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Dendroécologie et génétique d'une population de hêtre (*Fagus sylvatica*) en marge chaude de l'aire de répartition de l'espèce

Adib Ouayjan

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Pour obtenir le grade de Docteur

École doctorale Science et Environnement

Spécialité : Écologie évolutive, fonctionnelle et des communautés

Dendroécologie et génétique d'une population de hêtre (*Fagus sylvatica*) en marge chaude de l'aire de répartition de l'espèce

Dendroecology and genetics of a beech (*Fagus sylvatica*) population at the species' warm range margin

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Summary

Modern climate change is expected to cause a decline of forest tree populations that reside at the current low-latitude margin of species' ranges. Warming and a changing water balance stress are expected to result in reduced tree growth and reproduction and increasing mortality. This doctorate thesis investigates the demographic and genetic structure of a natural beech (*Fagus sylvatica*) population located in a climate refugium at the species' xeric range margin in SW France. This population persists on the slopes of a karstic canyon along the Ciron River (Gironde), a place that already harboured beech during the past glacial period. The overall goal of the present thesis is to better understand how this refugial population has managed to persist through past climate changes and how it responds to recent global warming.

The first thesis chapter assesses the genetic structure and diversity of the entire adult tree population ($n = 932$) to infer its postglacial history. The study reveals that the stand consists of two genetic clusters with different levels of diversity, which are likely to reflect an ancient local population that is successively being colonized by immigrant genotypes. The second thesis chapter investigates the mating system and patterns of pollen movement within the population by analysing seed progenies from selected mother trees ($n = 30$). It shows that predominant mating between genetically related neighbours has resulted in a very strong spatial genetic structure, a phenomenon that helps explain the observed slow admixture of the two genetic clusters present in the population. The third thesis chapter performs an extensive dendroecological analysis based on a third of the adult beech population ($n = 317$), plus 79 Pedunculate oaks (*Quercus robur*) sampled for comparison. Tree-ring studies and modeling based on climate projections reveal that beech growth has been so far relatively slightly affected in an increasingly xeric climate conditions. A strong increase in radial growth has been shown for beech between 1860 and 1920 that ceased later on. Then growth has declined imperceptibly since the 1980s without showing any accentuated decreasing according to the future climate scenarios data of the region. Fine-scale analyses including carbon stable isotopes show great among-tree heterogeneity in performance (in terms of growth and water use efficiency) that is partly driven by the fine-scale topography of the refugial habitat and might also be influenced to a small extent by the tree genotype.

Its combination of dendroecological and molecular ecological research approaches has enabled the thesis to attain important insights into the special character of the Ciron beech population and its performance within a constraining abiotic environment. Such insights represent valuable background information for the conservation and management of this and other refugial forest tree populations in a rapidly changing climate.

Résumé

Le changement climatique devrait causer un déclin des populations d'arbres forestiers résidant à des faibles latitudes, en marges chaudes de la distribution de l'espèce. En effet, le réchauffement et le stress dû au changement de l'équilibre hydrique devraient entraîner une réduction de la croissance et de la reproduction des arbres, et une augmentation de la mortalité. Cette thèse de doctorat étudie la structure démographique et génétique d'une population naturelle de hêtre (*Fagus sylvatica*) située dans un refuge climatique, en marge chaude de la distribution de l'espèce dans le sud-ouest de la France. Cette population persiste sur les pentes des gorges karstiques le long d'une rivière, le Ciron (Gironde), un lieu qui hébergeait déjà des hêtres pendant la dernière période glaciaire. L'objectif général de la présente thèse est de mieux comprendre comment cette population de refuge climatique a réussi à persister à travers les changements climatiques passés et comment elle pourrait répondre au réchauffement climatique.

Le premier chapitre de thèse évalue la structure et la diversité génétique de l'ensemble de la population d'arbres adultes ($n = 932$) afin d'inférer son histoire postglaciaire. L'étude révèle que la population se compose de deux clusters génétiques avec différents niveaux de diversité. Cela peut refléter une population locale ancienne qui a été successivement colonisée par des génotypes d'immigrés. Le deuxième chapitre de la thèse étudie le système d'accouplement et les modèles de mouvement du pollen au sein de la population. Cela était possible en analysant les progénitures de graines provenant d'arbres mères sélectionnés ($n = 30$) tout le long de la population. L'étude montre que l'accouplement prédominant entre voisins génétiquement apparentés a entraîné une structure génétique spatiale très forte. Ce phénomène aide à expliquer le brassage lent des deux clusters génétiques présents dans la population. Le troisième chapitre de la thèse consiste en une analyse dendroécologique basée sur un tiers de la population adulte de hêtres ($n = 317$), plus 79 chênes pédonculés (*Quercus robur*) échantillonnés pour la comparaison. Les études sur les cernes annuels et la modélisation basée sur les projections climatiques révèlent que la croissance du hêtre a été relativement peu affectée par des conditions climatiques de plus en plus sèches. Une forte augmentation de la croissance radiale a été démontrée pour le hêtre entre 1860 et 1920 qui a atteint un plateau plus tard. Ensuite, la croissance a légèrement diminué depuis les années

1980, et cela ne sera probablement pas accentué à l'avenir d'après les scénarios climatiques futurs de la région. En outre, les analyses à des échelles fines, y compris les isotopes, montrent une grande hétérogénéité de performance entre les arbres en termes de croissance et d'efficacité d'utilisation d'eau. Cela est en partie expliqué par la topographie locale de la vallée refuge, et pourrait également être influencé, dans une faible mesure, par le génotype des arbres.

La combinaison des deux approches de recherche, la dendroécologie et l'écologie moléculaire, a permis à cette étude d'atteindre des meilleures connaissances sur cette population particulière de hêtres dans la vallée du Ciron et sur sa performance dans un environnement abiotique contraignant. Ces idées représentent des informations de base précieuses pour la conservation et la gestion de cette population et d'autres populations d'arbres forestiers dans un climat en évolution rapide.

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General introduction

Recent climate change is now amply considered to be driven primarily by anthropogenic emissions of greenhouse gases (IPCC, 2013). Warming of the climate system is unequivocal, many of the changes observed since the 1950s are unprecedented over decades to millennia, and global temperatures are predicted to continue to rise through the coming decades. The accumulation of proof on global warming has drawn considerable attention to the fate of trees and forests (e.g. Dale et al., 2001; Millar et al., 2007; Petit et al., 2008; Lindner et al., 2010; Allen et al., 2010; Jump et al., 2017). Forests cover almost 30% of the land surface in tropical, temperate, and boreal regions. They store 45% of terrestrial carbon, contribute 50% of terrestrial net primary production, and can sequester large amounts of carbon annually (Bonan, 2008). They provide manifold services to natural systems and man including provision of food, regulation of the hydrologic cycle, protection of soil resources, refuges for biodiversity, etc. Forest trees are commonly foundation species of their communities and ecosystems that strongly influence forest structure and microclimate, and trigger fundamental ecosystem properties such as productivity, nutrient and water balance (Ellison et al., 2005). Their response to a changing climate hence has wide-ranging consequences for ecosystems and human well-being.

Three possible fates are expected for forest tree populations in a changing environment: extinction; persistence in situ through adaptation to new environmental conditions at the current growing sites; and migration to new places that offer the required conditions (Aitken et al., 2008). There are few recorded cases of species extinction during the late Quaternary that are clearly attributable to climate change (Jackson and Weng, 1999; Barnosky et al., 2004). Trees are also known to be capable of rapid microevolutionary adaptation (Petit and Hampe, 2006; Savolainen et al., 2007). And there is ample evidence for past range dynamics of tree taxa in response to climate changes (Davis and Shaw, 2001; Petit et al. 2008). However, modern climate warming occurs so rapidly that there is increasing concern whether ongoing environmental changes could outpace the response capacity of many tree populations (Jump et al., 2009; Corlett and Westcott, 2013; Sittaro et al., 2017). Numerous tree species are in fact undergoing range contractions because the formation of new populations at high-latitude range limits occurs more slowly than population extinction at low-latitude limits (Murphy et al., 2010; Zhu et al., 2012). This decline is typically induced by increasing mortality and a

decrease in growth and reproduction driven by higher temperatures and/or drought (van Mantgem et al., 2009; Allen et al. 2010; Carnicer et al., 2011).

For woody species, range shifts are the most noticeable, and best documented, response to Quaternary climate changes (Petit et al., 2008; Willis and MacDonald, 2011). Some species have completely shifted their range to new latitudes since the Last Glacial Maximum (LGM, 19-26.5 kyr BP; Clark et al., 2009). More commonly, however, some areas of the extant species range have harboured populations through Quaternary glacial and interglacial stages, functioning as climatic refugia that enabled long-term population persistence in regions that were otherwise inhospitable (Hewitt, 2000; Petit et al., 2003). Climate refugia are now amply recognized to have played a critical role for the long-term persistence of biodiversity through past periods of major climatic transitions (Gavin et al., 2014), suggesting that they could also be important in mitigating the impact of future global warming (Keppel et al., 2012). Today, many long-term climate refugia are located near the low-latitudinal periphery, or rear edge, of species' distribution range (**Figure 1**; Hampe and Petit, 2005). Their populations are often of great importance from an evolutionary point of view, because they exhibit a unique genetic composition as a consequence of their prolonged persistence in relative isolation. Thus, an extinction of these populations implies a drastic reduction for species' genetic diversity and evolutionary history (Hampe and Petit, 2005).

Climate refugia are areas with “physiographic settings that can support once prevalent regional climates that have been lost (or are being lost) due to climate shifts” (Dobrowski, 2011, p. 1023). An important quality that determines the long-term suitability of climate refugia consists in their ability to decouple local climate trends from those occurring at regional scale. Such a decoupling can arise as a consequence of topography, smaller-scale terrain effects, edaphic particularities, or vegetation structure and physiognomy (Hampe and Jump, 2011; Morelli et al., 2016; see **Figure 2**). It generally helps decrease climatic variability while creating steep microclimatic gradients over short distances (Dobrowski, 2011). These two characteristics of refugia – temporal stability and spatial heterogeneity - enable tree populations to encounter suitable microenvironments with relative ease even during major climate transitions (Hampe and Jump, 2011). One of the most widely distributed generators of refugial climates is the presence of water bodies in the landscape. Springs, ravines, lakes,

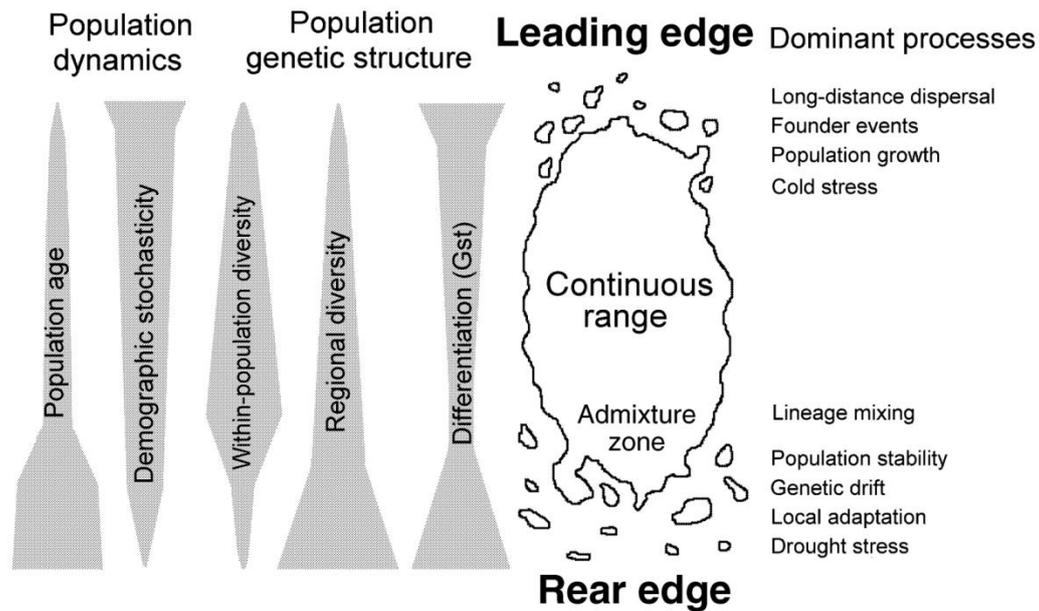


Figure 1. Schematic representation of leading and rear-edge populations under climate change. Paleocological and genetic evidence suggests that rear-edge populations may be extremely important in the conservation of long-term genetic diversity and that more attention should be given to climate change impacts on these populations. Illustration taken from Hampe and Petit (2005).

and mires are effective thermoregulators with diverse effects on the environmental conditions of adjacent areas (Caissie, 2006). Consequences such as lower and considerably more stable soil and air temperatures, elevated water availability, or modified air turbulence regimes can extend over scores or hundreds of metres from the water body itself, especially if they are further amplified by changes in terrain and vegetation (Dobrowski, 2011).

Climate refugia can help mitigate negative effects of unfavourable regional climate on tree populations. However, their limited size and resulting low carrying capacity as well as their scattered distribution across the landscape also pose additional constraints on population performance (Hampe and Jump, 2011). In fact, refugial tree populations are most often small and so isolated that regional population dynamics cannot easily compensate local extinction events. A rapidly growing number of studies have assessed relationships of refugial populations with spatial variation in current climate (Keppel et al., 2012; Hylander et al., 2015). Nevertheless, it still remains poorly understood which intrinsic dynamics have enabled long-term refugial populations to persist locally under the constraints of their climate-driven confinement (Hampe and Jump 2011). We ignore for instance how patterns of reproduction

can influence key components for long-term population persistence such as the maintenance of genetic diversity, adaptive potential and ultimately evolution (Moracho et al., 2016).

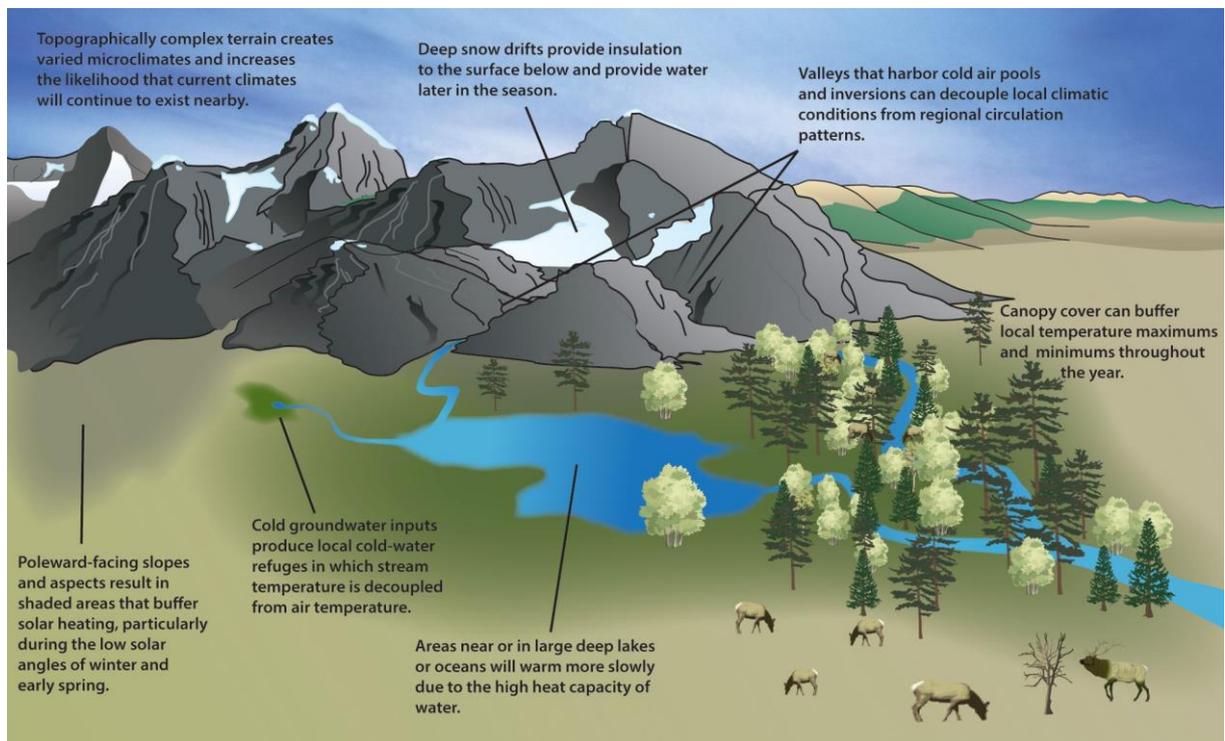


Figure 2. Different examples of climate refugia, or areas that are likely to experience reduced rates of climate change. Illustration taken from Morelli et al. (2016).

Similarly, we know little about how the microclimatic variation typically of refugial environments influences tree physiology and spatio-temporal patterns of tree growth and mortality. On the other hand, past survival of refugial tree populations does not imply that they are immune to threats from modern climate change. Hence, careful studies are required to better understand the functioning of refugial tree populations and to anticipate their responses to anthropogenic climate warming (Keppel and Wardell-Johnson, 2012).

1 OBJECTIVES, RESEARCH APPROACHES AND STRUCTURE OF THE THESIS

The main objective of this doctorate thesis is to investigate the functioning and fate of a long-term refugial beech (*Fagus sylvatica* L.) population located near the species' xeric range margin in SW France. For this purpose, I adopt two highly complementary research approaches rooted in molecular ecology and in dendroecology, respectively. Combining these

approaches allows addressing ecological, genetic, ecophysiological and bioclimatical aspects with the ultimate goal to achieve a better mechanistic understanding of the fine-scale environmental control of beech performance within its particular refugial setting.

Thus, molecular ecology tools allow for instance to reconstruct the population history and to evaluate how and why past population dynamics have generated the extent and distribution of genetic diversity that we observe today (Hewitt, 2004; Hu et al., 2009; Rajendra et al., 2014). Molecular analyses can also help identify the ecological mechanisms and processes responsible for contemporary patterns of mating, reproduction and effective regeneration (e.g. García et al., 2005; Gaüzère et al., 2013). In turn, dendroecological research approaches allow to infer individual variation and population-wide trends in tree growth and mortality and their relationships with past and current climate. Retrospective dendrochronological studies, including chemical analysis of tree rings such as stable isotope analyses, are a powerful way to assess the recent history of tree growth in a changing chemical or physical environment (Cook and Kairiukstis, 1990). Tree ring chronologies integrate the biological expression of the effects of climatic variability on growth (Di Filippo et al., 2013). They hence help determine the physiological response, performance and vigour of trees according to their immediate environment. Overall, the combination of molecular ecological and dendroecological approaches hence enables to address both short-term demographic processes as well as long-term population dynamics at a local scale.

