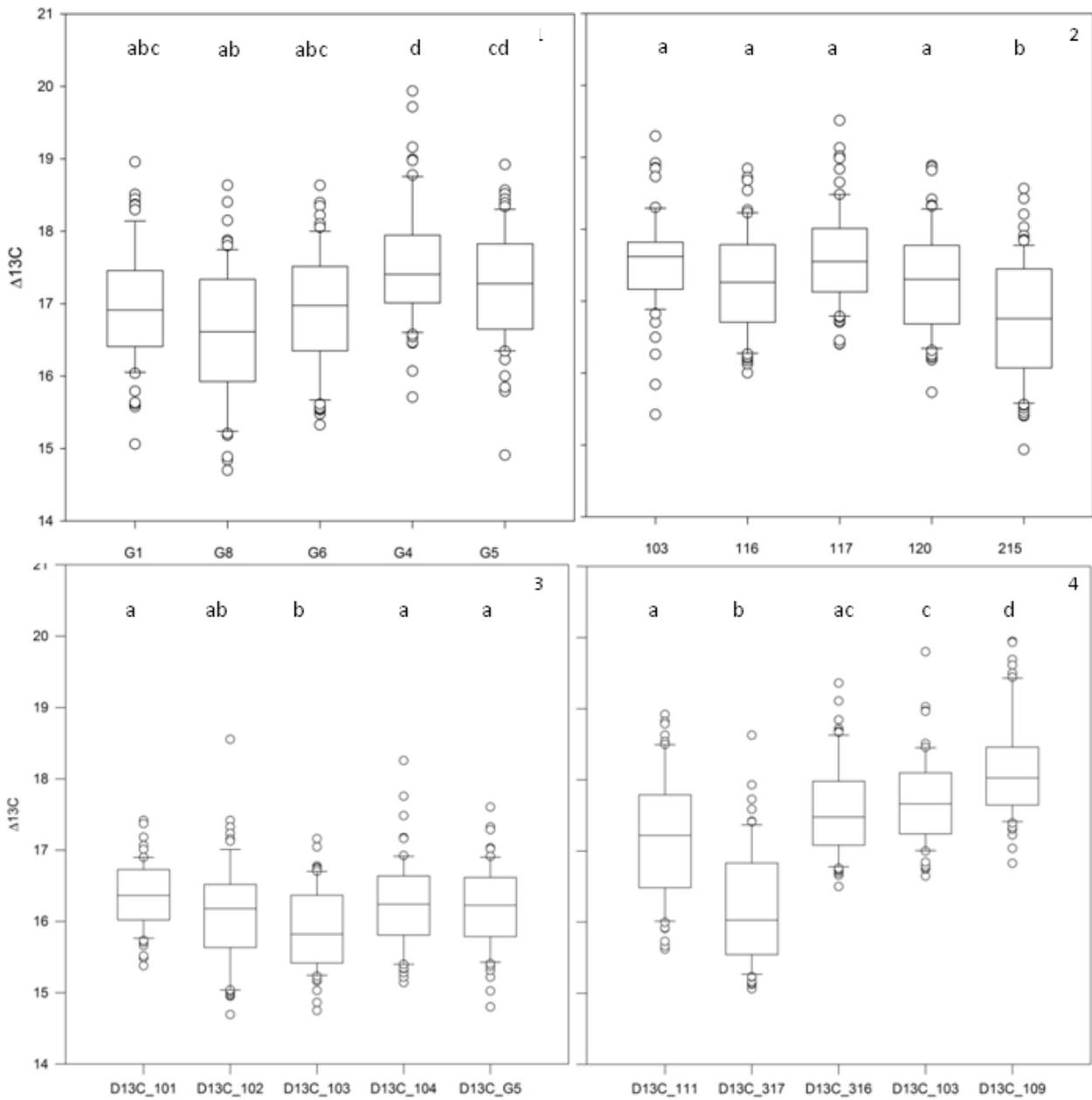


Figure 13: Box plot of $\Delta^{13}\text{C}$ of each different trees of each site 1: Zoolithenhole, 2: Pian del Cansiglio; 3:Collelongo; 4: Pian della Limina. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. Different letters represent differences (ANOVA, Tukey's post hoc test, p-value < 0.05).

4-1-4. Stable carbon Isotopes and sites climatic conditions

To assess the relation between site climatic parameters and growth, also to evaluate the relation of $\Delta^{13}\text{C}$ and site climate, we analyzed the whole study period (1950-2013) and two sub-period 1930-1975 and 1976-2013. In the analysis, we considered the monthly maximum average temperature and the monthly precipitations from June of the previous year to September of the current year.

In Zoolithenhöhle during the entire study period (Figure 14.1.A), carbon isotope discrimination was

positively correlated with temperature of April and precipitation of July and August and negatively with the max temperature of July. In the first period (1950-1975, Figure 14.1.B), carbon discrimination was positively correlated to July precipitation and negatively to max temperature of July and August. In the last period April and July temperatures of the previous year and July precipitations had a positive correlation with $\Delta^{13}\text{C}$, which was negatively correlated to April precipitations.

In VEN1 during the whole study period, $\Delta^{13}\text{C}$ was positively correlated to June and August precipitations and April temperature, at contrary a negative correlation was found with June temperature (Figure 14.2.A). From 1950 to 1975, June temperature and precipitations of the previous winter were instead positively correlated to carbon discrimination (Figure 14.2.B). In the last period precipitations did not have correlation with $\Delta^{13}\text{C}$, but we observed positive correlations with spring temperature and negative one with July one (Figure 14.2.B).

In ABR1, there were positive effects of maximum temperature in April and May of the current year and July and October of the previous year on carbon isotope discrimination. Also precipitations were correlated to $\Delta^{13}\text{C}$, positively those of June of the current and previous year and July of the previous year, while $\Delta^{13}\text{C}$ was negatively correlated to precipitations of April of the current year and October of the previous year (Figure 14.3.A). In the first sub-period, we observed positive correlations with precipitations of June of the current and previous year and December of the previous year. In this period, current August and previous June temperatures were negatively related to carbon discrimination, while October ones had a positive correlation (Figure 14.3.B). In the last period since 1976 (Figure 14.3.B), we observed positive correlations of $\Delta^{13}\text{C}$ with spring temperature and those of July and October of the previous year. The precipitation of July and October of the previous year had a positive and negative correlations with carbon discrimination, respectively.

In the southern site, during the whole study period there was a significant negative effect of temperature of January on carbon isotope discrimination (Figure 14.4.A). The same correlation was found in the first sub-period (1950-1975, Figure 14.4.B), besides the correlation with previous November and September precipitation which were positive and negative, respectively. In the last period (1976-2013, Figure 14.4.B), $\Delta^{13}\text{C}$ was positively correlated to current and previous July and previous August temperature.