The thesis is divided into three chapters:

- **Chapter 1.** Recent studies have shown that beech was already present in the area of the extant population before the LGM (de Lafontaine et al., 2014a), and that this population exhibits a peculiar genetic composition (de Lafontaine et al., 2013). These findings led the authors to conclude that the area served as glacial refugium for the species. The first chapter of my thesis focuses on the postglacial history of this population and the consequences for its present-day genetic structure and diversity. Based on an exhaustive genotyping of the adult population, it reveals the existence of two gene pools that are likely to correspond to the remainder of the glacial population and to individuals stemming from immigration,

respectively. The different histories of the two groups have resulted in divergent levels of genetic diversity.

- **Chapter 2.** The effectiveness of mating and gene dispersal within refugial tree populations is likely to be determined by the interplay between the intrinsic attributes, spatial distribution and ecological neighbourhood of the reproducing trees (e.g. Ghazoul, 2005). As a consequence, individuals are expected to vary greatly in their mating system and patterns of pollen dispersal (e.g. Bontemps et al., 2013; Chybicki and Burczyk, 2013; Gaüzère et al. 2013; Sánchez-Robles et al. 2014). This variation may have significant implications for individual fitness and ultimately the persistence of the entire population. The second chapter of the thesis describes patterns of mating and gene flow in the target population and investigates their ecological drivers. Analysing seed families of 30 mother trees, it shows that pollen-mediated gene flow is remarkably limited and mating occurs largely between closely related trees. In accordance, the population exhibits an unusually strong and far-reaching spatial genetic structure.

- **Chapter 3.** At the time of their formation, the tree rings register all the factors that condition the life of a tree, whether internal factors (health status, genetic potential etc.) or external factors (climate, competition etc.). In retrospect, dendroecology allows to reconstruct the evolution of growth, as an expression of the vitality of trees, or of certain parameters of wood, as records of past physiological functioning. Therefore, such studies provide access to past population trends since the earliest date of available tree-ring and allow to describe the most likely evolution in the future (Douglass, 1929; Fritts, 1976; Badeau et al., 1996; Mérian and Lebourgeois, 2011; Di Filippo et al., 2012; Keller et al., 2017; Latte, 2017). Finally, the third (and most extensive) chapter examines the ecological and climatic drivers that affect growth dynamics and water use efficiency of the relict beech population compared with *Quercus robur*, and gives its likely evolution after climate change. It reveals a recent slight decline in beech growth, which should not be much amplified by future climate change, and a sharper growth decrease of the oak population. The year-to-year growth variations are mainly related to the water balance of the growing season. Occasionally, some spring late frosts strongly reduced radial growth, which underlines the inherent complexity of climate-tree growth relationships.

2 STUDY SYSTEM

2.1 The species

European beech is one of the major broadleaf forest trees in Europe (Leuschner et al., 2006a; **Figure 3**) and of great importance both economically and ecologically. The species is a prominent resource for European forestry (Dittmar et al., 2003) and significantly contributes to carbon sequestration, soil conservation and water cycles (Bascietto et al., 2004; von Wühlisch, 2012; Houston Durrant et al., 2016). Beech tends to form mono-specific stands in large parts of its distribution range (Leuschner et al., 2006b). It is a monoecious, allogamous, anemogamous and zoochorous species that develops through a wide range of edaphic and climatic conditions (Leuschner et al., 2006b). Beech is able to grow on a wide variety of sites without being constrained by soil acidity, soil nutrition or humus type (Bolte et al., 2007), whereas it tends to avoid sites with very dry soils or with flooding or high groundwater levels (Ellenberg, 1988). The species is thus sensitive to the dryness of the soil and the atmosphere, with high temperatures highlighting the adverse effects of droughts (Lebourgeois et al., 2005). As a consequence, climate-based projections suggest that beech could be adversely affected by future climate change, especially in lowland forests and in the southern part of its range (Geßler et al., 2007; Meier et al. 2011; Cheaib et al, 2012).

In France, beech occupies 9.3% of the forest area dedicated to wood production (IFN, 2008). The species is mainly present in the north-east, the Alps, the Massif Central and the Pyrenees. Beech stands also occupy an important place in lowland areas in northwest France (**Figure 3**). On the other hand, lowland beech stands are rare and isolated in Southwestern France (Timbal and Ducouso, 2010).

The major glacial refugia and routes of postglacial expansion of beech are relatively well known thanks to extensive palaeoecological and phylogeographical investigations (Magri et al., 2006; **Figure 3**). Evidence for current trends in rear-edge populations is not fully conclusive. Populations of beech in Italy and Spain have been reported to be threatened by increasingly rare recruitment and declining growth of adult trees as temperatures increase (Jump et al., 2006; Peñuelas et al., 2007; Piovesan et al., 2008). On the contrary, a recent large-scale dendrochronological study showed that highest drought sensitivity and lowest resistance are

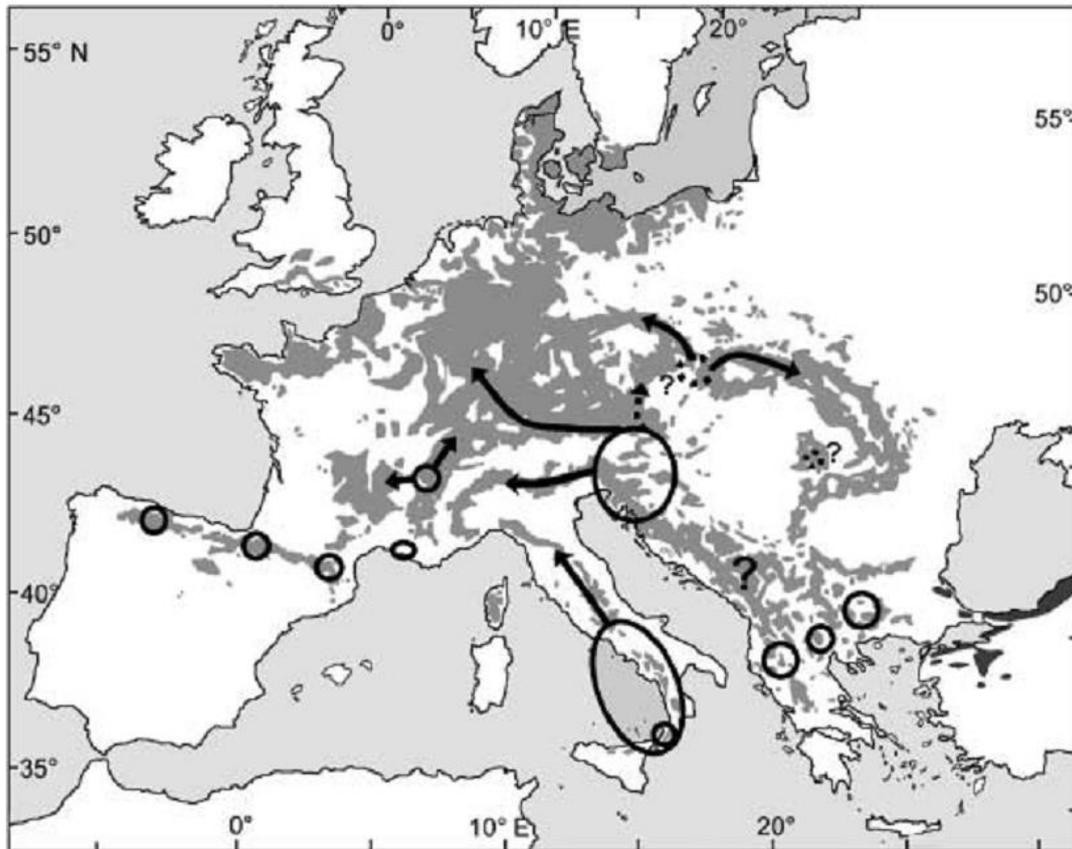


Figure 3. Tentative location of refuge areas for *Fagus sylvatica* during the Last Glacial Maximum and main colonization routes during the postglacial period according to Magri et al. (2006).

found in beech populations from the range core and not those from the rear-edge (Cavin and Jump, 2016), possibly as a consequence of the latter's refugial habitats. Similarly, beech populations from the SE range margin did not show noteworthy signals of short-term drought stress even during the European extreme year 2003 (Fotelli et al., 2009). And recruit mortality in a SE French beech population was relatively low and appeared to be driven rather by light availability than by drought (Oddou-Muratorio et al., 2011).

2.2 The study site

The field study site of this thesis is located within the Ciron valley near the commune of Bernos-Beaulac, 50 km south-east of Bordeaux (44 ° 22 '52 "North, 0 ° 15 '25 "West). With an area of 1311 km², the Ciron catchment basin originates in Lubbon, in the north east of the Landes department at 150 meters altitude, crosses part of the Lot et Garonne and joins the Garonne in Barsac (Gironde), 35 km upstream of Bordeaux at an altitude of 7 meters (SMABVC,

2011). The Ciron valley harbours the largest beech population in the region with around 7000 individuals (**Figure 4**) according to an exhaustive inventory performed in 2013 and 2014 by the Syndicat Mixte d'Aménagement du Bassin Versant du Ciron (SMABVC). Local naturalists testify that the Ciron beech forest has declined sharply during the last decade: About 16 years ago, islets of beech were present over more than 30 km along the Ciron, whereas today the species only occurs over a ca. 7 km stretch along the river banks (Guinberteau, 2011).



Figure 4. Spatial distribution of beech trees in the Ciron valley according to the inventory performed in 2013 and 2014 by the Syndicat Mixte d'Aménagement du Bassin Versant du Ciron (Genet, 2014). Red dots indicate the adult trees (circumference > 100 cm; with beechnuts during the fructification in 2013), green dots indicate the subadult trees (circumference between 30 and 99 cm; without beechnuts during the fructification in 2013) and yellow dots indicate the juvenile trees (circumference < 30 cm).

The Ciron beech population is restricted to riparian forests on the flanks of the Ciron river karst gorges, a site with particularly favourable microclimatic and edaphic conditions within the surrounding landscape. Within the study site, beech shares its habitat with other mesic tree species such as Pedunculate oak (*Quercus robur*), hornbeam (*Carpinus betulus*), large-leaved lime (*Tilia platyphyllos*), and black alder (*Alnus glutinosa*) (Timbal and Ducousso,

2010). The area harbours a very high biodiversity of flora, fauna and fungi, including many species of mountainous origin and/or associated with beech (E Silva et al., 2012).

A unique feature of the Ciron beech population is that it has probably been in place since (at least) the last interglacial period, according to 38 kyr and 42 kyr old fossil charcoal records found at the place of the extant population (de Lafontaine et al., 2014a; but see also the debate between Huntley, 2014, and de Lafontaine et al., 2014b). This history could also explain its relatively peculiar genetic composition, distinct from that of other populations in the region (de Lafontaine et al, 2013). The combined evidence suggests that the Ciron beech population would have been exposed to stronger and more sustainable climatic constraints than its congeners from the main distribution range (Jansson and Dynesius, 2002), without the possibility of escaping by migration. This particular feature renders the Ciron beech population a highly suited natural laboratory for research on the ecology of refugial tree populations (Hampe and Jump, 2011; Woolbright et al., 2014).

Chapter 1

Genetic structure and diversity within a refugial population of beech (*Fagus sylvatica*) in the Ciron valley

1 INTRODUCTION

Many temperate and boreal tree species maintain relict populations near the low-latitude limit of their current distribution range that have persisted roughly in situ through the numerous climate transitions of the Quaternary (Gavin et al., 2014). These populations are of great importance from an evolutionary point of view, because they exhibit a particular genetic composition and evolutionary differentiation as a result of their prolonged persistence in isolation (Hampe and Petit, 2005). Thus, their extinction implies a drastic reduction in species' overall genetic diversity. Relict populations have survived in climate refugia: sites that preserve suitable habitats through periods when the regional climatic conditions do not allow the existence of the species (Gavin et al., 2014; Hampe and Jump, 2011). This is possible because their topography, edaphic conditions or hydrology help uncouple the local climate from regional trends (Dobrowski, 2011). Such an uncoupling is not complete, however, and extant relict populations have probably been exposed to considerable climatic changes since the Last Glacial Maximum (LGM, ca. 19-26.5 kyr BP; Clark et al., 2009). They also have experienced considerable demographic turnover and eventually secondary contacts with other populations. However, detailed empirical accounts of the postglacial history and dynamics of relict tree populations remain very rare, a research gap that constrains our understanding of their eventual response to past, ongoing and future climate changes.

Three possible fates are expected for forest tree populations in a changing environment: i) extinction; ii) migration following their ecological niches; or iii) persistence and adaptation to the new conditions at the current places (Aitken et al., 2008). Relict populations fall, by definition, in the third category as they have neither gone extinct nor undergone significant migrations in the past. Empirical evidence for local adaptation in postglacial relict tree populations remains however scant (but see Benkman, 1999; Hampe and Bairlein, 2000; Kollmann and Pflugshaupt, 2001). Their typically small size should impose constraints on evolutionary adaptation (Willi et al., 2006), favouring instead inbreeding, genetic drift and reduced fitness. But trees are also known to be capable of maintaining high levels of diversity and experiencing comparatively rapid microevolution (Petit and Hampe, 2006; Savolainen et al., 2007). In addition, they often experience extensive long-distance gene flow (Kremer et al., 2012). Although relict populations usually are isolated at ecological timescales, periods of

intense population and range dynamics such as shortly after the LGM could moreover catalyse secondary contacts of different lineages (Hewitt, 2001). Overall, all the aforementioned processes could have left their imprints in the present-day genetic diversity and structure of relict tree populations. The challenge resides in filtering out the signals of past processes that have commonly been overridden by more recent population dynamics. Particularly suited and well-documented study systems are thus needed to gain detailed insights into the historical processes that have shaped the genetic composition of relict tree populations.

This study takes advantage of such a well-known and suited study system: an isolated relict beech (*Fagus sylvatica*) population occurring at low altitude in the Ciron valley ca 55 km southeast of Bordeaux (SW France). The population grows over 7 km along a karstic canyon, whose humid and cool microclimate allows its persistence in a region that is otherwise too xeric for beech. Recent analyses of fossil charcoal remains showed that exactly the same place acting today as an interglacial (or warm-stage) refugium already served as a glacial (or cold-stage) refugium for beech (de Lafontaine et al., 2014a). Such a shift in function has to my knowledge not been empirically documented for other sites or tree species. The long-term persistence of beech in the Ciron valley is further supported by the particular genetic composition of the population (de Lafontaine et al., 2013) as well as by the existence of a remarkably high local diversity of other organisms known to be associated with beech (de Lafontaine et al., 2014a). The genetic data point moreover to a possible existence of secondary gene immigration in the stand: Some parts of the population, but not others, are genetically distinct from other beech populations in the region (**Figure 5**; de Lafontaine et al., 2013). In addition, the Ciron population harbours remarkably high levels of genetic diversity given its size and putative isolation (Konnert, 2004).

Using the Ciron beech stand as an empirically validated model for long-term relict tree populations, I genotyped the entire adult population and explored its genetic structure and diversity with the aim to: (i) identify the existence and distribution of genetic groups within the stand, (ii) explore the consequences of the observed genetic structure for within-population patterns of diversity, and (iii) derive insights into the postglacial history of the stand.

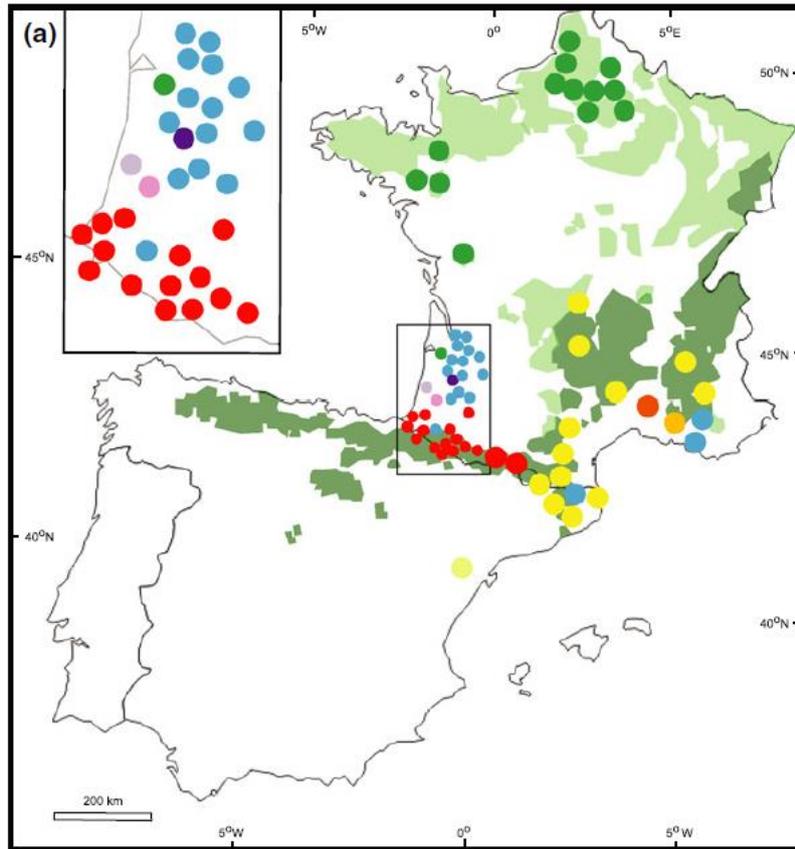


Figure 5. Geographical patterns of European beech (*Fagus sylvatica*) genetic structure inferred by BAPS Bayesian clustering according to de Lafontaine et al. (2013). Sampled populations are indicated by circles and different colours indicate assignment to distinct clusters. The distribution ranges of beech at low and high elevation (i.e., below and above 600 m a.s.l.) are shown in pale green and dark green, respectively. The violet circle indicates the central part of the Ciron beech population (termed ‘Ciron B’ in the original paper), whereas the two blue circles directly to its left and right indicate samples collected in the lower and the upper part of the population (termed ‘Ciron A’ and ‘Ciron C’), respectively. (Note that the ‘Ciron B’ sample was not distinguished by a similar analysis performed with the software STRUCTURE.).

2 MATERIALS AND METHODS

2.1 Study species and site

Common beech (*Fagus sylvatica* L., Fagaceae) is a late-successional and often dominant forest tree distributed through much of central Europe. It is a monoecious, anemogamous and allogamic species that occurs across a wide range of edaphic and climatic conditions (Leuschner et al., 2006). Beech survived the Quaternary cold stages in glacial refugia located in southern Europe, from where it expanded northwards when the climatic conditions became

more favourable (Magri et al., 2006). Today, beech is largely restricted to mountain ranges in its southern range parts (including the Dinaric Alps, the Appenine Mountains, the Massif Central and the Pyrenees). In the lowlands of Southwestern France, the species is absent except for a few small and isolated stands that occur along some rivers (Timbal and Ducouso, 2010). The largest population in this region is that of the Ciron valley (Gironde) with an estimated population of ca. 1000 reproductive individuals (Timbal and Ducouso, 2010). It has been suggested that the Ciron beech population may have suffered an important decline in extension, as a survey from 1992 had still detected the species over a total length of 35 km along the Ciron river (Timbal and Ducouso, 2010). On the other hand, a recent demographic survey revealed that the population shows a healthy demographic structure with abundant regeneration (Syndicat mixte d'aménagement du bassin versant du Ciron, unpublished data).

Two further beech populations, St. Symphorien with 18 adult trees and Roquefort with ca. 200 adults, are located at 14 and 40 km, respectively, in ecological settings similar to the Ciron population (Timbal and Ducouso, 2010). Extensive beech forests grow on the northern slopes of the Pyrenees at ca. 150 km.

2.2 Field sampling and DNA extraction

The sampling was conducted between March 2014 and June 2015. We mapped all putatively adult and close to adult beech trees in the Ciron valley ($n = 932$). Based on previous field observations, we used a threshold value of 70 cm for considering a tree as reproductive. We also included a small number of smaller trees that actually carried (very few) beechnuts. We collected different plant tissues that were stored in silica gel until genetic analyses. In most individuals, we were able to collect buds or leaves (harvested by hand or using a pruning pole), but in some cases the inaccessibility of the leaves or the buds forced us to take cambium disks using a cookie cutter (diameter 0.8 cm). DNA extraction was performed following the protocols of the Qiagen DNeasy 96 Plant Kit and the Invisorb DNA Plant HTS 96 kit/C.

2.3 SNP genotyping

All sampled trees were genotyped using 117 single nucleotide polymorphism (SNP) markers from the set described in Lalagüe et al. (2014) (for the specific loci see Ouayjan and Hampe, 2018). The loci were combined into three multiplexes and sequenced on an iPLEX

Gold genotyping kit (Sequenom, San Diego, CA, USA) at the Genomic and Sequencing Facility of Bordeaux (France) following the procedure described in Chancerel et al. (2013). SNP data were analysed using a hierarchical clustering method based on the Ward algorithm (Ward, 1963) as implemented in the Galaxy tool VIClust (Garnier-Géré, P., BioGeCo, unpublished script). I excluded four loci with poor performance during the clustering procedure and nine further loci due to lack of polymorphism, resulting in a final set of 104 loci for our analysis.

2.4 Genetic structure and diversity analysis

Structure - Bayesian clustering of the genetic data was performed using STRUCTURE v.2.3.4 (Pritchard et al., 2000). I ran STRUCTURE with K ranging from 1 to 5 and with 10 runs for each K value. I used a burn-in period of 50 000 iterations with 200 000 MCMC repetitions after burn-in, assuming allele frequencies to be correlated among populations and an admixture model of population structure. No prior information was used to assist the clustering. I selected the K value that best describes the data from the change in likelihood (delta K) as proposed by Evanno et al. (2005). The results of genotype clustering were analysed using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) and CLUMPACK (Kopelman et al., 2015).

Diversity - I calculated the diversity indices expected heterozygosity (H_E), allelic richness (R) and heterozygote deficit (F_{IS}). These estimates were calculated first for the overall population and then for different groups of trees for which the STRUCTURE analysis had returned a given probability to belong to one of the two identified genetic clusters (see RESULTS for details). We distinguished a total of eight tree groups whose sample sizes varied between 55 and 165 (see **Table 1**). Furthermore, I tested for heterozygote deficit relative to Hardy–Weinberg expectations by permuting alleles among individuals within samples. All diversity analyses were performed in FSTAT 2.9.3 (Goudet, 2001).

3 RESULTS

Structure - The STRUCTURE analysis revealed the existence of two distinct genetic clusters as the most likely scenario (**Figure 6**). The spatial illustration shown in **Figure 7** indicates that one of these clusters (shown in orange) was most dominant in individuals

located near the centre of the population and the second cluster (shown in blue) was more common towards the downstream and the upstream extremes of the population. Relatively few individuals were, however, strongly assigned to either one or the other cluster. Both clusters were roughly equally frequent in the population (global average across individuals: 0.52 for the blue vs. 0.48 for the orange cluster) (see also **Table 1**).

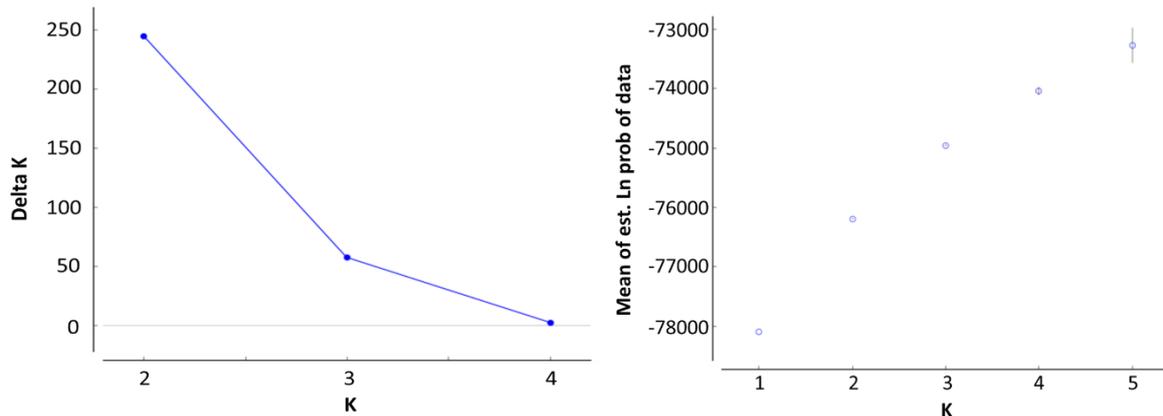


Figure 6. Optimal value of K according to Evanno et al. (2005) as implemented in STRUCTURE. The magnitude of delta K (on left) and mean likelihood $L(K)$ and variance per K (on right) as a function of K (source: STRUCTURE HARVESTER (Earl and vonHoldt, 2012)) suggests the existence of two major clusters as the most likely scenario.

Table 1. Tree groups defined according to their probability assignment to the orange cluster identified by STRUCTURE. Q score indicates the interval of probability chosen for each group and N is the number of individuals assigned to the group.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Q lower limit	0.000	0.125	0.250	0.375	0.500	0.625	0.750	0.875
Q upper limit	0.125	0.250	0.375	0.500	0.625	0.75	0.875	1.000
N	55	135	165	160	143	103	102	69

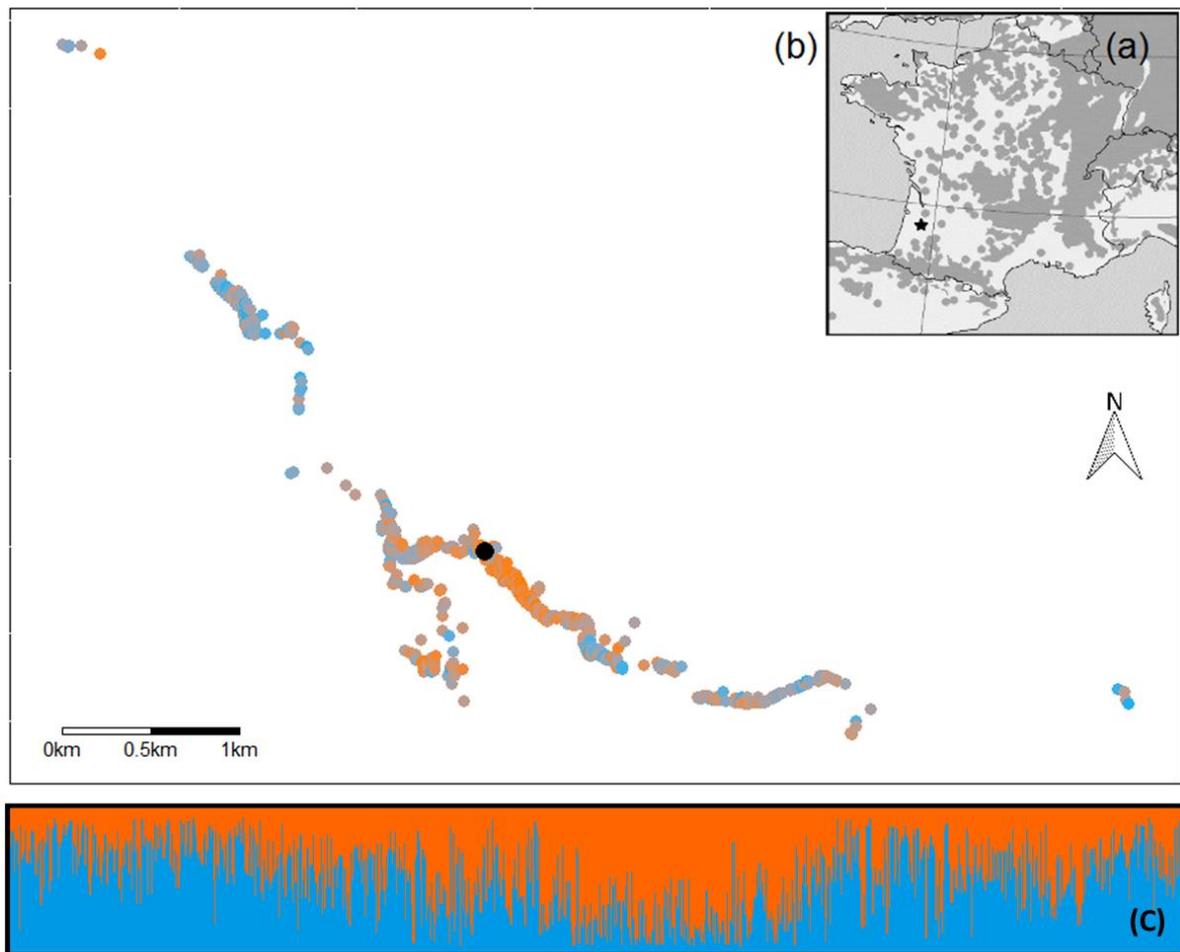


Figure 7. (a) Distribution area of *Fagus sylvatica* in France, according to EUFORGEN, with a black star indicating the study area in the Ciron valley. (b) Spatial distribution and genetic character of individual beech trees in the population. Each tree is indicated by a dot and the colour of the dots indicates their probability to belong to the orange or the blue genetic cluster identified by STRUCTURE. Black dot indicates the position of two *Fagus* charcoal records dated at 42 kyr and 32 kyr BP, respectively (de Lafontaine et al., 2014a). (c) Bar plots showing STRUCTURE ancestry proportions for $K = 2$ clusters. Each individual is represented as a line segment that is vertically partitioned with two colours representing the individual's estimated proportions of ancestry in each cluster. The segments are ordered according to the longitudinal position of the individuals (i.e., in from downstream to upstream on the map).

Diversity - Population-wide expected heterozygosity (H_E) was 0.292 and allelic richness (R) 1.984 while heterozygote deficit (F_{IS}) was 0.070 and did not significantly differ from zero. Genetic diversity indices for each of the eight tree groups are presented in **Figure 8**. All three diversity indices showed a similar, asymmetrically hump-shaped pattern. Values increased from group 1 (dominated by the blue cluster), achieved their maxima around groups 3 and 4 and descended again - more strongly - towards group 8 (dominated by the orange cluster)

which showed the lowest overall values. A significant heterozygote deficit was detected in groups 2 to 6 but not in the remaining ones.

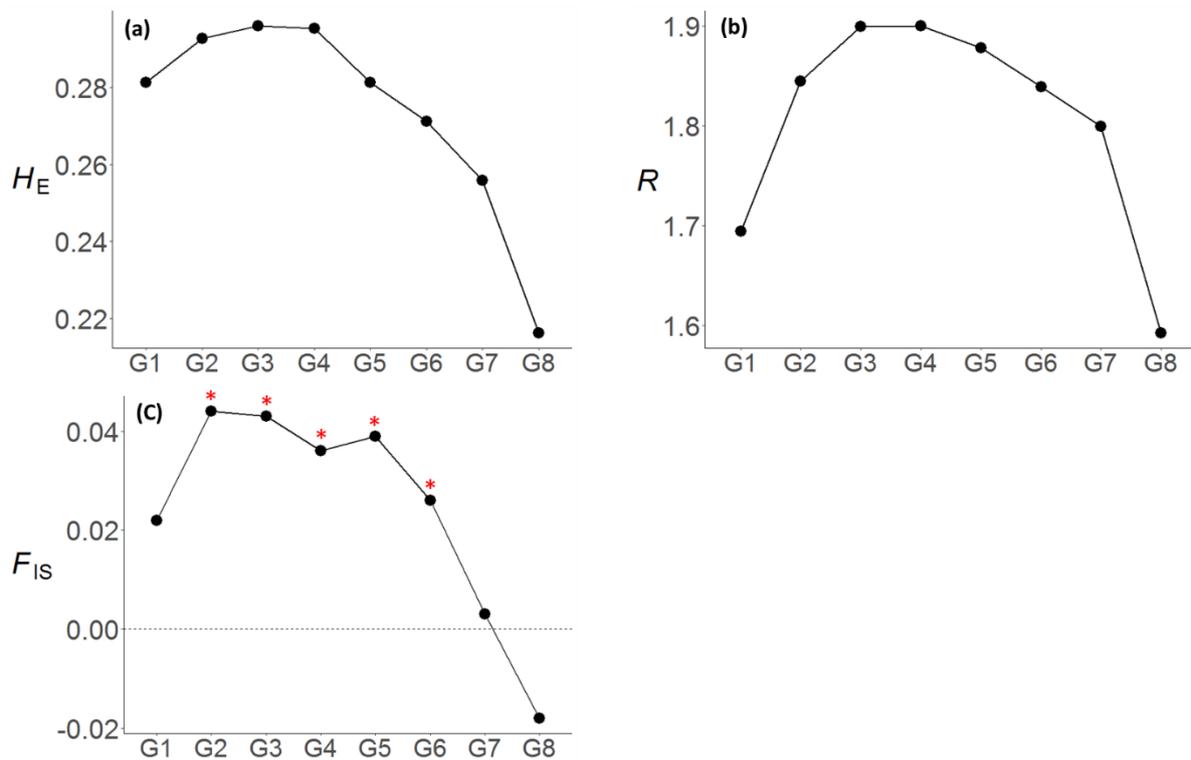


Figure 8. Genetic diversity indices calculated for each of the eight tree groups (G1 to G8) defined according to their STRUCTURE assignment. (a) Expected heterozygosity (H_E); (b) allelic richness (R); and (c) heterozygote deficit (F_{IS}). Red stars shown in (c) indicate significant differences from zero (at $P < 0.05$) for each group according to 2000 permutations.

4 DISCUSSION

4.1 Genetic structure and its evolution

The present study fully confirms the preliminary results of de Lafontaine et al. (2013) that had pointed to the possible existence of distinct genetic groups within the adult beech population of the Ciron. My STRUCTURE analysis based on the entire adult population clearly identified two distinct genetic clusters. One of them (shown in orange in **Figure 7**) was most prominent in the central part of the population, that is, the area (termed ‘Ciron B’) where de Lafontaine et al. (2013) had sampled the singular genotypes shown in **Figure 5** of this chapter and de Lafontaine et al. (2014a) had found beech charcoal remains dating back to ca. 32 and

42 kyr BP, respectively. Both results, taken together, suggest that the orange genetic cluster is likely to represent the extant remainder of the beech population that has putatively persisted in the Ciron area since the last interglacial period (see also the debate between Huntley (2014) and de Lafontaine et al. (2014b) upon the inference of a glacial refugium of beech in the Ciron area). The second, blue cluster was more prominent towards the downstream and upstream ends of the population. These areas correspond to the places where de Lafontaine et al. (2013) had detected genotypes that resemble those of other beech populations in the region (samples termed 'Ciron A' and Ciron C' in this paper). This genetic similarity suggests that the blue cluster would correspond to genotypes originating from outside the 'refugial' Ciron population core and arrived through immigration after the LGM. Such immigration could have occurred through the arrival and establishment of foreign beech seeds, which can in principle be transported over long distances by birds such as the Eurasian jay (*Garrulus glandarius*; Kunstler et al., 2007). However, it seems far more likely that the arrival of external genotypes would have happened through incoming pollen fertilizing local trees (Petit et al., 2005; Kremer et al., 2012). A similar 'pollen-driven' migration has also been described from the postglacial range expansions of the European white oaks, where some species colonized certain areas by hybridizing with other species that had previously arrived (Petit et al., 2003). Be the immigration through seeds or through pollen, the current genetic structure of the Ciron population reflects in any case a clear situation of secondary admixture between two distinct gene pools (Barton and Hewitt, 1985).