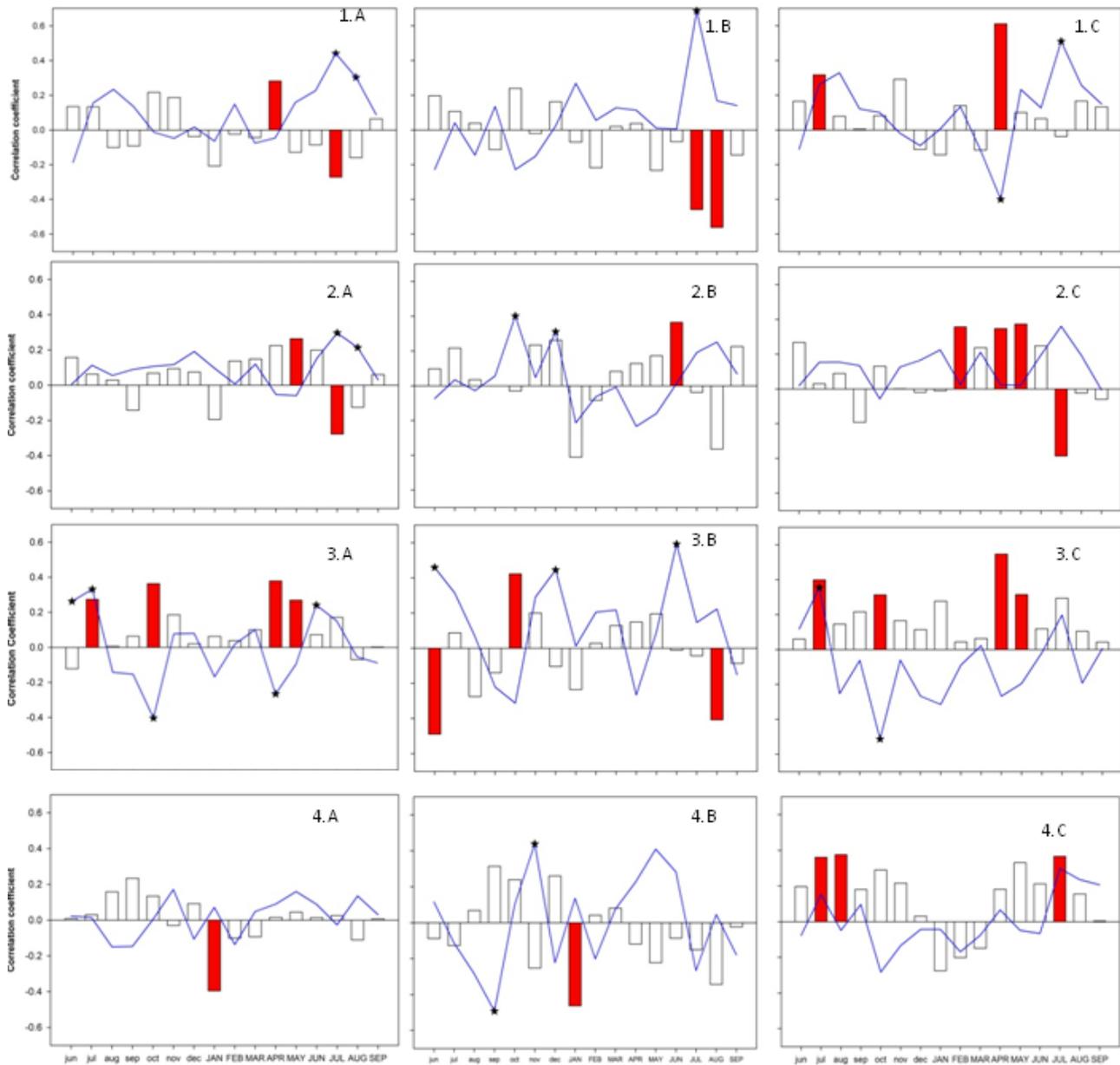


Figure 14: Relation between climatic parameters and $\Delta^{13}\text{C}$. Correlation coefficient were calculated using bootstrapped correlations, and the statistical significance was fixed at $p\text{-value} \leq 0.05$. Numbers represent site: 1 = ZOO; 2 = VEN; 3 = ABR; 4 = CAL. Letter are referred to different period: A=1950-2013; B=1950-1975; C=1976-2013. Vertical bars represent correlation coefficients between RWI and monthly max temperature, red ones are significant. Blue line represents correlation coefficients between precipitation and RWI, black stars represents significant coefficients.

4-1-5. Effect of forest management on tree physiology and growth

4-1-5.1. Zoolithenhole

The growth of ZOO was the results of the irregular shelterwood system which is named 'Femelschlag' in German (Figure 9.1A – B). The cycle of this management system is usually 120-140 years. The sampled trees were 136 ± 7 years old, so they are the seedlings established at the end of the previous rotation.

According to site chronology, we divided the isotope series in two periods (1950-1980 and 1981-2013) and we calculated the correlation with basal area increment of each tree (Table 5) and a mean correlation for the site. The correlation between growth and $\Delta^{13}\text{C}$ was not constant in all the samples trees. Considering the whole studied period we observed three significant correlation in trees G1, G8 and G6. Separating the two phase of the stand, during the release phase this correlation was significant for four trees (G1, G8, G6 and G5). During maturity phase, correlation coefficient was lower than in release phase in most trees except for tree G4. Furthermore, we observed always a negative correlation coefficient in all the phases (Table 5).

Table 5: Correlation (Pearson method) between BAI and $\Delta^{13}\text{C}$. n .years represents number of years considered in the analysis

$\Delta^{13}\text{C}/\text{BAI}$	1950-2013			1950-1980			1981-2013		
	release and maturity phase			release phase			maturity phase		
	r	p-value	n. years	r	p-value	n. years	r	p-value	n. years
G1	-0.601	<0.01	64	-0.528	<0.01	31	-0.388	0.03	33
G8	-0.428	<0.01	64	-0.599	<0.01	31	-0.117	0.518	33
G6	-0.598	<0.01	64	-0.622	<0.01	31	-0.476	<0.01	33
G4	-0.15	0.237	64	-0.225	0.224	31	-0.513	<0.01	33
G5	0.021	0.867	64	-0.493	<0.01	31	-0.278	0.118	33
Site	-0.381	<0.01	64	-0.563	<0.01	31	-0.276	0.12	33

4-1-5.2. Cansiglio Forest

Cansiglio Forest is the results of the applied uniform shelterwood system with a turn of 120-140 years, which represented the typical management method of this area. As in Zoolithenhöhle, the sampled trees were the seedlings established at the end of the previous rotation. Overall, in Cansiglio forest there was a very low thinning every 20 years (the last in 1970, data by National Forest Service).

According to site chronology and managers information, we considered two periods in maturity phase before and after 1970, which corresponds to the last thinning, and we calculated the correlation between basal area increment and $\Delta^{13}\text{C}$ of each tree and site in these two stages (Table 6). The correlation between growth and isotope discrimination was not constant in all the samples trees. Through the whole maturity phase, we observed three significant correlation in trees Can2(116), Can4(120) and Can5(215). But separating maturation phase into two stages (before and after 1970) we observed significant correlation in four trees; Can1(103), Can2(116), Can4(120) and Can5(215). However, in Cansiglio there was a significant correlation between BAI and $\Delta^{13}\text{C}$ in last 43 years of maturity.

Table 6: Correlation (Pearson method) between BAI and $\Delta^{13}\text{C}$. n .years represents number of years considered in the analysis

	1950-2013			1950-1970			1971-2013		
	Whole maturity phase			Maturity phase(before 1970)			Maturity phase(after 1970)		
$\Delta^{13}\text{C}/\text{BAI}$	r	p-value	n. years	r	p-value	n. years	r	p-value	n. years
Can1(103)	-0.172	0.174	64	0.280	0.219	21	-0.449	<0.01	43
Can2(116)	-0.440	<0.01	64	0.061	0.793	21	-0.521	<0.01	43
Can3(117)	0.176	0.129	64	0.224	0.304	21	0.075	0.588	43
Can4(120)	-0.471	<0.01	64	-0.333	0.141	21	-0.504	<0.01	43
Can5(215)	-0.240	0.037	64	0.030	0.893	21	-0.304	<0.01	43
Site	-0.177	0.162	64	0.146	0.527	21	-0.340	0.020	43

4-1-5.3. Collelongo

Collelongo stand is a transition high forest composed by the oldest standards and the best stools of the coppice released during the conversion. As many beech stands in the Central Apennines, this forest has been managed under the coppice system up to mid 20th century. Following the suspension of fuel wood harvesting, the conversion into high forest has been undertaken.

The correlation between growth and $\Delta^{13}\text{C}$ was not steady in all the samples trees. In the whole studied period we observed three significant correlation in trees Col1(101), Col3(103) and Col5(105) (Table 7). Separating two phase of the stand, during the release phase this correlation was significant for three trees (Col1, Col2 and Col4). Through maturity phase, the correlation coefficient was significant for tree Col1, Col3 and Col5. At the stand level, there was a significant correlation between BAI and $\Delta^{13}\text{C}$ during the whole study period and the maturity phase of the trees (Table 7).

In this site, we observed different response related to tree origins (old standard and best stools).

Considering the oldest standard we found a significant positive correlation between BAI and $\Delta^{13}\text{C}$ during the whole study period (1950-2013, $r= 0.389$, $p\text{-value} <0.01$) increasing during the maturity phase (1978 - 2013, $r= 0.639$, $p\text{-value} <0.01$) without finding any relation in the period after the conversion (1950-1978, $r= 0.074$, $p\text{-value}= 0.691$). In case of the best stools we found a negative correlation only after the conversion (1950-1978, $r= -0.614$, $p\text{-value} <0.01$) (Figure 15).

Table 7: Correlation (Pearson method) between BAI and $\Delta^{13}\text{C}$. n .years represents number of years considered in the analysis

	1950-2013			1950-1978			1979-2013		
	Release and maturity phase			Release phase_Conversion to high stand			Maturity phase		
$\Delta^{13}\text{C}/\text{BAI}$	r	p-value	n. years	r	p-value	n. years	r	p-value	n. years
Col1(101)	-0.337	<0.01	64	-0.488	<0.01	26	-0.317	0.059	38
Col2(102)	-0.11	0.39	64	-0.438	0.025	26	-0.011	0.948	38
Col3(103)	0.481	<0.01	64	0.043	0.831	26	0.53	<0.01	38
Col4(104)	0.136	0.283	64	0.456	0.019	26	-0.09	0.604	38
Col5(105)	0.291	0.021	64	0.228	0.262	26	0.608	<0.01	38
Site	0.323	<0.01	64	0.176	0.362	26	0.445	<0.01	38

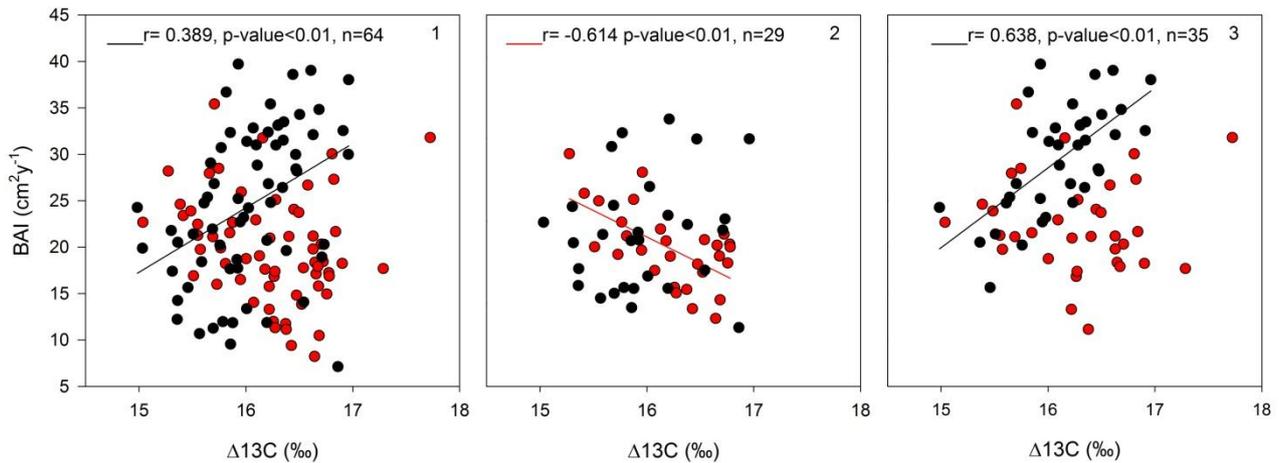


Figure 15: Relation between mean values $\Delta^{13}\text{C}$ of best stools (red point, tree 101 and 102) and oldest standard (black point, trees 103,104 and 105) with BAI during the whole study period (1), from 1950 to 1978 (2) and after 1978 (3).