As briefly outlined above, the two clusters were not randomly distributed but showed a certain spatial gradient from the centre towards the extremes of the population. Such a gradient appears remarkable in a wind-pollinated tree species, whose typically extensive long-distance gene flow (Kremer et al., 2012) should rapidly blur local-scale genetic clines. One possible explanation for its persistence could be that the arrival of the blue cluster would be quite recent (e.g. as a consequence of silvicultural activities). However, I also observed that most of the analysed trees show a notable admixture of ancestry proportions and only few are strongly assigned to the orange cluster (see **Table 1**). Such a widespread mixture of coancestries could hardly arise from a very recent immigration. Therefore it appears far more likely that the observed spatial gradient has been favoured by an unusually limited local gene

flow combined with a remarkably stable population structure, a phenomenon that was recently described by Ouayjan and Hampe (2018; see chapter 2 of this thesis). In other words, the present-day genetic structure of the Ciron beech population appears to reflect a secondary admixture process occurring in slow motion. It appears noteworthy in this context that the observed fine-scale genetic structure (**Figure 7**) presents little evidence for eventual beech tree plantations or movements of plant material. This apparent absence of significant human imprints could be related with the traditional small-scale structure of the local land ownerships – the Ciron population extends over no less than 50 (private or communal) properties – and the limited industrial exploitation of hardwoods in the region. Finally, a relatively stable persistence of the Ciron beech forest in historical times can also be inferred from the detailed map of Cassini (Dupouey et al., 2007), which indicates that the area was mostly covered by broadleaf forests in the early 18th century.

The molecular analysis alone does not allow to infer when the immigrants arrived and launched the secondary admixture. Inspecting the STRUCTURE results provides, however, a further interesting insight. If the hypothesized scenario of a secondary immigration through pollen dispersal and subsequent crossing with local genotypes is correct, the resulting first-generation offspring should exhibit coancestry proportions of roughly 50% between the two clusters. Several generations of backcrosses between immigrants would subsequently be needed to produce trees that are strongly assigned to the ‘immigrant cluster’ - which is moreover likely to be constituted by genotypes from different external populations and hence genetically rather heterogeneous (see also below). Such backcrosses between immigrants would moreover be very unlikely until a certain number of them had successfully established in the population (Currat et al., 2008; Lepais et al., 2009), even if the backcrossing were asymmetric and favoured the immigrant gene pool (e.g. Petit et al., 2003; Brock, 2004; for other species). The immigrant trees would moreover need to grow near each other to ensure regular mating between them, given the observed dominance of short-distance gene dispersal in the Ciron population (Ouayjan and Hampe, 2018). The fact that one observes a small but non-negligible amount of trees strongly assigned to the blue cluster in spite of all these constraints suggests therefore that quite a number of generations have most likely passed since the first arrival and effective establishment of immigrants in the Ciron area.

The available molecular and fossil records do not enable a detailed reconstruction of beech expansion across the Landes de Gascogne (de Lafontaine et al., 2013, 2014a). Yet we certainly may assume that the species would have been rare and scattered in the region shortly after the LGM, constrained both by low temperatures and scarce precipitation. Fossil and genetic data from other parts of Europe (Magri et al., 2006) suggest that i) beech colonized central Europe relatively late, ii) the dynamism and extent of population expansions varied greatly among regions, and iii) the species expanded preferably along mountain chains. Taken together, these observations might suggest that the Landes de Gascogne possibly did not experience extensive changes in beech abundance after the LGM but rather a gradual increment and permanent establishment in the relatively scattered areas that provided suitable climatic and edaphic conditions. The expansion process would at latest have finished with increasing human activities, which have been shown to constrain the geographical distribution of beech in the region (E Silva et al., 2012).

4.2 Consequences for within-population patterns of genetic diversity

Geographically peripheral populations such as those in climate refugia commonly exhibit lower genetic diversity and higher genetic differentiation than central populations (Eckert et al., 2008). Secondary contact and admixture could hence be a way for such populations to increase their standing genetic variation, which would in turn help reduce their risk of inbreeding depression and maintain their adaptive potential (Willi et al., 2006; Aitken et al., 2008). Contrary to the first expectation, global diversity levels in the Ciron population do not appear to be particularly low (expected heterozygosity: $H_E = 0.29$, compared to 0.31-0.34 reported for SNPs by Seifert [2012] and 0.27 reported by Pluess et al. [2016]; heterozygote deficit: $F_{IS} = 0.07$, compared to -0.06 to 0.01 in Seifert [2012] and 0.005 in Pluess et al. [2016]), although the choice of SNP markers renders direct comparisons between studies difficult. (Note that global allelic richness actually is irrelevant in this context because the study used only polymorphic loci). The gene immigration that putatively underlies the blue cluster could go a long way in explaining the relatively high global diversity of the Ciron population.

The analysis of the eight STRUCTURE-based tree groups revealed a hump-shaped distribution for all diversity measures with marked decreases in the outermost classes (**Figure**

8). Such an overall pattern is expectable as my classification procedure highlights the consequences of a Wahlund effect (Wahlund, 1928). More surprising is, however, the clearly discernible asymmetry that I detected: Trees with strong coancestry of the orange cluster consistently showed lower values than those strongly assigned to the blue cluster. Two mutually non-exclusive explanations can be envisaged for this marked asymmetry: i) If my hypothesis upon the origin of the two clusters is correct, the orange cluster corresponds to the historically small and isolated population of the Ciron glacial refugium whereas the blue cluster reflects a gene pool (rather than a 'population' *sensu stricto*) formed by immigration events that are likely to originate from more than one source population. This multi-source origin implies that the immigrant gene pool would exhibit higher levels of diversity upon its (successive) arrival than the local population. ii) Any incorporation of immigrant genotypes into the Ciron population by means of incoming pollen must have passed through an initial hybridization event with local genotypes, whose effect would moreover have been amplified by the subsequent expansion of the immigrant genotypes (Excoffier et al., 2009). As a consequence, even present-day trees with strong coancestry of the blue cluster are very likely to still contain some imprints of the past admixture (Currat et al., 2008; see also Sankararaman et al., 2014 for a similar phenomenon in humans). Unfortunately, the effects of each of the two mechanisms are very difficult to tease apart without the use of simulations.

The observed asymmetry between the blue and the orange cluster is most pronounced for expected heterozygosity and heterozygote deficit (a parallel that nicely underpins the validity of the observed trend) than for allelic richness. Such a trend can be expected when a gene pool - here: the blue cluster - expands following a foundation event, because heterozygosity is more quickly re-established than allelic richness (Cornuet and Luikart, 1996; see also Widmer and Lexer, 2001). In this sense, the observed heterozygote excess of the orange cluster trees might perhaps even be interpreted as a signal that this cluster still continues to decrease in abundance (Cornuet and Luikart, 1996). Finally, it appears noteworthy that my results are at odds with those of de Lafontaine et al. (2013), who observed the highest levels of expected heterozygosity within the central group of their study (Ciron B). This apparent contradiction indicates that these authors probably sampled an area dominated

by genetically intermediate trees, rather than trees with strong coancestry of the orange cluster.

Overall, numerous effective immigration events were most probably necessary to leave the first significant traces in the overall population structure and diversity of the Ciron population (Currat et al., 2008; Lepais et al., 2009), but the present-day structure of the population clearly demonstrates the long-term success of this immigration process. One can speculate whether immigrant individuals might have had a reproductive advantage compared to local individuals owing to their genetic composition (see e.g. Hampe et al., 2013). Such an advantage would have been most likely if the ancient local gene pool had been very small and affected by genetic erosion, inbreeding and resulting fitness decline. Again, modelling or simulation studies would be required to test the probability of such a scenario. On the other hand, I am not aware of specific empirical evidence from beech that would point to the eventual existence of fecundity differences between clusters in the Ciron population. But I believe that the present study, albeit purely descriptive, nicely illustrates that the Ciron beech population offers interesting opportunities for future research on cryptic refugia (Provan and Bennett, 2008; Parducci et al., 2012; Tzedakis et al., 2013) and on the postglacial fate of refugial populations and their members (Anderson et al., 2006; Sexton et al., 2011; Edwards et al., 2014).

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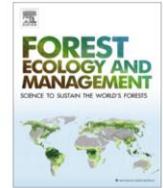
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Chapter 2

Extensive sib-mating in a refugial population of beech (*Fagus sylvatica*) growing along a lowland river



Extensive sib-mating in a refugial population of beech (*Fagus sylvatica*) growing along a lowland river



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ABSTRACT

Many temperate and boreal tree species have small and scattered populations near the low-latitude margins of their range that have persisted over extended periods of time in microrefugia. It remains poorly understood how patterns of mating and gene flow influence key components for their long-term persistence such as effective population size or genetic diversity. Yet such information is critical for designing effective protection measures and informing programs for the conservation of forest genetic resources.

Here we investigate the mating system and pollen dispersal of a long-term refugial population of beech (*Fagus sylvatica*), a major European forest tree. This population stretches over ca. 7 km along the ravines of a small lowland river in SW France. We exhaustively mapped the adult population ($n = 932$ trees) and collected seed families ($n = 450$ seeds) from 30 mother trees spread across the stand. All individuals were genotyped at 113 SNP markers and submitted to paternity analyses. We estimated various mating parameters and tested whether among-individual variation was related with the ecological neighbourhood of the mother tree.

A unique father could be identified for all seeds analysed. We detected neither pollen immigration nor selfing events. Instead, we observed extensive mating between neighbouring trees that resulted in a very steep decline of the pollen dispersal kernel and a low effective number of fathers (median $N_{ep} = 7.9$). In turn, male fecundity was tightly related with the distance between mates. The adult population showed an exceptionally strong and far-reaching spatial genetic structure indicating that neighbouring trees are most often sibs. The predominant mating between these neighbours suggests that patterns of reproduction and pollen flow are not constrained by cross-compatibility issues. Instead, dense conspecific neighbourhoods even tend to bar long-distance pollen flow by saturating the stigmas of mother trees through pollen swamping. The observed patterns are a direct consequence of the refugial habitat that imposes the population's linear distribution and whose dense vegetation represents a further obstacle to pollen dispersal. The elevated capacity of sib-mating might be a microevolutionary consequence of the population's long-term persistence in isolation. The resulting spatial genetic structure and the critical role of tree density in assuring an effective reproduction have important consequences for the design of forest genetic resources programs as well as for the conservation management of refugial and other riparian tree populations.

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1. Introduction

The long-term survival of populations in climate refugia during the Quaternary cold stages has been critical for the present-day composition of the world's temperate and high-latitude tree floras (Gavin et al., 2014). Today, the remainders of these populations often form outposts near species' low-latitudinal range limit that persist in particularly favourable habitats, commonly called

microrefugia (Dobrowski, 2011). These stands are important conservation targets and highly informative natural laboratories for investigating how tree populations can persist within a hostile greater environment (Hampe and Petit, 2005; Woolbright et al., 2014). Refugial populations are often so isolated that local extinctions cannot easily be buffered by regional meta-population dynamics. Their performance and viability therefore depend strongly on their inherent population characteristics in addition to the constraints imposed by their environment (Hampe and Jump, 2011). While the latter has attracted the interest of a rapidly growing research activity (Dobrowski, 2011; Keppel et al., 2012), the intrinsic dynamics of refugial populations and their role for

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population persistence still remain poorly understood. Thus, we virtually ignore how patterns of mating and pollen flow influence key components for long-term population persistence such as effective population size, genetic diversity and its spatial structure. Yet such information is critical for designing effective protection or restoration measures as well as for informing programs for the conservation of forest genetic resources (Lefèvre et al., 2013; Fady et al., 2016).

Refugial tree populations are often small owing to the constraints of their environment, and their effective size is likely to be even smaller due to the extensive inequality in fecundity that is typical of trees (Oddou-Muratorio et al., 2005). In such a context, the mating system and spatial patterns of gene flow play a crucial role for long-term population viability and evolution. A wealth of studies on small and isolated plant populations has documented that these tend to show reduced allelic richness, gene diversity, heterozygosity, and individual fitness (Leimu et al., 2006; Aguilar et al., 2008; Eckert et al., 2008). Yet tree populations are comparatively little affected by anthropogenic landscape fragmentation (Kramer et al., 2008; but see Jump and Peñuelas, 2006). Trees share life history characteristics that render them particularly resistant to the erosion of genetic diversity, including a long life span, great fecundity, widespread self-incompatibility, and a great propensity for long-distance gene dispersal (Petit and Hampe, 2006; Kremer et al., 2012). These characteristics might help refugial tree populations with maintaining their viability over extended periods of time in spite of their confinement (Leonardi et al., 2012; Moracho et al., 2016).

Pollen is the primary vehicle for gene flow in many trees, especially those pollinated by wind (Petit et al., 2005). Patterns of pollen dispersal are intimately linked with the mating system, which can be characterised by analysing the pollen cloud of seed-producing trees to quantify i) the rate of selfing (s), ii) the extent of correlated paternity among the seeds produced by a mother tree (r_p), and iii) the amount of pollen stemming from outside the population (m) (Ritland, 2002; Gaüzère et al., 2013). Numerous studies have examined among-population variation in the mating system to understand the impact of population size, density or related factors on mating parameters (e.g. in the context of habitat fragmentation; see Aguilar et al., 2008). An increasing number have also analysed how mating parameters differ among individuals within populations owing to fine-scale variation in their abiotic and biotic environment. Such studies commonly report extensive variation (e.g. García et al., 2005; Tamaki et al., 2009; Gaüzère et al., 2013; Sánchez-Robles et al., 2014) driven by the ecological settings surrounding the pollen recipient, or the 'ecological maternal neighbourhood' (García et al., 2005). Only a few studies have however investigated all three mating system components and related pollen dispersal distances at the individual scale and determined their drivers, especially for anemophilous species (but see de Lucas et al., 2008; Gaüzère et al., 2013; Sánchez-Robles et al., 2014).

In refugial tree populations, mating system and patterns of pollen flow may be constrained both by intrinsic population features as well as by habitat constraints. The long-term persistence of refugial populations at roughly stable size can result in marked family structures, which may in turn affect the cross-compatibility of individuals (Vekemans and Hardy, 2004). On the other hand, refugial populations are commonly located near water bodies that mitigate high temperatures and drought, such as lakes, bogs or rivers, and they are often further sheltered by ravines or dense vegetation (Hampe and Jump, 2011). The spatial configuration of such specific habitats often results in populations that have a highly scattered or a linear distribution. This imposes important additional constraints on patterns of pollen flow and further favours the formation of small-scale mating clusters and a rapid disconnection of more

distant individuals (Ghazoul, 2005; Moracho et al., 2016). The long-term persistence of refugial populations suggests that they have been able to cope with such problems, but through which biological mechanisms remains unclear.

Here we assess the mating system and contemporary pollen flow in a long-term refugial population of beech (*Fagus sylvatica* L.), a major forest tree species in Europe. This population grows along a lowland river in SW France, a region whose dry summers relegate beech otherwise to high-elevation forests in the Pyrenees. A singular feature of our target population is that exactly the same place that currently represents an interglacial (or cool-humid) refugium of beech already served as a glacial (or warm-humid) refugium for the species during the Late Quaternary (de Lafontaine et al., 2014). Using this beech stand as an empirically validated model for long-term refugial tree populations, we performed an extensive paternity analysis of freely pollinated seed families produced during a medium masting event in order to: (i) examine the composition of individual pollen clouds and assess levels of selfing, correlated paternity and pollen immigration; (ii) quantify the distances and directionality of effective pollen dispersal; and (iii) address effects of the ecological neighbourhood on patterns of mating. Based on our results, we infer the major ecological processes involved in forming the spatial distribution of genetic diversity in our stand and derive recommendations for the conservation of refugial tree populations and the sampling of forest genetic resources.

2. Materials and methods

2.1. Study species and site

European beech (*Fagus sylvatica* L.) is a late-successional forest tree widely distributed across central and western Europe. The species is monoecious and highly outcrossing with selfing rates usually below 10% (Merzeau et al., 1994; Wang, 2003; Gaüzère et al., 2013). Flowering occurs in April and May, shortly after bud burst. Reported average within-stand pollen dispersal distances typically span a few scores of metres (Piotti et al., 2012: 80–184 m; Gaüzère et al., 2013: 35–63 m). Beech is a masting species whose annual seed production shows strong links with climate (Piovesan and Adams, 2001). Successful recruit establishment appears to occur primarily following mast years (Oddou-Muratorio et al., 2011).

The study was conducted in the Ciron valley (44°23 N, 0°18 W, 60 m a.s.l.), situated some 60 km southeast of Bordeaux, SW France. Beech occurs over approximately 7 km along a karstic canyon formed by the river (Fig. 1). Some scattered individuals grow also outside the ravine, typically in the vicinity of sources. Two further beech populations with 18 and a few hundred adult trees, respectively, are located at 14 and 40 km in ecological settings similar to the Ciron population (Timbal and Ducousso, 2010). Extensive beech forests grow on the northern slopes of the Pyrenees at ca. 150 km.

A recent palaeoecological study identified charcoal fossils of beech at the site of the extant Ciron beech stand and dated the oldest ones at 42 kyr BP (de Lafontaine et al., 2014). This evidence, together with a peculiar genetic composition of the population (de Lafontaine et al., 2013), led the authors to conclude that the extant stand is very likely to represent the remainder of a Quaternary cold stage refugium. On the other hand, the Ciron beech stand was proposed in 2006 by the French Ministry of Agriculture to serve as a conservation genetic unit for the species and is one of four French beech populations included in an ongoing national program for assisted migration.