4-1-5.4. Pian della Limina

The active management of state property until the first half of the twentieth century was based on high intensity cuttings over a wide area. Consequence of this management was a general impoverishment of forest. In the second half of the last century there was a contraction of forest utilization. Since this period, in public forests, the management system turned to classical silviculture (selection system) applied to small area (Marziliano et al. 2011).

According to BAI series of this study site, we considered three periods and for each one we calculated the correlation between BAI and $\Delta^{13}\text{C}$ of single trees. At tree level we did not find any significant correlation between the two variables. However at stand level we found correlations in the period between 1971 and 1993 periods ($r = -0.591$, $p\text{-value} < 0.01$) and since 1994 till 2013 ($r = 0.456$, $p\text{-value} = 0.04$)

Table 8: Correlation (Pearson method) between BAI and $\Delta^{13}\text{C}$. n .years represents number of years considered in the analysis

$\Delta^{13}\text{C}/\text{BAI}$	1950-2013			1950-1970			1971-1993			1994-2013		
	r	p-value	n. years									
Cal1(111)	-0.0309	0.808	64	0.266	0.244	19	0.0406	0.854	25	0.365	0.113	20
Cal2(317)	-0.0435	0.735	64	0.376	0.102	19	-0.132	0.547	25	-0.328	0.159	20
Cal3(316)	-0.0668	0.6	64	-0.16	0.489	19	-0.0553	0.802	25	0.123	0.605	20
Cal4(103)	0.0967	0.447	64	0.12	0.605	19	-0.272	0.21	25	-0.108	0.649	20
Cal5(109)	0.195	0.123	64	-0.0254	0.913	19	-0.181	0.409	25	0.35	0.13	20
Site	0.115	0.366	64	0.331	0.166	19	-0.591	<0.01	25	0.456	0.04	20

4-2- Relation between tree crown characteristics with $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$ and iWUE

Results of past forest management were the tree characteristics. In the Italian sites (Pian del Cansiglio, Collelongo and Pian di Limina) we observed a great effect of crown depth on $\delta^{13}\text{C}$ that explained 77% of the variability of $\delta^{13}\text{C}$ (Rsqr=0.777, p-value<0.01, Figure 16A). Trees with deeper crown showed in more negative values of $\delta^{13}\text{C}$. Crown characteristics affected $\Delta^{13}\text{C}$ (Rsqr=0.773, p-value=0.01, Figure 16.B). Intrinsic water use efficiency was significant related to crown depth only in CAL1 (Rsqr=0.702, p-value=0.04, Figure 16.C) and pooling all the sampled trees (Rsqr=0.553, p-value=0.01, Figure 16.C).

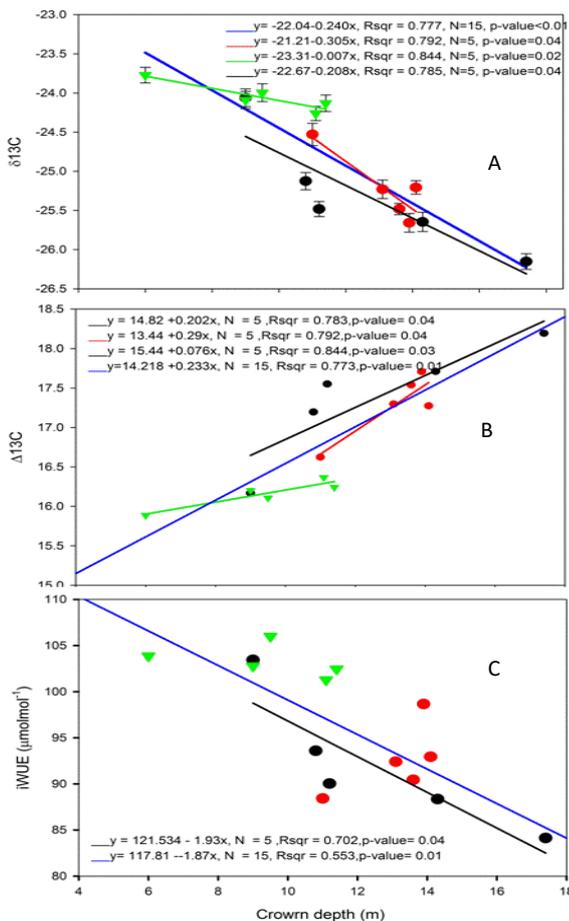


Figure 16: Relation of crown depth with mean values of $\delta^{13}\text{C}$ (A), $\Delta^{13}\text{C}$ (B) and iWUE (C). Green triangle:ABR1; Red point: VEN1; Black point: CAL1; each point represents a sampled trees; The significant relation are represented by lines with the corresponding equation, coefficient of correlation and statistical significance. The blue line is the regression considering all the 15 trees.

4-3- Temporal trends of $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , iWUE and BAI

To evaluate trend in the variables $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , iWUE and BAI we considered first the whole period (1950-2013) then two periods corresponding to the low temperature increase period (1950-1975) and high temperature increase period (1975-2013), during this last period almost all the trees were in the maturity phase.

In the whole study period (i.e. from 1950 to 2013), the $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, the C_i and the iWUE were different in the Collelongo site (ABR1) respect to the other sites (ANOVA, $P < 0.05$; post hoc Tukey's test) (Figure 17). In this site we measured the less negative value of $\delta^{13}\text{C}$, consequently we estimated lower carbon discrimination ($\Delta^{13}\text{C}$) and internal concentration (C_i) and a higher intrinsic water use efficiency (iWUE). Differences in BAI were not found among the sites.

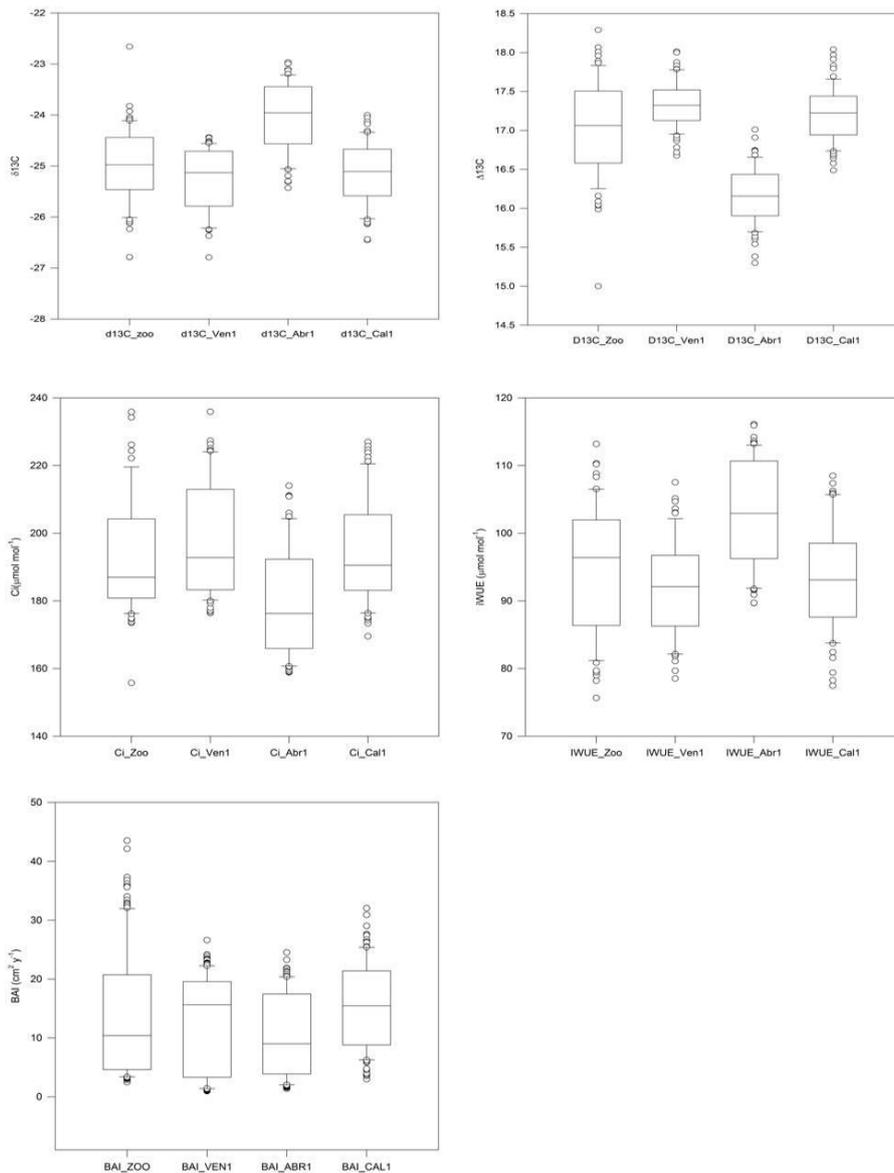


Figure 17: $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , intrinsic water use efficiency (iWUE) and Basal area increment (BAI) of beech trees in four forest latitudinal sites during the period 1950-2013. significantly different values between Abr1 and the other sites ($P < 0.05$; post hoc Tukey's method after ANOVA) except in BAI series.

In all our study sites we observed a quadratic relation between $\delta^{13}\text{C}$ and time, but the shape of the regression is different between Zoolithenhöhle and the Italian sites (Figure 18.A.1). In the period before 1975 only the German site showed a significant trend (Figure 18.A.2), with values ranging from -24.047 to -25.637 ‰. In the second phase (1976-2013) all the sites showed high variation of $\delta^{13}\text{C}$ and significant relations between $\delta^{13}\text{C}$ and time (Figure 18.A.3). Comparing the different response among the sites we observed that ZOO was different from CAL1 (ANCOVA, Tukey Contrasts, p-value =0.001) and VEN1 (ANCOVA, Tukey Contrasts, p-value =0.03).

Carbon discrimination ($\Delta^{13}\text{C}$) (Figure 18.B.1) showed a parabolic pattern only in Germany showing initially a decrease till 1975 then an increase phase till nowadays. Significant decrease of $\Delta^{13}\text{C}$ were found in ZOO and VEN1 (Figure 18.B.2). During the post maturity phases we observed a positive trend of $\Delta^{13}\text{C}$ in ZOO, VEN1 and ABR1. Furthermore we observed a different response between ZOO and VEN1 (ANCOVA, Tukey Contrasts, p-value =0.01).

C_i increased significantly in all four sites (Figure 18.C.1) with a quadratic relation between C_i and time. In the pre-maturity phase only VEN1 and ABR1 showed a significant increase of the value (Figure 18.C.2) while during the post-maturity phase all sites showed a significant increment (Figure 18.C.3). The variation of C_i from 1975 to 2013 was different only between ZOO and CAL1 (ANCOVA, Tukey Contrasts, p-value <0.01).

In all the four sites the intrinsic water use efficiency (iWUE) was increasing but showing a different pattern between the German site (quadratic relation), where iWUE tend to stabilize, and the Italian ones (linear relation) (Figure 18.D.1). During post maturation phase, we observed a different variation of iWUE between CAL1 and ZOO (ANCOVA, Tukey Contrasts, p-value <0.01).

Among the sites only ABR1 presents a clear pattern of the BAI (Figure 18.E.1), showing before an initial increase (Figure 18.E.2) and after 1975 a stable trend (Figure 18.E.3). Furthermore, we observed significant increment trend of BAI in the pre maturity phase of ZOO (Figure 18.E.2).

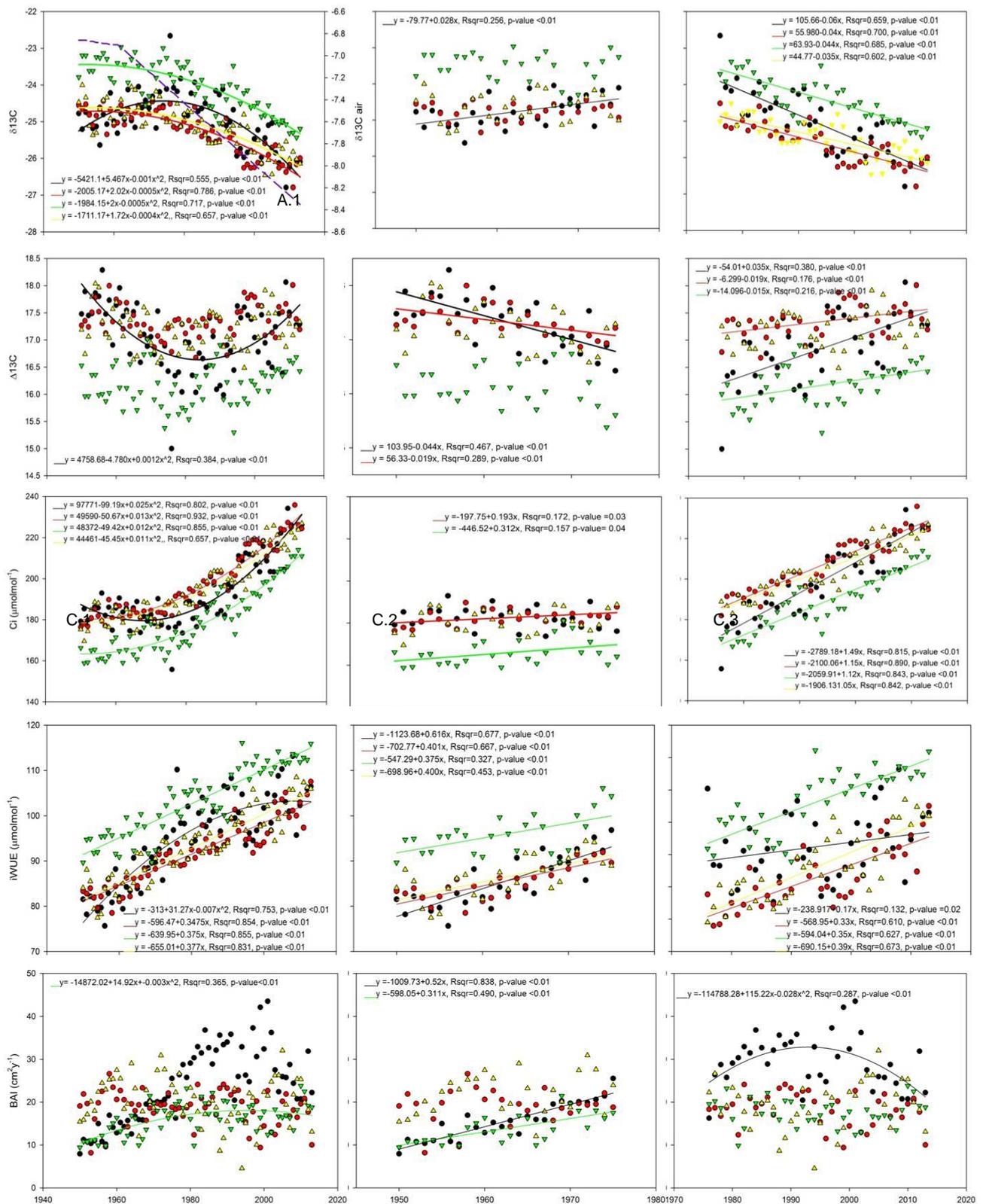


Figure 18. Chronologies of $\delta^{13}C$ (A), $\Delta^{13}C$ (B), Ci (C), iWUE (D) and BAI (E) at different reference periods: 1: 1950-2013 (1), 1950-1975 (2) and 1976-2013 (3). In A.1. violet line represents $\delta^{13}C$ of the air. Black circles = ZOO, red circles= VEN1, green triangles= ABR1 and yellow triangles =CAL1. The significant trends are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

4-4- $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , $i\text{WUE}$ and BAI relationships with atmospheric CO_2 concentration

Relation of $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , $i\text{WUE}$ and BAI with atmospheric CO_2 concentration were analyzed and results are shown in Figure 19.

Also this analysis was performed considering pre (1950-1975) and post-maturation phases (1976-2013) which corresponds also to high frequency of extreme climatic events as drought periods .

In all the sites we observed a clear relation between atmospheric CO_2 concentration and $\delta^{13}\text{C}$. However we observed different trend in ZOO from the other sites (Figure 19.A.1). Infact, only in this site we observed an increase of $\delta^{13}\text{C}$ in the pre maturation phase (Figure 19.A.2). Instead, during post-maturation phase all the sites showed a decreasing trend (Figure 19.A.3). The ZOO response at the variation of atmospheric CO_2 concentration was different between VEN1 (ANCOVA, Tukey Contrasts, p-value = 0.01) and CAL1 (ANCOVA, Tukey Contrasts, p-value <0.01).

Carbon isotopic discrimination showed parabolic pattern in ZOO and VEN (Figure 19.B.1). Initially both sites showed a decreasing trend (Figure 19.B.2), after that $\Delta^{13}\text{C}$ increased. During the post maturity phase, no relation was found in CAL1 and the response of $\Delta^{13}\text{C}$ to atmospheric CO_2 concentration was different between ZOO and VEN1 (ANCOVA, Tukey Contrasts, p-value = 0.01) (Figure 19.B.3).