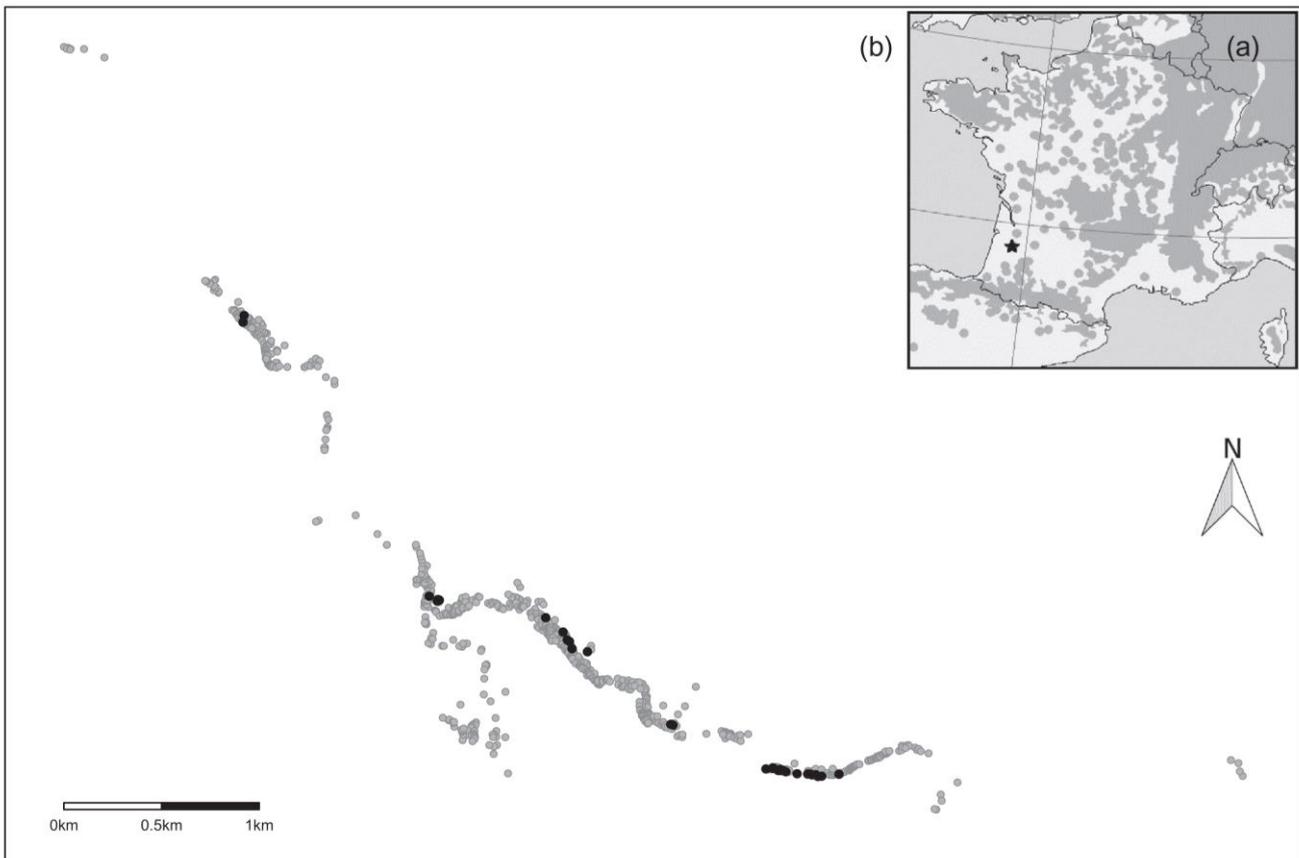


Fig. 1. (a) Distribution area of *Fagus sylvatica* in France according to the European Forest Genetic Resources (EUFORGEN) program with a black star indicating the study area. (b) Map of the investigated population. Grey circles indicate the adult trees sampled for paternity analyses ($n = 932$), black circles the trees from which seed families were collected ($n = 30$).

2.2. Field sampling

We exhaustively mapped all putatively reproductive trees of the population and measured their stem circumference at breast height. Based on previous field observations, we used a threshold value of 70 cm for considering a tree as reproductive. This circumference corresponds to an average tree age of 68 years (Ouayjan and Bert, unpublished data) and is a relatively conservative estimate for age of maturity in beech (Gaüzère et al., 2013). We also included a few smaller individuals on which we spotted some scattered beechnuts. From each mapped and tagged tree, we collected some plant tissue (buds, leaves or cambium) and stored it immediately in silicagel for later DNA isolation. A mast fruiting event of medium intensity occurred in autumn 2013 (the first after 2006). In October of this year, we collected up to 30 beechnuts on or, if impossible, beneath the canopy of 30 trees from across the Ciron valley (see Fig. 1). These seeds were likewise stored in silicagel until DNA isolation.

2.3. SNP genotyping

Genomic DNA was isolated from leaves, buds, cambium and seeds using the protocols of the Qiagen DNeasy 96 Plant Kit and the Invisorb DNA Plant HTS 96 kit/C. Genotyping was performed with 117 single nucleotide polymorphism (SNP) markers from the set described in Lalagüe et al. (2014) (see Table S1, Supporting information). The loci were combined into three multiplexes and sequenced on an iPLEX Gold genotyping kit (Sequenom, San Diego, CA, USA) at the Genomic and Sequencing Facility of Bordeaux

(France) following the procedure described in Chancerel et al. (2013). SNP data were analysed using a hierarchical clustering method based on the Ward algorithm (Ward, 1963) as implemented in the Galaxy tool VIClust (Garnier-Géré et al., unpublished). We excluded four loci with poor performance during the clustering procedure and ended up with in a final set of 113 loci.

We successfully genotyped all sampled trees ($n = 932$). A pilot analysis of seeds from seven haphazardly chosen mother trees using genetic diversity accumulation curves of multilocus genotypes indicated that a sample of 10 seeds per mother adequately describes the genetic diversity of seed families (see Fig. S1, Supporting information). We genotyped 15 seeds per tree, resulting in an overall sample of 450 seeds.

2.4. Data analysis

2.4.1. Pollen flow and mating patterns

Before starting the paternity assignment, we checked and removed all offspring whose multilocus genotypes did not match their putative mother tree (indicating sampling errors committed in the field) using CERVUS 3.0 (Kalinowski et al., 2007). The final progeny array consisted of 395 seeds (mean \pm SD per mother tree: 13.2 ± 1.6). We used categorical paternity assignments as implemented in CERVUS 3.0 to identify candidate father trees. This approach was facilitated by the exhaustive sampling of candidate fathers and the very high exclusion probability of our SNP markers (0.999998) (Marshall et al., 1998). The pollen donor of each seed was assigned by likelihood ratios assuming the strict confidence

criterion (95%). We performed simulations with the following parameters: number of offspring genotypes = 500,000, number of candidate fathers = 932, proportion of candidate fathers sampled = 0.95, mistyping rate = 0.0005 and proportion of loci typed = 0.9936. These parameters correspond to the adult population size of the Ciron valley plus an incoming gene flow of 5%, which seems reasonable given our study context and the empirical knowledge on isolated populations of wind-pollinated trees (Kremer et al., 2012). The mistyping rate was chosen after careful preliminary tests as the most reasonable compromise to account for the existence of genotyping errors while minimising the risk of false assignments given the large number of candidate fathers.

We used the results of the categorical paternity analysis and the spatial distances between the mother trees and the identified father trees to estimate the kernel and median distance of pollen dispersal. Our ability to assign all seeds to a single pollen donor (see Section 3.1 for details) enabled us moreover to directly infer the rates of selfing (s) and immigration (m) from the assignment data instead of relying on indirect mating model based approaches. We used the spatially explicit KINDIST approach as implemented in the software POLDISP 1.0c (Robledo-Arnuncio and García, 2007) to estimate levels of within-family and among-family correlated paternity (r_{pwf} and r_{paf} respectively) and the number of effective parents (N_{ep}) for each mother tree. Note that r_{pwf} is directly linked to N_{ep} as $N_{\text{ep}} = 1/r_{\text{p}}$. The latter estimate quantifies the number of male trees actually participating in the pollination of a given mother tree. Finally, we calculated the diversity indices expected heterozygosity (H_E) and heterozygote deficit (F_{IS}) for each seed family using FSTAT 2.9.3 (Goudet, 2001). On the other hand, we derived the distribution of male fecundities from the categorical paternity assignments.

2.4.2. Ecological correlates of pollen flow and mating patterns

We addressed the effects of three characteristics of the ecological neighbourhood on the pollen cloud of mother trees: (i) The size of the mother tree as estimated from the circumference at breast height. (ii) The density of conspecific neighbours. For this aim, we estimated the number of trees within a radius of 20 m (a threshold value chosen after testing different distances and finding them tightly correlated); the 20 m radius corresponds roughly to the average width of the canyon on each side of the Ciron river. (iii) The geographical ‘centrality’ of the mother tree within the population. For this aim, we calculated the mean distance from the target tree to every other tree within the Ciron valley. The centrality can be considered a measure of plant density at the scale of the entire population and thus complements the previous variables focusing on the fine-scale neighbourhood.

We used general linear models (GLM) to assess effects of the ecological neighbourhood on the H_E , F_{IS} and the (log transformed) median pollen dispersal distance of seed families, as well as on the among-family correlated paternity (r_{paf}). Previous tests confirmed that these variables all matched the underlying statistical assumptions. A generalised linear mixed model (GLMM) with a gamma distribution was used for the effective number of fathers (N_{ep}). The absence of selfing and immigration (see Section 3.1) rendered corresponding analyses superfluous. All models included the three predictor variables mother tree size, density of conspecific neighbours, and mother tree centrality.

Finally, we tested whether male fecundity was related with the distance of father trees to their mates. For this aim, we calculated the median distance of each identified father tree to all 30 mother trees and used this variable as predictor in a generalised linear model (GLM) with male fecundity as response variable. All linear models were constructed in R version 3.3.1 (R Development Core Team, 2016).

2.4.3. Spatial genetic structure (SGS) of the adult population

We assessed the fine-scale SGS among the adult trees using Nason’s kinship coefficient F_{ij} (Loiselle et al., 1995) as implemented in SPAGeDi 1.4 (Hardy and Vekemans, 2002). Confidence intervals (95%) were obtained by permuting individual locations 10,000 times. Our extensive sample allowed us to choose short distance classes (10 m) over a wide range (1000 m) while securing the reliability of kinship estimates according to the recommendations of Hardy and Vekemans (2002) and Cavers et al. (2005). We also calculated the S_p statistic of Vekemans and Hardy (2004) and tested its difference from zero with 10,000 permutations.

3. Results

3.1. Mating system and pollen dispersal

The paternity analysis assigned a unique pollen donor to each of the 395 genotyped and undiscarded seeds. This complete assignment allowed us to very robustly estimate the frequency distribution of mating events and associated distances of pollen dispersal. All individual tree-level estimates of mating parameters are specified in Table S2 of the Supporting information. We did not detect a single selfing or immigration event, while the median effective number of fathers was 7.9 (range: 1.9–925.9). The mean among-family correlated paternity (r_{paf}) value of -0.004 (range: -0.012 to 0.002) indicated that fathers comprised a roughly representative sample of the overall adult population. Expected heterozygosity (H_E) averaged 0.23 (range 0.19–0.26) while heterozygote deficit (F_{IS}) values averaged -0.155 (range -0.329 to 0.006) and differed from zero in 17 of the 30 trees.

The shape of the pollen dispersal curve was leptokurtic (population median distance: 34.8 m). By far most mating events occurred between neighbouring trees (Fig. 2), although a few pollen dispersal events exceeded 1000 m and spanned up to 3970 m (Fig. 3). Observed pollen dispersal distances were much shorter than might have been expected based on the availability of father trees (Fig. 3). We did not detect any signs of preferential upstream or downstream pollen dispersal flow when taking into account the availability of mates (Fig. 3; two-sample Kolmogorov-Smirnov test on the observed distance distributions of upstream and downstream matings weighed by the distance distribution of potential mates: $D = 0.14$, $P = 0.73$).

We identified a total of 142 father trees. The distribution of male fecundities was highly unbalanced with most father trees siring only one or two offspring and a handful of trees contributing greater numbers of seeds (Fig. 4).

3.2. Ecological correlates of the pollen cloud

The ecological neighbourhood of mother trees influenced some of the measured mating parameters (Table 1). In particular, seed families of mother trees surrounded by a high density of conspecifics tended to share a lower effective number of fathers and to originate from mating events spanning shorter distances. In turn, seed families of mother trees located near the population centre tended to exhibit a higher expected heterozygosity than those from mothers near the margins. We did not detect any effects of the mating environment on levels of among-family correlated paternity or heterozygote deficit.

On the other hand, we observed that male fecundity was tightly and negatively related with the distance between father and mother trees (Table 2).

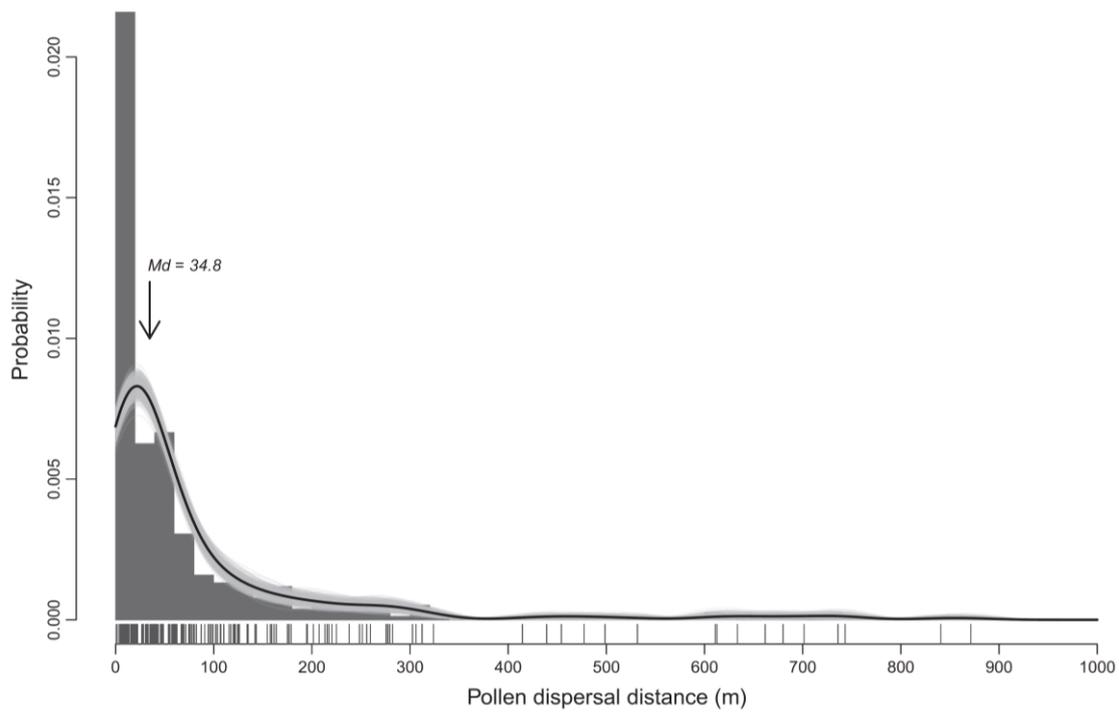


Fig. 2. Observed fine-scale frequency distribution of pollen dispersal distances for *Fagus sylvatica* estimated via paternity analysis. The line indicates the nonparametric smoothing spline fit to the empirical distance distribution together with bootstrapped estimates ($n = 100$ randomisations). The arrow indicates the population-level median dispersal distance. Individual pollination events are indicated by vertical lines under the plot.

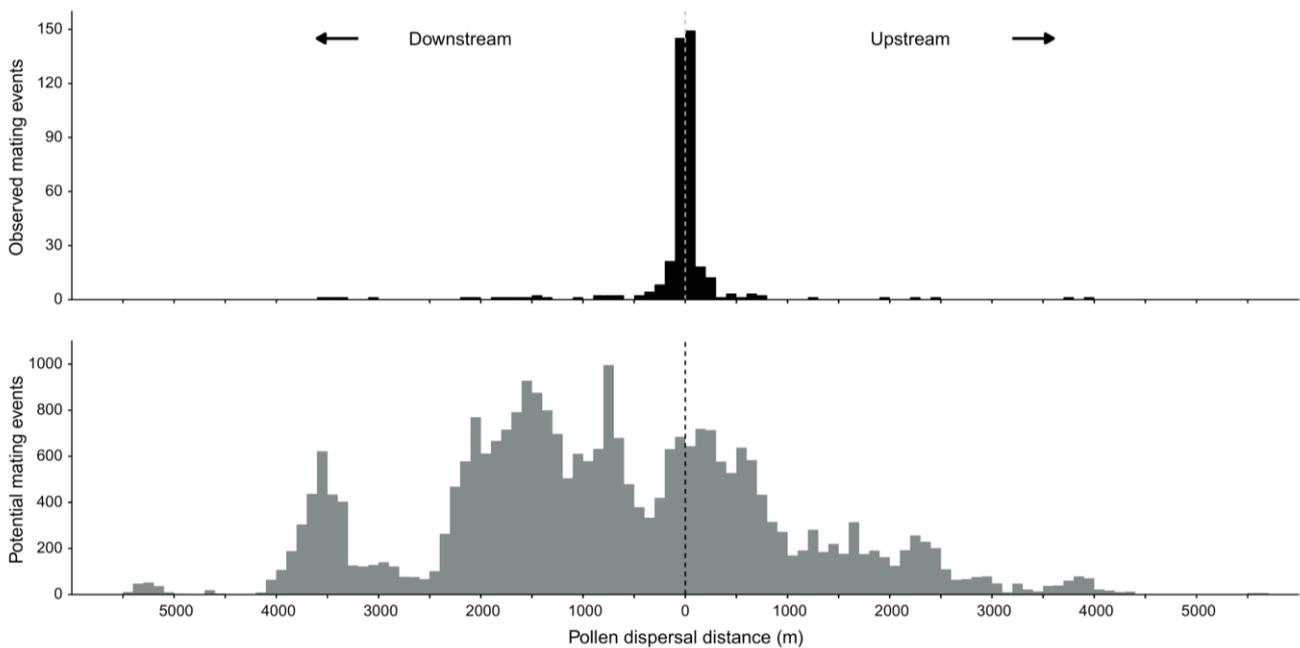


Fig. 3. (a) Observed population-wide frequency distribution of pollen dispersal distances for *Fagus sylvatica* according to paternity analysis. (b) Potential population-wide frequency distribution as estimated from the distances of mother trees to each potential father tree in the population. The zero on the x axis indicates the location of mother trees and values to the left and to the right refer to pollen from fathers located either downstream or upstream.

3.3. Spatial genetic structure of the adult population

The adult population exhibited a marked and far-reaching spatial genetic structure with maximum F_{ij} values as high as 0.13

(Fig. 5). F_{ij} declined sharply over the first ca. 40 m and more slowly beyond this distance but remained statistically significant up to a distance of 410 m. The Sp statistic or regression slope of F_{ij} on \log (distance) was 0.018, significantly greater than zero ($P < 0.001$).

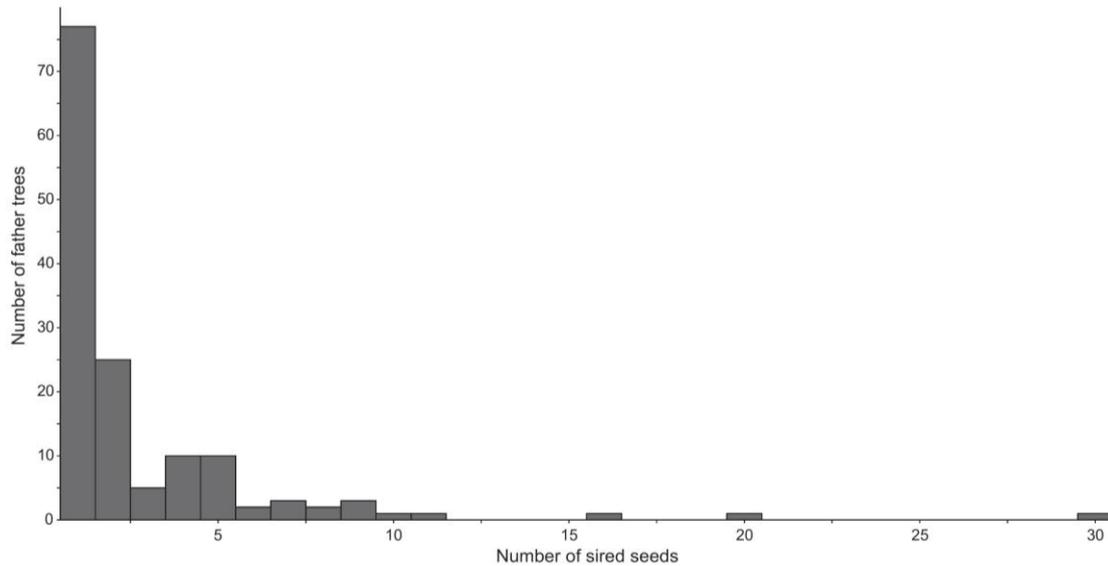


Fig. 4. Distribution of *Fagus sylvatica* male fecundities as derived from paternity analysis.

Table 1

Effects of the ecological neighbourhood on different mating parameters of individual *Fagus sylvatica* mother trees: effective number of fathers (N_{ep}), among-family correlated paternity (r_{paf}), median pollen dispersal distance (D), expected heterozygosity (H_E), and heterozygote deficit (F_{IS}). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$.

	N_{ep}			r_{paf}			D			H_E			F_{IS}		
	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	5.0658	1.8959	*	-3.30e-03	5.50e-03	ns	5.2857	1.0936	***	1.94e-01	2.46e-02	***	-2.26e-02	9.38e-02	ns
Tree size	-0.0019	0.0061	ns	-3.26e-06	1.79e-05	ns	-0.0007	0.0035	ns	-2.56e-05	8.00e-05	ns	-4.76e-04	3.05e-04	ns
Density	-0.2883	0.0873	**	-1.19e-04	2.53e-04	ns	-0.1428	0.0503	**	-5.40e-05	1.13e-03	ns	-6.43e-03	4.32e-03	ns
Centrality	0.0003	0.0008	ns	6.60e-07	2.41e-06	ns	-0.0005	0.0004	ns	2.56e-05	1.08e-05	*	-3.13e-06	4.11e-05	ns

Table 2

Effects of the spatial distance between father and mother trees on male fecundity.

	Estimate	SE	P
Intercept	1.415e+00	1.426e-01	***
Distance	-3.414e-04	8.309e-05	***

4. Discussion

4.1. Gene movements into and within the study site

A first striking result of our study is that we were able to identify a unique father tree for every single of the almost 400 seeds

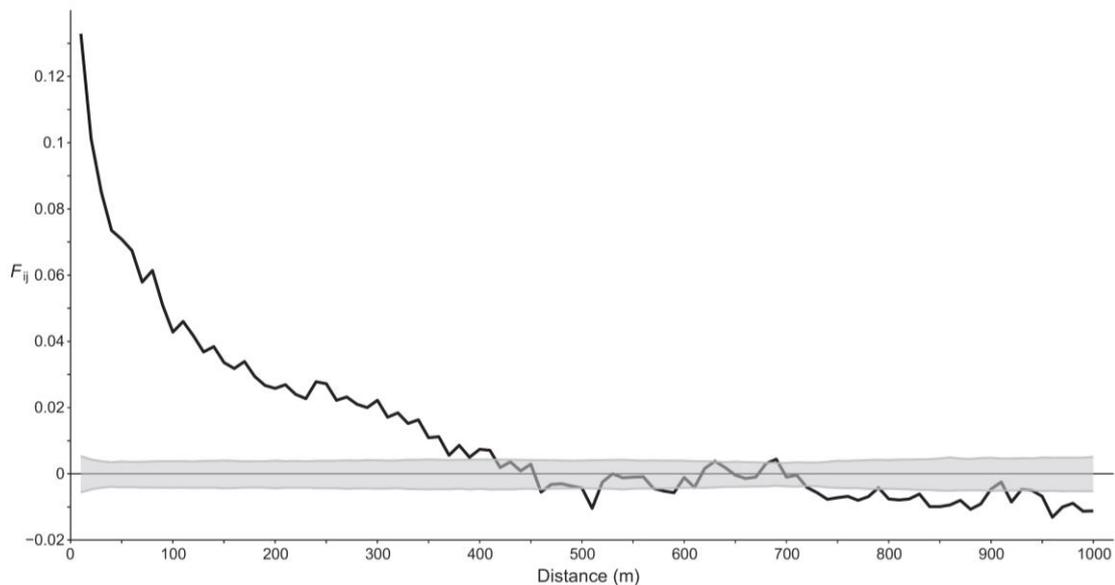


Fig. 5. Spatial genetic structure present in the adult *Fagus sylvatica* population. Observed values are shown with their upper and lower 95% confidence intervals.

that we analysed. We are not aware of any other study on free-pollinated, natural tree populations that would have achieved a complete assignment. The result underpins the exclusion power of the used SNP marker set and the exhaustiveness of our adult tree sampling. It may also have been favoured by the nature of our markers themselves because, for a di-allelic locus, highly heterozygous candidate fathers are difficult to exclude from any paternity assignment (Jones et al., 2010). Note, however, that the heterozygosity of our candidate fathers was rather moderate (P_{HT} : mean = 0.25, maximum = 0.41). Overall, our result clearly indicates very low levels of pollen immigration into the Ciron population despite the fact that two other populations with a few hundred reproductive trees exist at some km distance and extensive beech forests occur in the Pyrenees, ca. 150 km south of our target stand. Even assuming the existence of some limited cryptic gene flow, our results are in stark contrast with many other studies on isolated stands of wind-pollinated tree species that have reported regular effective pollen dispersal spanning several to many km (reviewed in Kremer et al., 2012). However, such studies are typically performed in very small populations that are considerably more likely to suffer from a limited availability of cross-compatible pollen than the Ciron stand (Hampe et al., 2013; see also Lesser and Jackson, 2013). Interestingly, another recent landscape-scale study of long-term refugial Pedunculate oak (*Quercus robur*) populations likewise revealed infrequent long-distance pollen flow and a pronounced spatial genetic structure (Moracho et al., 2016). Hence such populations could indeed tend to behave differently than small and isolated tree populations of more recent origin.

Secondly, we did not detect any selfing event. Beech is typically described to be predominantly outbreeding, although Wang (2003) detected up to 13% of selfed seeds on individual mother trees in locally isolated stands in Germany and Gaüzère et al. (2013) even up to 48% in a marginal beech population located in SE France. Similarly, Moracho et al. (2016) detected up to 40% of selfing in acorn families from long-term refugial Pedunculate oak populations. Gaüzère et al. (2013) reported that selfing was favoured by the density of the vegetation surrounding the mother tree and concluded that it could be driven by pollen limitation at a fine spatial scale. Such an interpretation would be in line with observations of Lagache et al. (2013) that the tendency of two related oak species to hybridise was likewise driven by the availability of conspecific pollen in the immediate neighbourhood of mother trees. Such results imply that the absence of selfing in the Ciron beech population would be favoured by the high local density of adult trees (which clearly exceeds that of Gaüzère et al., 2013; see Fig. 1). Overall, our finding contradicts the hypothesis of Moracho et al. (2016) that the breakdown of self-incompatibility might be favoured in long-term isolated tree populations.

The frequency distribution of pollen dispersal distances was characterised by a strong dominance of mating events between neighbours. Median distances were considerably shorter than those reported in other studies on beech (Piotti et al., 2012; Gaüzère et al., 2013), and most mother trees counted with a low number of effective fathers (median N_{ep} = 7.9; Gaüzère et al., 2013: mean N_{ep} = 12.3–29.2). In turn, father trees growing close to our target mothers exhibited a higher male fecundity than those growing farther away. The dominance of short-distance mating should be exacerbated by the linear distribution of the Ciron population, which implies that the number of available mates declines very rapidly beyond the width of the Ciron canyon. Long-distance pollen dispersal could be further hampered by the area's dense vegetation, a phenomenon that has been reported for beech by Piotti et al. (2012) and Millerón et al. (2012). In this sense, the pollen dispersal kernel that we report here can be considered to be a direct consequence of the Ciron population's refugial habitat. Only two other studies have to the best of our knowledge assessed

patterns of pollen flow in riparian tree populations with approaches that render them directly comparable to our study (Ahmed et al., 2009; Rathmacher et al., 2010). Both were conducted in more open landscapes and with lower population densities, and both reported considerably more frequent mating between distant individuals than we found.

The dominance of small-scale mating clusters should explain to a large extent the exceptionally strong and far-reaching spatial genetic structure of the Ciron population (Fig. 5; Vekemans and Hardy, 2004). F_{ij} values of up to 0.13 for the shortest distance classes indicate that most adjacent trees actually are sibs. The frequent mating between these neighbours would then indicate that patterns of reproduction and pollen flow are not constrained by cross-compatibility issues in the Ciron stand - a very unusual behaviour in forest trees (Petit and Hampe, 2006). On the other hand, two more phenomena are likely to have further exacerbated the remarkable spatial genetic structure in the Ciron stand: i) relatively inefficient seed dispersal (Oddou-Muratorio et al., 2011; Millerón et al., 2013), and ii) the presumably very stable spatial distribution of the refugial stand that would have allowed a slow but consistent build-up of extensive family structures.

4.2. Ecological correlates of individual variation in pollen clouds

Most mating parameters showed great among-individual variation, a phenomenon that appears to be rather the rule than the exception in tree populations (García et al., 2005; Tamaki et al., 2009; Gaüzère et al., 2013; Sánchez-Robles et al., 2014). The linear models revealed that both the effective number of fathers and the median distance of pollen dispersal towards a given mother tree tended to decline in dense conspecific neighbourhoods. The first result is at odds with classical expectations that more effective fathers should (co-)exist at higher population density (Sork and Smouse, 2006). Our opposite trend further confirms, in line with the results discussed previously, that tree density does not constrain reproduction in the Ciron population (see also Gaüzère et al., 2013). Instead, trees actually appear to saturate the stigmas of their neighbours and to thwart the arrival of pollen travelling over longer distances. This so-called pollen 'swamping' (Lagache et al., 2013) would explain our second finding that the shortest pollen dispersal distances occurred in dense conspecific neighbourhoods (see also Sork and Smouse, 2006). Extensive pollen swamping would further contribute to hampering mating events between distant individuals in addition to the previously outlined spatial distribution of mates *per se* and the dense vegetation structure of our study area.

Finally, we found that expected heterozygosity tended to be lowest in seed families near the centre of the Ciron population. This population-wide trend is difficult to explain. A comparison of the 100 most central and the 100 most peripheral adult trees showed the same trend ($H_{E\text{ central}} = 0.25$, $H_{E\text{ peripheral}} = 0.29$), suggesting that the observed variation in seed families reflects that of the adult population. But the historical development of this counterintuitive trend in the adult population requires further investigation.

4.3. Implication for the conservation of forest genetic resources

Tree populations from species' current low-latitude range periphery are now considered important conservation targets as they can contain important adaptive variants conferring resistance to a future warmer and drier climate (Hampe and Petit, 2005; Fady et al., 2016). The unusually widespread capacity of sib mating that we observed in the Ciron population might be a microevolutionary consequence of its long-term persistence in isolation. Our results provide important guidelines for optimising conservation and restoration programs in this and other long-term refugial tree

stands. For instance, the strong family structure of the adult trees and the nature of individual pollen clouds imply that collecting beechnuts from mother trees scattered throughout the Ciron population will maximise the genetic uniqueness of seed families while focusing on mother trees growing at relatively low density near the periphery will maximise their genetic diversity. Perhaps the most important implication of this study is to highlight the importance of securing plant densities high enough to allow effective mating and reproduction (rather than maximising for instance the spatial extent of populations). The Ciron population performs well in its refugial confinement in part because the extensive mating between nearby sibs allows the production of an abundant crop of viable seeds (see also Moracho et al., 2017). The well-balanced demographic structure of the stand (Ouayjan and Hampe, unpublished data) indicates moreover the existence of widespread and effective recruitment. This high inherent regeneration capacity of the Ciron beech population will hopefully enable it to successfully continue its long-term refugial persistence under a future warmer and drier climate.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foreco.2017.07.011>.

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Chapter 3

Dendroecology of a refugial beech (*Fagus sylvatica*) population at the species' warm range margin in southwestern France

1 INTRODUCTION

Climate change and concomitant increased drought events are expected to affect ecosystem functioning and services at multiple scales (Garcia et al., 2014). Dendroecology, or the study of long-term trends in radial tree growth allows to understand its relation to natural and anthropogenic factors, providing a solid basis for the historical assessment of the vitality of forest tree species and the role of ecological and climate factors (Badeau et al., 1996). Many studies have focused on the impact of recent climate change on beech (*Fagus sylvatica*), one of the most important forest species in Europe (e.g. Dittmar et al., 2003; van der Werf et al., 2007; Kramer et al., 2010; Gillner et al., 2013; Weber et al., 2013; Cavin and Jump, 2016). One remarkable result is a decrease in adult tree growth for populations residing at the warm range margin of the species at low latitude, that have been linked to the effects of drought associated with climate change (Jump et al., 2006; Piovesan et al., 2008). Beech is a drought sensitive species (Granier et al., 2007) and therefore should be particularly affected by the decline of the water balance as temperatures increase. However, Cavin and Jump (2016) also observed in an extensive dendrochronological study of beech along a latitudinal gradient that the highest drought sensitivity and lowest resistance are found in populations from the range core and not those from the rear-edge. These authors argued that their observation could be due to the fact that rear-edge populations are growing in refugial areas that provide a relatively stable local climate. In any case, the response of trees to climate change is affected by numerous other environmental factors that vary both at global scale (e.g., atmospheric CO₂) and at local scale (e.g., topography, atmospheric pollution, soil nutrients, and biotic interactions). These need to be taken into account in order to better understand the role of environmental changes in growth trends and the physiological response of trees within populations at the southern range margin (Charru et al., 2010; Ruzicka et al., 2017)

In temperate regions, many trees produce each year a growth ring whose width depends on the environmental conditions and the physiological processes specific to the tree. The thickness of the ring varies in response to different endogenous (biotic factors such as age, longevity, sensitivity and genetics) and exogenous factors (climate, soil, exposure, pathogen, stand dynamics and anthropogenic actions) (Cook, 1985). Thus, I consider a series of tree rings as a linear aggregation of several signals, which can be expressed as follows:

$$R_t = C_t + A_t + \alpha D1_t + \alpha D2_t + \epsilon_t, \text{ with}$$

R_t , observed series of tree ring widths of the year t

C_t , climatic signal (high frequency signal linked to interannual climate variations)

A_t , age trend (variations of low frequencies related to the age of the tree)

$\alpha D1_t$, impulse linked to local endogenous perturbation (silviculture, senescence of dominant trees etc.)

$\alpha D2_t$, impulse linked to exogenous perturbation to the stand (fires, storms, pathogens, insect outbreaks etc.)

ϵ_t , unexplained part of interannual variations, related to each individual (physiology and genetics etc.).

On the other hand, the current increase in temperatures, atmospheric CO₂ concentrations and nitrogen deposition can potentially affect trees growth. The increase of atmospheric CO₂ in particular is expected to favour tree growth, as it facilitates the physiological processes of carbon fixation, and potentially leads to an increase in plant water use efficiency (WUE), that is, the amount of carbon gained by plants per unit water lost (Franks et al., 2013). A popular method of studying water use efficiency is to use the stable carbon isotope composition of tree rings that allows to track the trend of WUE through the last decades (Bert et al., 1997). Such approaches reconstruct historical changes in WUE by looking backwards in time using individual tree ring series of big trees (Brienen et al., 2017).

The present study performs a dendroecological study based on tree-ring analyses and isotope analyses of WUE on a long-term refugial population of beech within the Ciron valley (department Gironde) in SW France. This population is likely to represent the remainder of a former glacial refugium that existed at exactly the same location, changing its function in the course of postglacial climate warming to become an interglacial refugium for the species at present (de Lafontaine et al., 2014 and chapter 1). The particularity of this study system makes it an instructive model for studying how marginal populations will respond to ongoing climate change. The impacts of climate change on rear edge population of beech have been recorded where beech is least suited to its environment (Jump et al., 2006; Piovesan et al., 2008). In such places beech is more exposed to global changes. However the studied population is present within a climate refugia, which is a site where the local conditions and the landscape

heterogeneity can buffer the effect of ongoing climate change. Thus, from that perspective and by using this beech stand as an empirically validated model for climate refugial tree populations, I performed dendroecological and dendroclimatological analyses with the objective to: **(i)** examine trends in tree growth and water-use efficiency through the past several decades, **(ii)** identify ecological drivers of tree growth and water-use efficiency, **(iii)** assess future trends in tree growth under predicted climate change.

2 SAMPLING DESIGN

The study site is spread linearly over 7 km on both sides of the Ciron River. This riparian forest is located on the slopes of the karstic canyon along the river with a maximal altitude around 30 m above the river level. The soil and the geological profile correspond to the limestone gorges where the Ciron and its tributaries have stripped the sand layer and flow directly onto the Miocene limestone bedrock (Genet, 2014).

Within the Gironde region the climate is temperate oceanic. The winters are mild and humid and the summers are relatively warm and dry. At local scales, we can notice that the winter is humid and mild and that the summer is dry with a deficit in water balance occurring from the beginning of May to the end of September (**Figure 9**, see subchapter 8 for more details about the climatic data and calculation).