During the whole study period, C_i increased in all the sites (Figure 19.C.1), but only in VEN1 was significant during the pre maturity phase (Figure 19.C.2). At all sites, from 1975 we observed a C_i increase and a difference in the response of ZOO from VEN1 (ANCOVA, Tukey Contrasts, p-value = 0.02) and CAL1 (ANCOVA, Tukey Contrasts, p-value = 0.001).

Intrinsic water use efficiency presented a different pattern in the German compared to the Italian sites (Figure 19.D.1). In ZOO, $i\text{WUE}$ increased faster in the pre maturity phase (Figure 19.D.2) than during the post maturity phase (Figure 19.D.3). Furthermore the response of $i\text{WUE}$ to variation of atmospheric CO_2 concentration was different between ZOO and ABR1 during pre maturity phase (ANCOVA, Tukey Contrasts, p-value = 0.01). However, during the last period ZOO and CAL1 (ANCOVA, Tukey Contrasts, p-value = 0.001) and ZOO and VEN1 (ANCOVA, Tukey Contrasts, p-value = 0.02) showed a different response to CO_2 concentration.

Variation of atmospheric CO_2 concentration had a positive effect on BAI of ZOO and ABR1 (Figure 19.E.1 and E.2) during pre-maturity phase.

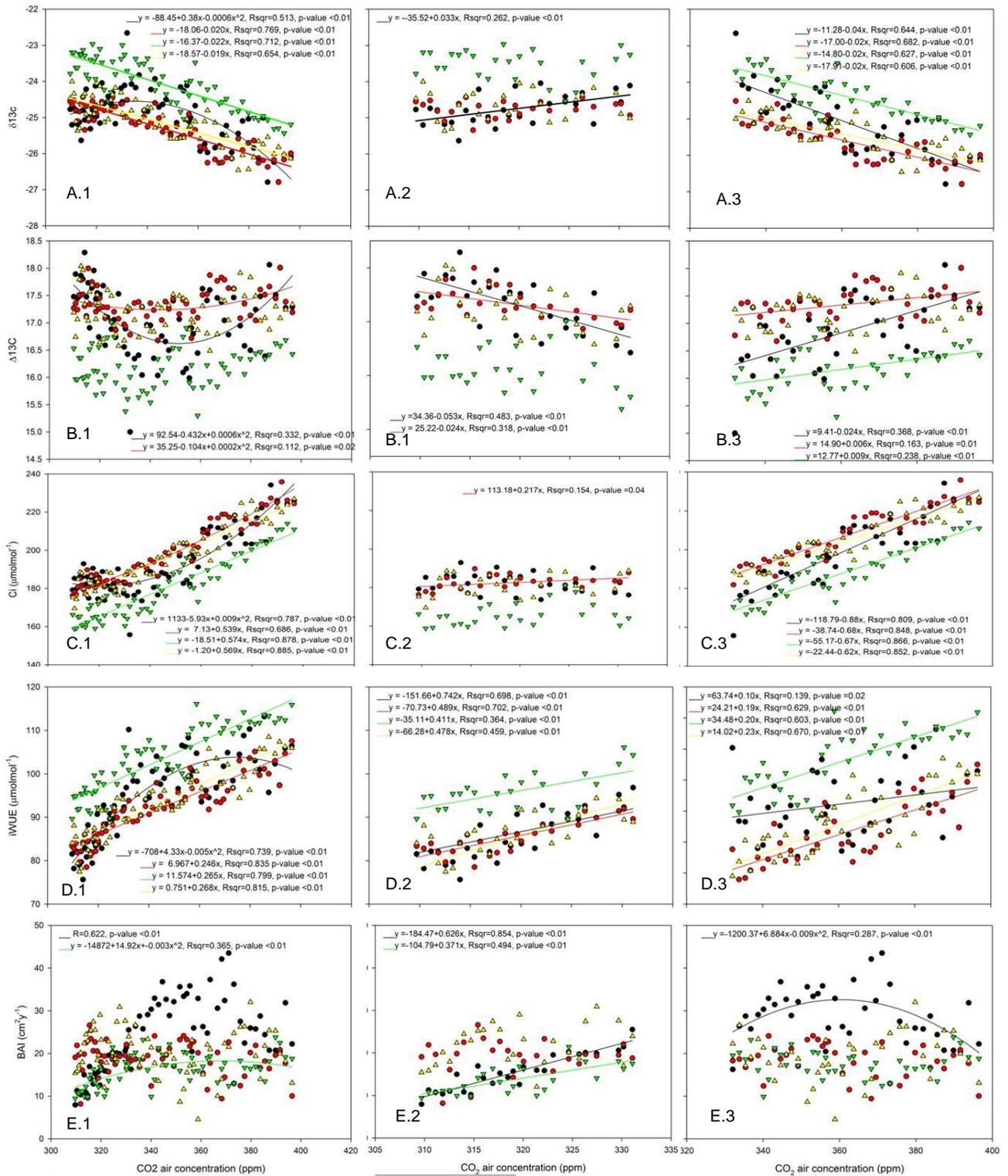


Figure 19. Relation of CO₂ air concentration with $\delta^{13}\text{C}$ (A), $\Delta^{13}\text{C}$ (B), C_i (C), $i\text{WUE}$ (D) and BAI (E) during different reference periods: 1: 1950-2013 (1), 1950-1975 (3) and 1976-2013 (3). Black circles = ZOO, red circles= VEN1, green triangles= ABR1 and yellow triangles =CAL1. The significant trends are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

It is clear that the only non-constant factors that control fractionation are C_i and C_a , the intercellular and ambient concentrations of CO₂. Furthermore, in order to understand the type of trees response to changing CO₂ concentration we calculated C_i/C_a and $C_a - C_i$. According to McCarroll et al. 2009,

changes in $\delta^{13}\text{C}$ assuming either a passive response (constant ca-ci) or an active response (constant Ci/Ca). Our results showed a constant ratio of Ci/Ca and increment of $\text{Ca}-\text{Ci}$ in all the study sites from 1950 till 2013. So we can hypothesize an active response of trees in our sites.

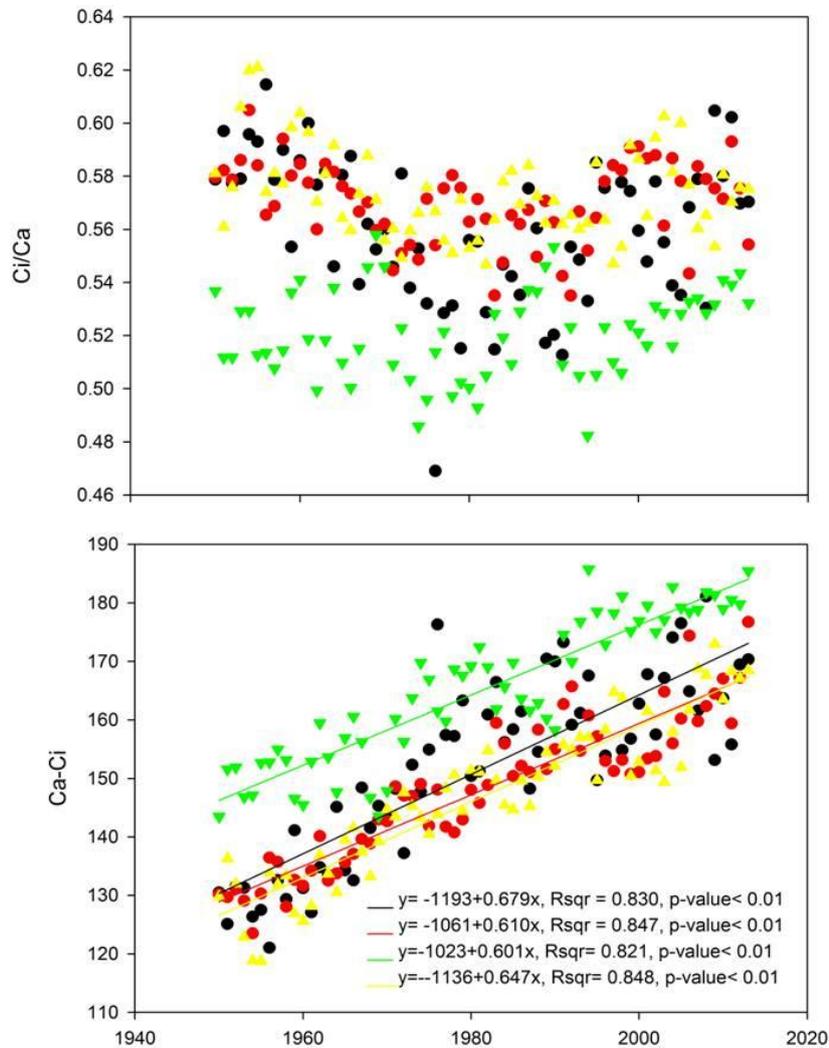


Figure 20. Ci/Ca and $\text{Ca}-\text{Ci}$ temporal pattern. Black circles = ZOO, red circles= VEN1, green triangle= ABR1 and yellow triangle =CAL1. The significant trends are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

4-5- Relationship between iWUE and BAI

For each site we evaluated the effect of iWUE on BAI during the whole study period and the two different climatic periods (1950-1975 and 1976-2013) phases. ZOO and ABR1 showed a positive correlation between intrinsic water use efficiency and BAI (Figure 21. A). During pre maturation phase only ZOO had a positive relation with iWUE (Figure 21.B), but after 1975 in any sites we did not observe significant relations.