I sampled 288 out of the 932 adult beech trees that I had analysed for the genetic studies described in chapters 1 and 2. Moreover, I added 25 subadult and 8 dead beech trees. Based on previous field observations, I used a threshold value of 70 cm for considering a tree as adult. Thus, a total of 321 beech trees were sampled all along the study site. In addition, 81 Pedunculate oaks (*Quercus robur* L.) were sampled within the study site.

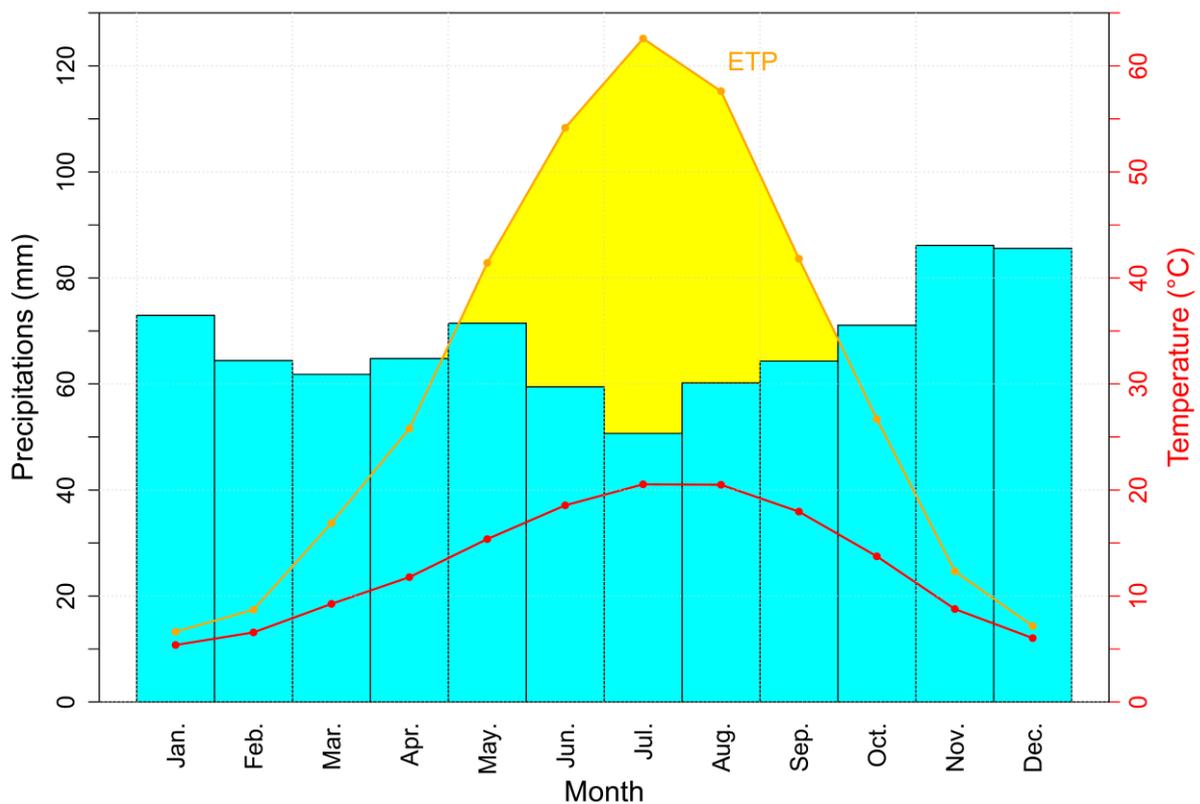


Figure 9. Climograph between 1897 and 2015 in the Ciron valley. The blue bars indicate the monthly average level of precipitation, the red curve indicates the mean temperature and the orange curve indicates the mean evapotranspiration (Thorntwaite, 1948). The yellow area shows a deficit in water balance.

This species is one of the dominant broadleaf species within the Ciron valley and allows to compare the growth of beech with another species under the same environmental conditions. On the other hand, I excluded from my analyses 4 cores of beech and 2 of oak due to the presence of break in the wood and to other technical problems. I hence ended up with an overall sample of 317 beech trees and 79 oaks distributed along the entire population (**Figure 10**).

One core was taken per tree using a 5-mm diameter increment borer and prepared using standard dendrochronology methods following the instructions of Lebourgeois and Mérian (2012). The coring was carried out parallel to contour lines of the terrain. I avoided any visible defects on the trunk (gellings, wounds etc.) and whenever possible also trees that presented multi-trunks or any shape deformation (leaning or tortuous trees).

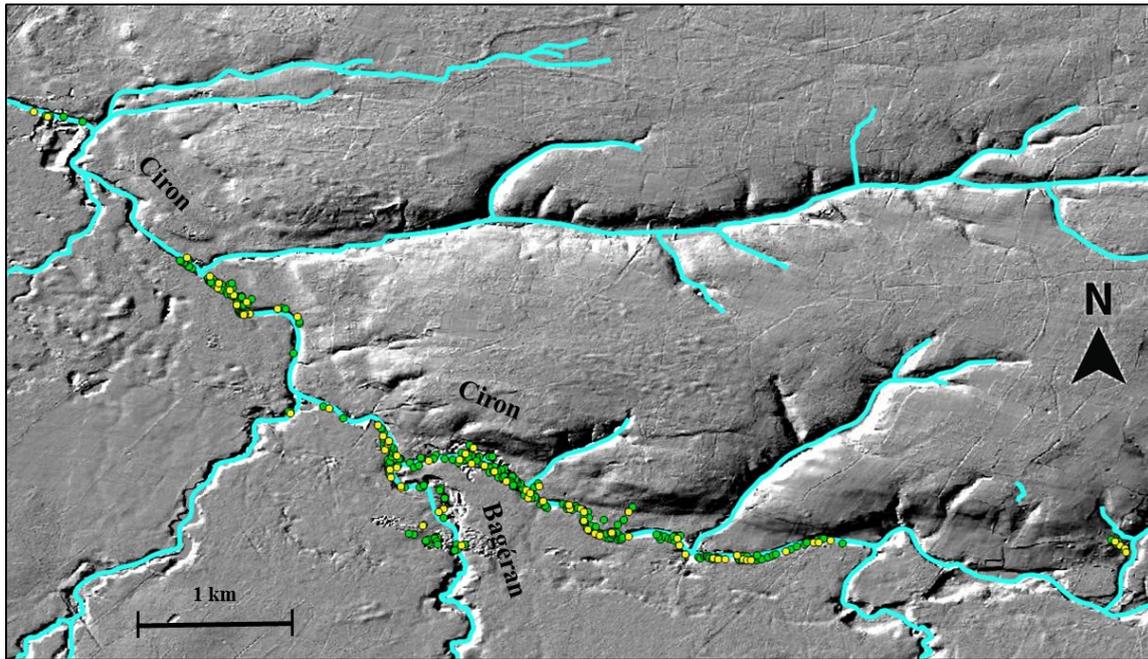


Figure 10. Trees sampled in the Ciron valley. Green dots indicate the 317 sampled beech trees and yellow ones indicate the 79 sampled oaks.

Sometimes the difficult access for some trees (strong slope, cliff etc.) forced me to be less demanding in my sampling (**Figure 11**). Then, I sampled 16 beech trees and 2 oaks with two trunks each. After the extraction of the core, the boreholes were filled with a mastic containing fungicide.

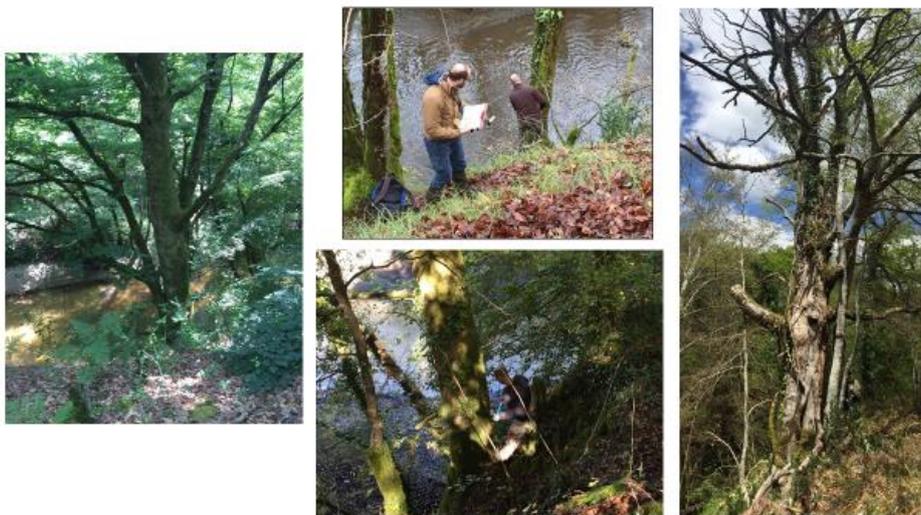


Figure 11. Photos taken during the sampling campaign.

In addition, a series of data were taken during the field sampling (**Figure 12**). For each tree, I recorded its circumference at breast height (C130) at 1.3 m above ground level. I also measured the following variables: 1) distance from the river, 2) altitude above the river, 3) slope of the growing place, 4) azimuth (classes north-east, south-east, south-west and north-west), 5) riverbank (right or left), and 6) topographical position (downslope, lower back slope, upper back slope and plateau, see **Figure 12**). The topographical classification was done in the field based on two criteria: the position of the tree in the valley and the water supply according to the slope. I considered a tree in a downslope position if it was located inside the valley near the river and if the water supply is greater than the water departure; a tree in the lower back slope if it was located inside the valley far from the river with the same amount of water supply and departure; a tree in upper back slope position if it was located inside the valley far from the river with a water supply lesser than the departure; and a tree on the plateau if it was located outside the valley with the same amount of water supply and departure.

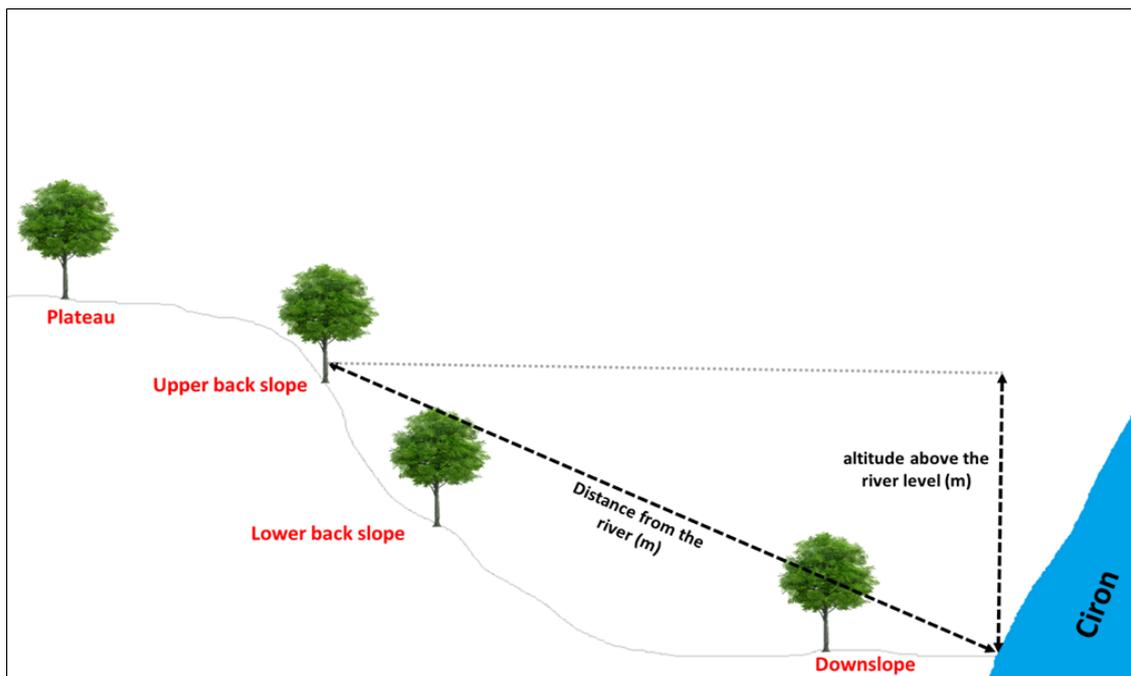


Figure 12. Station-specific data collected in the Ciron valley. Distance from the river (in meters), altitude above the river (in meters), and topographical position: plateau, upper back slope, lower back slope, downslope.

3 DENDROCHRONOLOGY METHODS

3.1 Sample preparation

Tree cores were glued if they were broken (this was common in oaks). Then cores were flattened with a blade in the laboratory. Beech have diffuse pore wood with little contrast between the initial and final wood and oak have wood with a porous initial zone with fairly identifiable tree rings. Moreover, the woody rays and the colour changes of the wood were sources of recurrent errors. Therefore, after flattening the cores tree rings were marked with a pencil under a binocular magnifier. This facilitated the counting of rings and measuring of their widths.

Once dried, cores were scanned at 1200 dpi (**Figure 13**). Then tree rings were counted and their width was measured using WinDENDRO version 2012 (Regent Instruments Inc.).



Figure 13. Photo of a beech core (A) and oak core (B) after flattening and marking the boundaries of tree rings, the bark was on the right.

3.2 Crossdating and pointer year calculation

A first version of the master chronology of tree-ring width as a function of years was calculated for each species using R version 3.3.1 (R Development Core Team 2016). A master chronology is an average tree-ring chronology of a species in a particular region that forms the reference against which new ring series may be compared and dated. The mean curve was used in WinDENDRO to do cross-dating. This step allows, by using some pointer years of high or low growth, to assign to each tree ring its actual year of formation. During the crossdating, I identified and eventually corrected some missing rings or false ring measurements. Then, a second version of the master chronology was calculated.

“Pointer years” are years where an abrupt growth, increase or decrease is recorded in a tree ring chronology relative to mean growth. They can be used to study the effect of

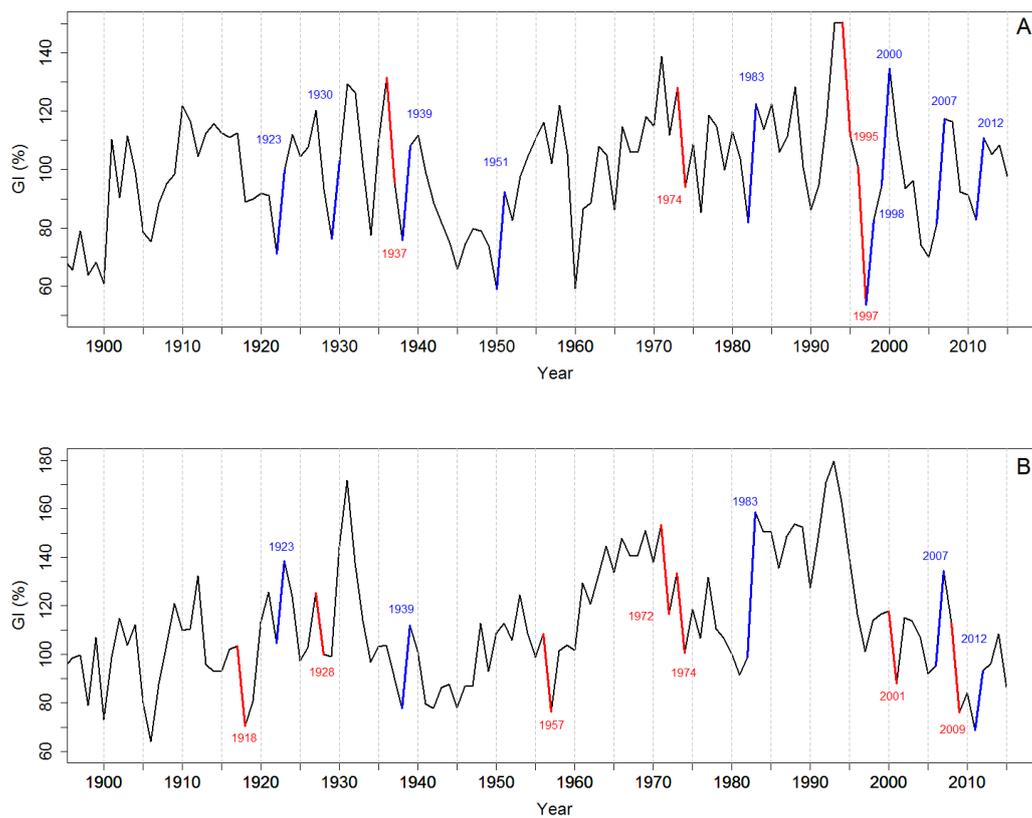


Figure 14. Master chronology as Growth Index (GI in %, i.e. subchapter 4 for GI calculation) in function of the year for Beech (A) and Oak (B) in the Ciron valley. Pointer years are indicated in the figure, with the years in red for the negative pointer years and in blue for the positive pointer years.

extreme climatic events (strong summer droughts, cold winter) on the growth of population in that year (Schweingruber et al., 1990). Pointer years were calculated from the raw data of crossdated tree-ring widths using the function *dendro* in the *dplR* package (Mérian, 2012a) in R version 3.3.1 (R Development Core Team 2016). This function uses the method developed by Becker (1989):

$$RD_{it} = \frac{RW_{it} + RW_{i(t-1)}}{RW_{i(t-1)}}$$

where RD_{it} is the relative difference in tree-ring width between the year t and the year $t-1$ and RW is the tree-ring width. A year t is considered as pointer year if it corresponds to the date at which at least 75% (threshold 1) of the trees show a relative change in growth of at least 10% (threshold 2) compared to the previous year.

A further cross-dating step with WinDENDRO was used for both species based on the second version of the master chronology as well as the pointer years (**Figure 14** and Appendix S1). This allowed to control all dating errors in each individual and to detect null tree rings in some particular years with an absence of tree ring production, increasing the representativeness of the master chronology of each species. Finally, a third check of crossdating on all the samples was done. Overall, much attention was paid to this step of iterative cross-dating, because no master chronology existed for beech and oak in South-West France.

3.3 Statistics of reference chronologies

We calculated two categories of growth series parameters: parameters at the population level and parameters at the individual level (Lebourgeois and Mérian, 2012). All stages of chronology building (and further analyses) were performed using R version 3.3.1 (R Development Core Team 2016) and the package dplR (Bunn, 2010).

The statistics crossdating coefficient, expressed population signal and signal-to-noise ratio were calculated at the population level.