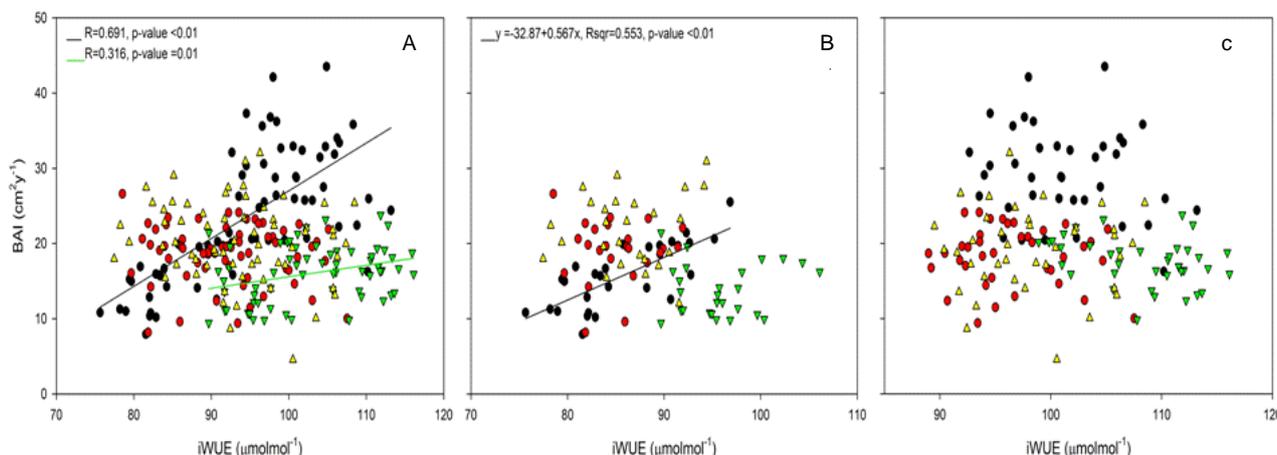


Figure 21 Relation between BAI and iWUE. Black circles = ZOO, red circles= VEN1, green triangles= ABR1 and yellow triangles =CAL1. A: 1950-2013; B:1950-1975; C:1976-2013. The significant trends are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

4-6- $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , iWUE and BAI relationships with Temperature and precipitation

The response to CO_2 variation of mean temperature of growing season was similar only between VEN1 and ABR1 Figure 22(ANCOVA, Tukey Contrasts, p-value <0.05).

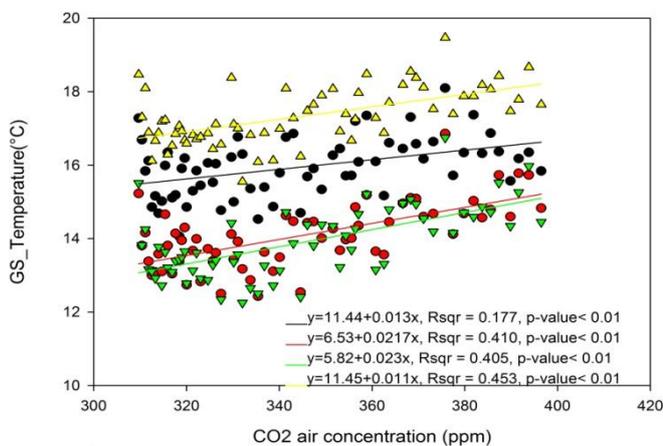


Figure 22: Relation between CO_2 and temperature. Growing season mean temperature (May-September); Black circles = ZOO, red circles= VEN1, green triangles= ABR1 and yellow triangles =CAL1. The significant relation are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

We analyzed the effect of temperature and precipitation during growing season (May-September) on $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i , iWUE and BAI (Figure 23, Table 9). Our results showed that temperature is correlated to $\delta^{13}\text{C}$ in all the sites except in Zoo, where is related to BAI, and to C_i and iWUE in all the sites. Precipitations has negative correlations with $\delta^{13}\text{C}$ in ZOO and CAL1, showed a positive correlation with $\Delta^{13}\text{C}$ in ZOO and a negative one with BAI in VEN; precipitations were related to C_i in ZOO and CAL1 and only in ZOO to iWUE.

Table 9: Correlation table between $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, BAI, Ci and iWUE with mean temperature of the current growing season (T_{GS})

	$\delta^{13}\text{C}$		$\Delta^{13}\text{C}$		BAI		Ci		iWUE	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
ZOO_T_GS	-0.115	0.366	-0.196	0.12	0.284	0.023	0.26	0.038	0.429	<0.01
VEN_T_GS	-0.585	<0.01	0.173	0.171	0.007	0.953	0.635	<0.01	0.557	<0.01
ABR_T_GS	-0.545	<0.01	0.167	0.187	0.1	0.431	0.607	<0.01	0.557	<0.01
CAL_T_GS	-0.452	<0.01	-0.015	0.908	-0.202	0.11	0.538	<0.01	0.52	<0.01
ZOO_P_GS	-0.455	<0.01	0.515	<0.01	-0.149	0.241	0.319	0.01	-0.295	0.018
VEN_P_GS	-0.197	0.122	0.231	0.069	-0.373	0.003	0.166	0.194	0.011	0.931
ABR_P_GS	0.088	0.49	0.058	0.651	0.075	0.557	-0.133	0.294	-0.198	0.116
CAL_P_GS	-0.327	0.01	0.195	0.132	0.241	0.062	0.335	<0.01	0.161	0.215

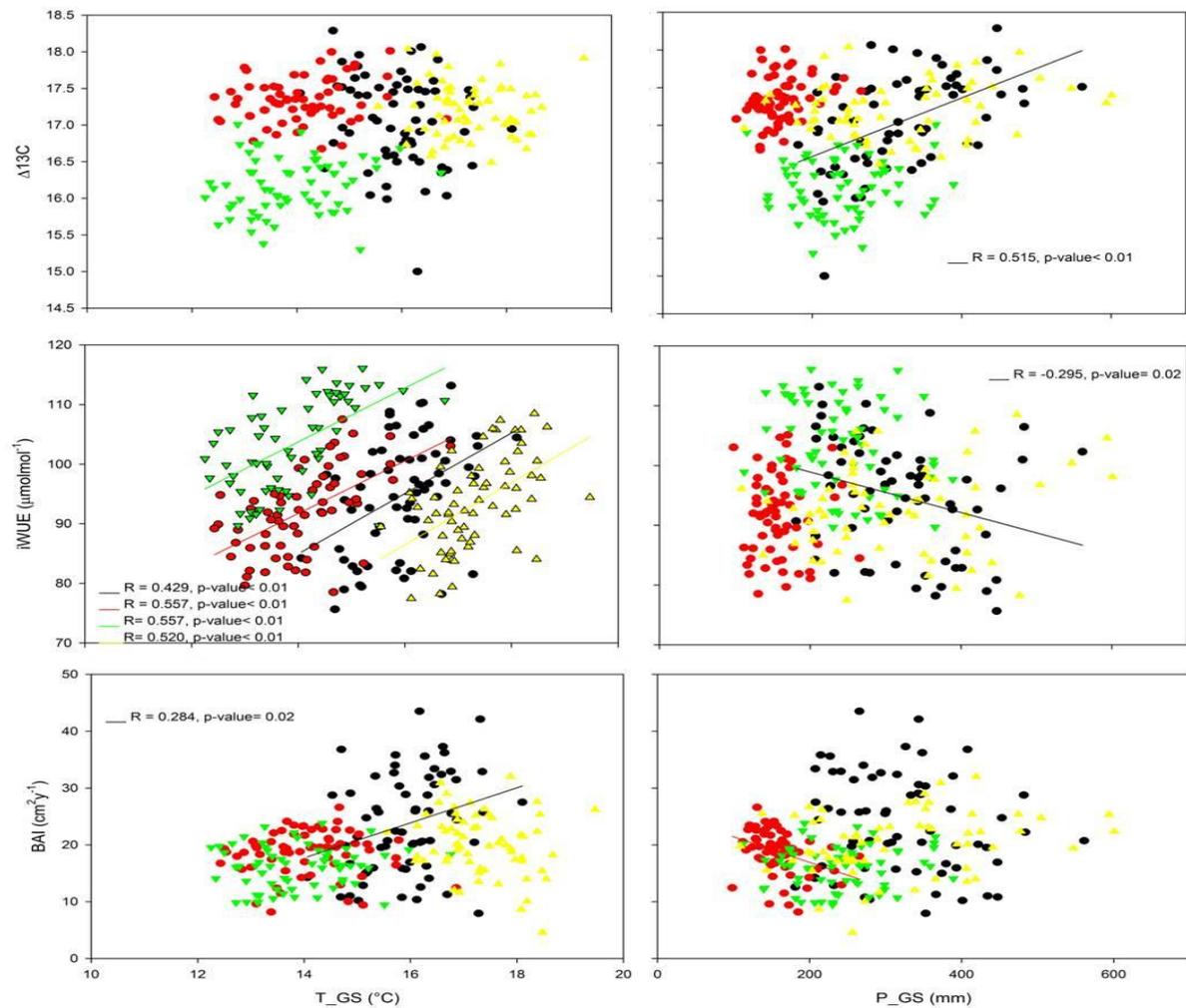


Figure 23. Relation between $\Delta^{13}\text{C}$, iWUE and BAI with temperature and precipitation. Growing season mean temperature (May-September); Black circles = ZOO, red circles= VEN1, green triangles= ABR1 and yellow triangles =CAL1.. The significant relation are represented by lines with the corresponding equation, coefficient of correlation and statistical significance.

5- Discussion

5-1- Climate effect on growth, Carbon isotope and iWUE

Considering the whole studying period and the two sub period (1950-1975;1976-2013), correlations between growth and climatic parameters were different. During the whole study period we observed a negative correlation of the temperature and precipitation (ABR1) of the previous summer with the growth of the current year, as occurred throughout Europe, e.g. in the Pyrenees (Gutiérrez 1988; Dittmar et al. 2003), Cantabria (Rozas 2001), Apennines (Piovesan & Schirone 2000), Alps (Di Filippo et al. 2007), French hills (Lebourgeois et al. 2005) and central Europe (Dittmar et al. 2003). This demonstrated, as in several deciduous species, that reserves are mobilized at bud break to sustain spring growth (Scartazza et al. 2013) and that a severe drought period affecting reserve accumulation and partitioning may also influence phenology and spring growth rate in the subsequent year (Bréda et al. 2006; Scartazza et al. 2013). In the coldest sites we observed a negative correlation between April temperature and growth, this can be explained with an induction of early bud break and consequently damages of late frost in May (Piovesan et al. 2008). In the ABR1 and CAL1, we observed negative and positive correlations of growth with summer temperature and precipitation, respectively. In the Mediterranean area, summer drought is a growth limiting factor and temperature has a greater importance than precipitation in causing this drought (Piovesan et al. 2008). The effect of summer temperatures was significant in the Apennine sites, but in recent times a similar effect appeared in ZOO and VEN1. In VEN1, during the less warm period growth was positively correlated to June temperature, but in the last period we observed an opposite signal. In addition, CAL1 the southernmost did not show much sensitivity to the summer drought, since it had abundant rainfall.

Going inside every sites with monthly analysis of temperature and precipitation, our results suggested a strong effect of spring temperature on carbon isotope discrimination ($\Delta^{13}\text{C}$) in Zoolithenhöhle, Pian del Cansiglio and Collelongo. We could not find any correlation with spring temperature in Pian di Limina which is the warmest site. Phenological changes differ from species to species, but some are substantial. In Mediterranean ecosystems, the leaves of most deciduous plant species unfold up to 16 days earlier and fall on average up to 13 days later than they did 50 years ago (Peñuelas & Filella 2001). A wide seasonal variations of canopy $\delta^{13}\text{C}$ was observed by Scartazza et al. 2013 at the Collelongo site, with an increase during the beginning of growing season. In this respect, the early onset of photosynthetic activities may have produced wood with higher values of $\Delta^{13}\text{C}$. The favourable conditions are related to soil water availability that in spring is very high.

In northern sites (Zoolithenhöhle and Pian del Cansiglio), humid conditions In central European sites the sensitivity to summer drought is underlined by positive correlation between $\Delta^{13}\text{C}$ and

precipitation of July and August.. In Zoolithenhöhle, we observed that precipitation of August was positively correlated with $\Delta^{13}\text{C}$ and negatively with growth. So probably trees were allocating carbon not in tree rings but in other sink (i.e. reserves and below ground biomass).

Higher precipitation and snow are typical for the autumn–winter period in the central Italian site (Collelongo). This results in high soil water contents mainly at the very beginning of the growing season. Crucial period for this site was May that represents the period when the growth starts. In this month we found a negative effect of precipitation that demonstrated a strong dependence of beech radial growth to leaf photosynthesis (Michelot et al. 2012).

Our results indicated a significant effect of CO_2 on temperature of the growing season (Figure 22). We investigated the general effect of temperature and precipitation during the growing season on basal area increment and physiological parameters. In all the Italian sites, we found a negative correlation between $\delta^{13}\text{C}$ and temperature which is related to the effect of CO_2 increment during the last half century (1950-2013). However, considering $\Delta^{13}\text{C}$, we did not find any correlation with temperature (Figure 23).

In our sites, as in many other studies (Saurer et al. 1995; Switsur & Waterhouse 1998), iWUE had positive relationships with temperature. Differently, growing season precipitation had a negative effect on water use efficiency only in Zoolithenhöhle (i.e. Saurer et al. 1995; Switsur & Waterhouse 1998) and no effect in other Italian sites. However, no effect was found for precipitation possibly due to its greater stability as compared with temperature (Silva et al. 2009). In Zoolithenhöhle, precipitation had a positive correlation with carbon isotope discrimination ($\Delta^{13}\text{C}$). Considering that in this site there is less precipitation respect to other studying sites (Table 1), probably the result is related to stomatal conductance and/or a more limited evapotranspiration.

Generally, during spring, warming was favourable to $\Delta^{13}\text{C}$ but during the summer warming made the climatic situation less favourable to the growth. So, also in front of an increase of iWUE we did not observe an increase in BAI.

5-2- CO_2 effect on in $\delta^{13}\text{C}$, iWUE and BAI

We observed that low frequency patterns in half century of physiological parameters of trees ($\Delta^{13}\text{C}$) are not only related to climate variation, i.e. temperature but most likely depend on assumptions of the plant physiological response to anthropogenic changes in the atmospheric CO_2 concentration (Treydte et al. 2009). In contrast, tree-ring $\delta^{13}\text{C}$ values from the industrial period (since ~AD1850) commonly exhibit a declining trend (Leavitt & Lara 1994; Raffalli-Delercé et al. 2004; Kitagawa & Matsumoto 1995) that is not related to changes in climate. Most of this decline is due to the incorporation of isotopically light carbon primarily released by the burning of fossil fuels (Freyer &

Belacy 1983). The concentration of CO₂ within the leaf is determined by the balance between the rate at which it enters, regulated through stomatal conductance, and the rate at which it is removed, which is photosynthetic rate. If *Ca* is stable, an increase in tree-ring δ¹³C represents a decline in stomatal conductance, an increase in photosynthetic rate, or some combination of the two (McCarroll et al. 2009). In this respect, the growing number of available tree-ring δ¹³C series suggests the response of trees is heterogeneous and often nonlinear (Waterhouse et al. 2004).

A passive response consists in the greatest decline in δ¹³C, in response to rising CO₂, that occur where trees display an entirely passive response to increased *Ca*, and do not alter either stomatal conductance or photosynthetic rate. In this case, for every incremental increase in *Ca*, there will be an equal increment in *Ci*, so that *Ca* - *Ci* remains constant. According McCarroll et al. (2009), our results confirmed an active response of trees to increase of atmospheric CO₂ concentration because of the increase in *Ca* - *Ci* which is often sufficient to maintain a nearly constant *Ci/Ca*. An increase in *Ca*-*Ci*, as an active response to increasing concentrations of atmospheric CO₂, can be seen also in terms of an improvement in the tree's water-use efficiency (McCarroll et al. 2009).

The iWUE increased by an average of 39% over the second half of the 20th century as expected from the fast build-up of atmospheric CO₂. The magnitude of change is in agreement with previous reports that found an increase of iWUE during the same period by using historical records in both leaves and tree rings (Peñuelas et al. 2008). Over the whole period of study (1950 till 2013), the iWUE increased ca. 50% in the Zoolithenhöhle, 37% in Pian del Cansiglio, 30% in Collelongo and 40% in Pian di Limina, which is in agreement with the 44% increase reported by in their study of the changes in tree-ring δ¹³C and iWUE of beech trees in northeastern France. Although in case of Zoolithenhöhle an important part of the change could be linked to the forest management in the increasing phase of BAI. Others have also found this increased iWUE in tree-rings (Feng & Epstein 1995; Bert et al. 1997) although some (Marshall & Monserud 1996) did not find such changes. In other cases, different responses have been reported, from 6% increase to a 43% decrease during the last 200 years (Paoletti & Gellini 1993; Woodward & Kelly 1995). The graph of iWUE plotted against atmospheric CO₂ concentrations in mature trees (Figure 19) shows that the rate of increase in iWUE was different between the German site and the Italian one, which shown a higher sensitivity from north to south during the maturity phase when cease the effect of management because of trees adaptedness involving both genetic and physiological mechanism (Robson et al. 2012), showing drought sensitivity of beech provenances along a gradient from northern to southern Central Europe and to the Mediterranean region (Peuke et al. 2006; García-Plazaola & Becerril 2000). Genetic variability is the results of the species biogeographical history (Robson et al. 2012). Beech was largely eliminated from Europe during the last glaciation, apart from isolated refugia maintained around the Mediterranean and in the Balkans (Magri et al. 2006; Magri 2008; Gömöry

et al. 2010), prior to subsequent post-glacial recolonisation from the South East (Giesecke et al. 2006). The diversity of beech has been widely studied using allozymes and more recently micro-satellite markers (Jump & Peñuelas 2007; Gömöry et al. 2010). Most of these studies report high genetic differentiation among populations and inbreeding within populations, but illustrate the capacity of beech to relatively rapidly evolve local adaptation (Jump et al. 2007).

However, despite higher iWUE, the expected increase in growth rates in response to rising atmospheric CO₂ (Long et al. 2004) were observed only in the Zoolithenhöhle, Collelongo. However, we could not find any significant increase of plant growth (BAI) over the study period in Pian del Cansiglio and Pian di Limina (Figure 21.A). So tree growth and iWUE have not risen as expected and they have remained stable or even declined in some areas, suggesting that other local stress factors have overridden the expected CO₂-induced growth increase (Poorter & Pérez-Soba 2001; Saurer et al. 2004; Waterhouse et al. 2004; Linares & Camarero 2012). Climate change and particularly warming-related drought, nutrient limitation and/or physiological long-term acclimation to elevated Ca have been proposed as potential factors that could limit the expected CO₂ fertilization effect (Hyvönen et al. 2006; Levanič et al. 2011). Probably, the increase of iWUE after silvicultural treatments is related to the increase of photosynthesis rate.

5-3- Forest management

In this study we used the last 64 years of data which in all sites that represented also the pre-maturation phase (increase phase) of the trees. Our results show the existence of a temporal effect on carbon isotope discrimination in the Zoolithenhöhle which may derive from more permissive site and silvicultural treatment. These age-related changes were not found in the Pian del Cansiglio, Collelongo and Pian di Limina, whose beech trees reached maturity earlier. In the 'age effect' or 'juvenile effect' phenomenon, wood formed during the early years of tree life is generally depleted in ¹³C in comparison with wood formed later (Francey & Farquhar 1982; Bert et al. 1997). The corresponding $\delta^{13}\text{C}$ increase varies around 1.5‰. However, the reported time span of this increase is highly variable: from the first 20 years of tree life (Freyer 1979) to 215 years (Mazany et al. 1980). This age effect may be attributed to two different causes: changes in micro-environmental variables during stand maturation and physiological changes linked to tree structural development (that could be also related to forest management). During stand maturation, canopy height, leaf area and tree density change. At the young stage, small trees may assimilate CO₂ coming in a larger proportion from soil emissions, and therefore isotopically lighter. Furthermore, among the physiological changes linked to tree structural development there are changes in hydraulic conductivity (McCarroll & Loader 2004) and bark re-fixation of respired CO₂ (Cernusak et al. 2001) that may add to this increase in $\delta^{13}\text{C}$.

It has also been shown that $\delta^{13}\text{C}$ increases with an increase in vapour pressure deficit (Farquhar et al. 1989) or irradiance (Francey & Farquhar 1982; Collet et al. 1993), two factors that change as trees grow.

The trend of $\delta^{13}\text{C}$ in Zoolithenhöhle was in agreement with the changes in BAI. Our result indicated a strong effect of forest management in this study site. The release phase of BAI was the result of a strong silvicultural treatment. In general the canopy is “organized” to maximize carbon fixation and thinning, after a drastic decline of leaf area index (LAI) modify the ratio between shade and light leaves (Aussenac 2000), increasing the amount of light leaves that have a higher photosynthetic rates (Matteucci et al. 1999; De Angelis & De Luca 1998). This higher photosynthetic rate produced a reduction of C_i in the canopy and a decrease of C_i/C_a producing an enrichment of ^{13}C (less negative $\delta^{13}\text{C}$ and lower $\Delta^{13}\text{C}$).

Consequently, an increase in light in the lower parts of the crown and a significant increase in the foliar mass of the remaining trees (Aussenac 2000) were reflected in the BAI increasing phase. After that canopy closed the gaps, the ratio between shade and light leaves changed again but this time with an increasing fraction of shade leaves, with a lower photosynthesis rate, that caused an increment of C_i . In this respect, more negative leaf $\delta^{13}\text{C}$ values are recorded in more closed canopies than in more open stands (Leavitt 1993; Buchmann et al. 1997), while strong interacting effects of water availability and light as depending on stand density on $\delta^{13}\text{C}$ have been shown for beech (Gebler et al. 2001). Contemporary we observed a decrease of BAI, so our results suggested a reduction of growth related to an overall lower photosynthesis rate.

The same stand response to silvicultural treatment was observed in Pian del Cansiglio, where the last thinning operation occurred in 1970. The duration of disturbance was shorter because of the low thinning. In this site we observed a negative correlation between $\Delta^{13}\text{C}$ and BAI. In the Collelongo forest, conversion from coppice-with-standards to high stand forest occurred releasing the older standard and the best stool of each stumps, without creating huge gaps. Among our sampled trees we found two old standards and three younger best stools. The response of this two group was not similar. The above mechanisms which were seen in Zoolithenhöhle and Pian del Cansiglio, could explain the observed significant correlation of $\Delta^{13}\text{C}$ with BAI after the cutting in best stools. During the first stage of their life, coppice-with-standards trees grow at a lower irradiance level (Duquesnay et al. 1998) and the conversion changed the light availability affecting the ratio between shade and light leaves. For older standard trees, a reverse situation is observed: in coppice-with-standards, old trees grow isolated in an open stand (Duquesnay et al. 1998), for this reason we did not observed any correlation between carbon isotope discrimination and BAI after the cutting. During the maturity phase the older standard shown a positive correlation between $\Delta^{13}\text{C}$ with BAI which is

linked to variations of the stomatal conductance (McCarroll & Loader 2004). In Pian di Limina, forest management was not occurring with a classical system. The sampled trees, big and very distant, are the results of high intensity cuttings over a wide area (Marziliano et al. 2011). The basal area increment is not associated to any silvicultural treatment and probably derived from micro environmental characteristic.

6- Conclusions

Sites specific climatic parameters had different effects on growth and tree physiology, as assessed through carbon discrimination.

Previous summer temperature affected the growth in the study sites, influencing the reserves accumulation, which are mobilized at bud break to sustain spring growth. Reserves accumulation is affected by a severe drought periods, that especially in Mediterranean area is related to temperature coupled to a lack of precipitation. However, the latter plays a crucial role only in the growth of ABR1 forest. During the whole study period, the current spring and summer temperature are negatively related to growth in the north and south sites, respectively. Furthermore, ABR1 represents a transition sites because has similar behaviour to both north and south sites, being located at south but at higher elevation. Since 1975, after increasing of temperature also in the northern site summer temperature are negatively correlated to growth.

Site climatic conditions affect also carbon discrimination ($\Delta^{13}\text{C}$). In northern sites a climatic control on stomatal conductance is demonstrated by positive and negative correlation of $\Delta^{13}\text{C}$ with summer precipitation and temperature, respectively. Furthermore, in the cold sites (ZOO, VEN1 and ABR1) we observed a positive relation between spring temperature and $\Delta^{13}\text{C}$, suggesting an "earliness effect" causing the early onset of photosynthetic activities allowing to trees to grow in favorable conditions (i.e. soil water availability).

In all the study sites we observed an increasing of temperature related to atmospheric CO_2 concentration increment. These increment of temperature was positively related to C_i and $i\text{WUE}$ and these relationships are the results of an active response of trees to CO_2 increment. Furthermore sensivity of $i\text{WUE}$ to increment of the concentration of CO_2 increased from North to South, showing the typical behaviour of species occupying large geographic ranges that respond to contrasting environmental conditions by genotypic variation and phenotypic plasticity.

Increasing temperature of growing season caused a continuous enhancement in $i\text{WUE}$ in all the sites. These results imply that the ratio between carbon assimilation and stomatal conductance has increased independently of the location of the forests, having as main causes changes in atmospheric CO_2 (C_a).

In all the sites, increasing of iWUE was not always related to growth increment as expected. Growth remained stable or even declined (VEN1), suggesting that other local stress and limiting factors have overridden the expected CO₂ induced growth increase (e.g. competition due to changing forest structure, distance from last management operations). In this respect, climate change and particularly warming related drought, nutrient limitation and/or physiological long term acclimation to elevated *Ca* have been proposed as potential factors that could limit the expected CO₂ fertilization effect.

Increasing of intrinsic water use efficiency was correlated to growth increment in the two sites with lower precipitations (ZOO and ABR1) and where appreciable silvicultural treatments were performed.

Silvicultural treatments increased the radiation intercepted by remaining trees, modifying the ratio between shade and light leaves, increasing the amount of light leaves that have a higher photosynthetic rates. This higher photosynthetic rate produced a reduction of *Ci* in the canopy and a decrease of *Ci/Ca* producing an enrichment of ¹³C (less negative $\delta^{13}\text{C}$ and lower $\Delta^{13}\text{C}$).

After that canopy closed the gaps, the ratio between shade and light leaves changed again but this time with an increasing proportion of shade leaves, with an overall lower photosynthesis rate, that caused an increment of *Ci* and lower growth rate.

In case of silvicultural treatments, iWUE is related to growth because increased the photosynthesis, soil water availability and nitrogen availability.

In conclusion, we observed that climate is changing (i.e. increase of temperature) and its effect of on growth and tress physiology is crucial, but a sustainable and adaptive forest management can have a central role in climate change mitigation and adaptation through preserving and enhancing forest carbon uptake.

7- References

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