- a. The crossdating coefficient (SR) corresponds to the extent of synchronism of the elementary series from which the master chronology is derived. It represents the ratio of the average sensitivity calculated directly on the master chronology (MS_m) to the average of the mean sensitivities calculated on the corresponding elementary series (MS_i), with

$$SR = \frac{MS_m}{MS_i}$$

A SR value close to 1 express maximum synchronism. Complete asynchronism is expressed by values that depend on the size of the analysed sample. For a group of n tree-ring series, a value of SR equal to $\frac{1}{\sqrt{n}}$ would correspond to a series whose ring-width would vary randomly (Munaut, 1966; Munaut 1978; Schulman, 1956). In my study system, for the 317 beech trees sampled $MS_m = 0.144$ and $MS_i = 0.289$, which means that $SR = 0.498$ and $\frac{1}{\sqrt{n}} = 0.056$. As SR is much bigger than $\frac{1}{\sqrt{n}}$, the variations are not

random. The same occurs with the 79 oaks, for which $MS_m = 0.145$ and $MS_i = 0.243$: $SR = 0,597$ and $\frac{1}{\sqrt{n}} = 0.112$ (**Table 3**), implying that the variations are not random. Weber et al. (2013) found a level of SR between 0.653 and 0.806 for beech in Switzerland, which is in accordance with my study.

- b. The Expressed Population Signal (EPS) quantifies the degree to which the chronology expresses the population chronology (Wigley et al., 1984). The EPS was defined as

$$EPS = \frac{r_{bt}}{r_{bt} + \frac{1 - r_{bt}}{N}}$$

where N is the number of cored trees per plot, and r_{bt} is the mean intertree correlation that quantifies the strength of the signal common to all trees (Briffa and Jones, 1990). Therefore r_{bt} estimates the strength of the common signal to all trees, and $1 - r_{bt}$ estimates its noise. EPS estimates the proximity between the theoretical population chronology and the chronology obtained by averaging the N sampled individual chronologies by reducing the uncommon variability of the N chronologies from $1 - r_{bt}$ to $\frac{1 - r_{bt}}{N}$ (Briffa and Jones, 1990). EPS ranges from 0 to 1 and yields 1 when the chronology mirrors the population signal. Though a specific range of EPS values constituting acceptable statistical quality cannot be given, Wigley et al. (1984) suggested a threshold of 0.85 as reasonable. For beech in the Ciron valley, $r_{bt} = 0.283$ and $EPS = 0.981$ and for Oak $r_{bt} = 0.264$ and $EPS = 0.938$ (**Table 3**). In both species, EPS values are higher than 0.85 and tend towards 1, reflecting low noise and a reliable estimate of the signal common to all trees. The EPS value of my study is in accordance with other studies in Europe for beech (Lebourgeois et al., 2005: **0.963 < EPS < 0.993**; Scharnweber et al., 2011: **0.96 < EPS < 0.98**; Weber et al., 2013: **0.884 < EPS < 0.939**; Cavin and Jump, 2016: **0.84 < EPS < 0.98**) and for oak (Rozas, 2005: **0.895 < EPS < 0.931**; Scharnweber et al., 2011: **0.94 < EPS < 0.98**).

- c. The Signal to Noise Ratio (SNR) is the proportion of explainable variation (due to climate or other causal factors) divided by the unexplainable or residual variation:

$$SNR = \frac{N_r}{1 - r}$$

where r is the average correlation between trees and N is the number of trees within a site chronology. This parameter is an expression of the strength of the observed common signal among trees (Wigley et al., 1984). SNR has no upper bounds and this is clearly a difficult quantity to interpret (Cook and Kairiukstis, 1990). In the Ciron valley, the value of SNR for the sampled is 52.09 beech and is 15.1 for oak (**Table 3**). This is in agreement with previous studies in Europe for beech (Dittmar et al., 2003: $7.9 < \text{SNR} < 15.1$; Lebourgeois et al., 2005: $25.8 < \text{SNR} < 141.4$) and for oak (Rozas, 2005: $8.55 < \text{SNR} < 13.57$).

On the other hand, the mean sensitivity, the first-order correlation (Fritts, 1976) and the Gini coefficient, (Gini 1912 in Biondi and Qeadan, 2008) were calculated for each individual tree of both species and averaged per population. The three indices were calculated via the function *dendro* within the dplR package (Bunn, 2010) in R version 3.3.1 (R Development Core Team 2016).

- d. The mean sensitivity (MS) measures the year-to-year variability and expresses the extent of the short-term changes affecting the width of the tree rings. It was calculated to estimate the average variation between the widths of two successive annual tree-rings (**Figure 15**). The average MS value was 0.289 for beech and 0.243 for oak (**Table 3**), consistent with previous studies for beech (Dittmar et al., 2003: $0.27 < \text{MS} < 0.36$; Lebourgeois et al., 2005: $0.199 < \text{MS} < 0.319$; Scharnweber et al., 2011: $0.2 < \text{MS} < 0.27$;

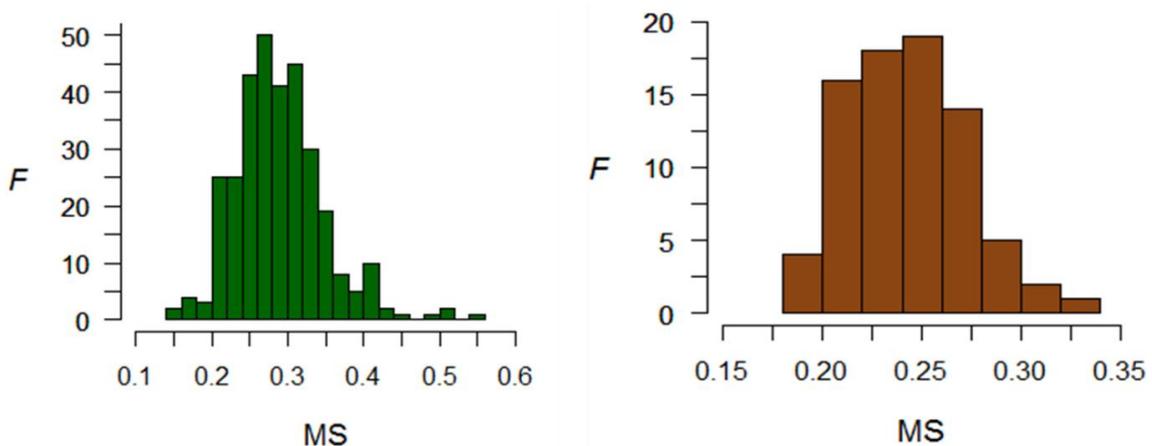


Figure 15. Histogram representing the frequency (F) of trees mean sensitivity (MS) for Beech (green) and Oak (brown) in the Ciron valley.

Weber et al., 2013: **0.222 < MS < 0.294**; Cavin and Jump, 2016: **0.15 < MS < 0.35**) and for oak (Rozas, 2005: **0.182 < MS < 0.196**; Scharnweber et al., 2011: **0.15 < MS < 0.21**).

- e. The first-order autocorrelation was calculated to estimate the interdependence between two successive tree-rings of the same chronological series, that is, to quantify the effect of persistence linked to the conditions leading to the formation of the ring of the year (t) on the setting of the next year's ring (t + 1) (Lebourgeois and Mérian, 2012). It corresponds to the correlation coefficient of the simple linear regression between the ring width series of a given tree and this same series shifted by one year. The average value of AR was 0.248 for beech and 0.213 for oak (**Table 3**), that is, smaller than those in previous studies for beech (Dittmar et al., 2003: **0.61 < AR < 0.79**; Lebourgeois et al., 2005: **0.301 < AR < 0.665**; Scharnweber et al., 2011: **0.554 < AR < 0.630**; Weber et al., 2013: **0.55 < AR < 0.70**; Cavin and Jump, 2016: **0.38 < AR < 0.84**) and for oak (Rozas, 2005, 2005: **0.567 < AR < 0.562**; Scharnweber et al., 2011: **0.593 < AR < 0.752**). However, AR values were significant for many trees in both species (**Figure 16**).

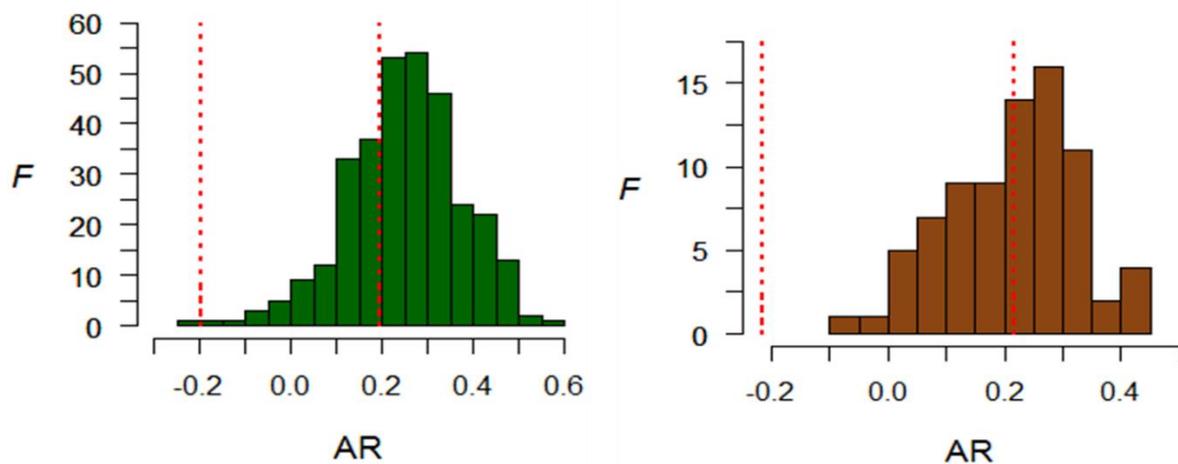


Figure 16. Histogram representing the frequency (F) of trees first-order autocorrelation (AR) for Beech (green) and Oak (brown) in the Ciron valley with their upper and lower 95% confidence intervals of the null distribution (red dotted line).

- f. For discrete data such as tree-ring widths data, the Gini coefficient (G) is the sum of the absolute values of the differences between all pairs of observations, weighted by the mean and the sample size (Biondi and Qeadan, 2008b). It varies between 0 (perfect equality between years) and 1 (one year of growth and all other with zero growth). The

higher the value, the greater is the inequality. We adopted in my analysis the equation taken from Weiner and Solbrig (1984):

$$G = \frac{1}{2n \sum_{i=1}^n x_i} \sum_{i=1}^n \sum_{j=1}^n |x_i - x_j|$$

The average value of G was 0.161 for beech and 0.141 for oak (**Table 3, Figure 17**). This indicates that the degree of heterogeneity in the ring series was low.

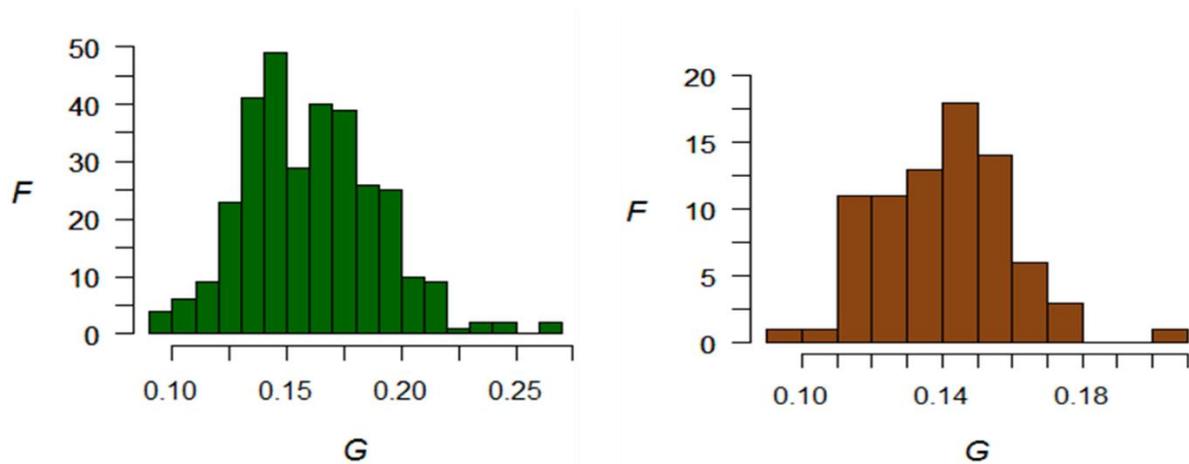


Figure 17. Histogram representing the frequency (*F*) of trees gini coefficient (*G*) for Beech (green) and Oak (brown) in the Ciron valley.

The high values of MS, EPS and SNR ratio of the tree ring series indicate that the ring width of beech and oak in my study system is a very sensitive parameter clearly reflecting the signal of exogenous influences. This is in accordance with previous studies, which showed high relevance of beech and oak tree rings for dendroclimatological analyses across Europe (Dittmar et al., 2003; Lebourgeois et al., 2005; Rozas, 2005; Scharnweber et al., 2011; Weber et al., 2013; Cavin and Jump, 2016). Values of all three parameters were higher for beech than for oak, indicating a greater sensitivity of the former species. This result is not surprising given that beech is known to be more drought sensitive than oak, exhibiting larger drought effects in the leaves, stem and roots (Scharnweber et al., 2011). The results of my study are comparable to those found for beech forests in Central Europe at low altitude (Dittmar et al., 2003). Conjointly, these studies specify sufficient water availability, especially during summer of the current and the previous year, as favourable for the formation of wide rings. However, in my case of study the influence of the previous year's growth upon the current year's growth

is lower than reported in the cited studies. This questions the importance of the climate variable of the previous year's growth on wood formation in my study system. Thus, the high values of mean sensitivity for beech and oak in the Ciron valley, could be interpreted as a high susceptibility to external influences, and the low values of first order autocorrelation as a low buffering capacity (Gillner et al., 2013).

3.4 Basal Area Increment

Tree ring width was converted to tree ring surface, or Basal Area Increment (BAI in cm²), according to the following standard formula:

$$\text{BAI} = \pi (R_t^2 - R_{t-1}^2)$$

where R is the radius of the tree (in cm) and t is the year of tree ring formation.

The reason for such converting is that as trees grow older and wider, annual ring width generally decreases along a cross-sectional radius, because of the geometrical constraint to add new wood layers over an expanding surface (Fritts 1976; Cook 1987). Converting tree ring width to tree ring surface is a proper way to overcome this problem. Indeed, given that the trunk has an approximately circular shape, a surface (two-dimensional) measure such as BAI or ring area represents overall tree growth (a volume, or three-dimensional measure) better than a linear (one dimensional) measure such as stem diameter increment or ring width (Biondi and Qeadan, 2008a). For multi-trunk trees, the BAI of each trunk of the same individual was calculated separately and summed over all trunks.

For a correct estimation of BAI and the cambial age, the radius must be taken into account from the pith. As the coring often misses the pith, it is necessary to estimate the distance between the last rings measured in the centre and the pith. This distance is estimated with a simple geometric system by using a transparent sight on which concentric rings are drawn. I attempted to find the ideal position of the core under this sight by making the rays of curvature of the rings coincide with those of the circles of the sight. This distance is then converted to the number of rings by dividing this length by the average width of the last five rings measured (Lebourgeois and Mérian, 2012). This step allows to estimate the cambial age for each core which is the years since pith formation.

3.5 Tree age estimation

The exact determination of the age of a tree can only be made at the level of the first growth from the seed. In general, this shoot is short and is at ground level, or only a few cm above this level. Thus, by counting tree rings at this level, this may correspond better to the number of years of growth from the seed. In most cases, the trees studied are not cut and their growth is studied using coring samples. This type of sampling does not give the exact age of the tree, because the core height is generally around 1.30 m. This prevents me from taking the rings formed during the time that the tree has set to reach the height of 1.30 m. It is therefore necessary to estimate the growth rate in height during the youth, to add the estimated number of years to the age of core in order to obtain the actual age of the tree (seed age). Here I used three approaches to solve this problem. The first approach was to count the number of tree rings at different heights of one same stem; the second approach was to estimate the growth rate of 6 small beech trees by measuring their height and determining their age with a micro core sample; and the third one was to estimate the actual number of years spent on two cores taken from the same tree, one at the base and one at a height of 1.30 m. All three approaches produced convergent results and indicated that it is necessary to add 10 years to the estimated age at the coring height of 1.30 m to estimate the real age of a beech in the Ciron valley. This result is consistent with the study of Trotsiuk et al. (2012) on a refugial beech forest in the Carpathian Mountains, who added 11 years.

The average age of the sampled beech trees is 99.2 years with a range from 32 to 205 years (**Table 3**). This is consistent with other studies on the same species (Dittmar et al., 2003: mean age of studied stands between 100 and 250; Lebourgeois et al., 2005: mean age of studied stands between 54 and 160; Jump et al., 2006: mean age of 110.5, 94.6 and 92.1 with range from 50 to 236, from 57 to 143 and from 50 to 119, respectively; Scharnweber et al., 2011: mean age of studied stands between 125 and 140; Weber et al., 2013: mean age of studied stands between 118 and 159; Cavin and Jump, 2016: maximum age around 250). My result is mainly close to those at the south margin of the species distribution (Dittmar et al., 2003: Italian and Spanish stands; Lebourgeois et al., 2005: stands in southwestern France; Jump et al., 2006). For the sampled oaks, the average age in 2015 is higher than that of beech (mean: 113.6 years, range from 47 to 245 years). In some case of study the maximal age can

go to 471 years old (Rozas, 2005: age range between 24 and 471) and in another case of study is quite similar (Scharnweber et al., 2011: mean age of studied stands between 130 and 210). Thus, in Scharnweber et al. (2011) the mean age of beech and oak stand located in the same site are similar in 2 of 3 study sites and different only in one.

Overall, a decrease in the frequency of trees older than ca. 70 years for beech and 80 years for oak was observed in the Ciron valley (**Figure 18**). This can be explained by the fact that these populations are little managed and regenerate naturally. This natural decline may indicate that mortality of established trees is likely to occur primarily at after 70 and 80 years old, respectively for beech and oak. This is confirmed by observation in the field and by personal communication (A. Ducouso, S. Irola and A. Quénu). Importantly, it should be noted that, the lack of subadult trees, younger than 30 years old for beech and 40 years old for oak,

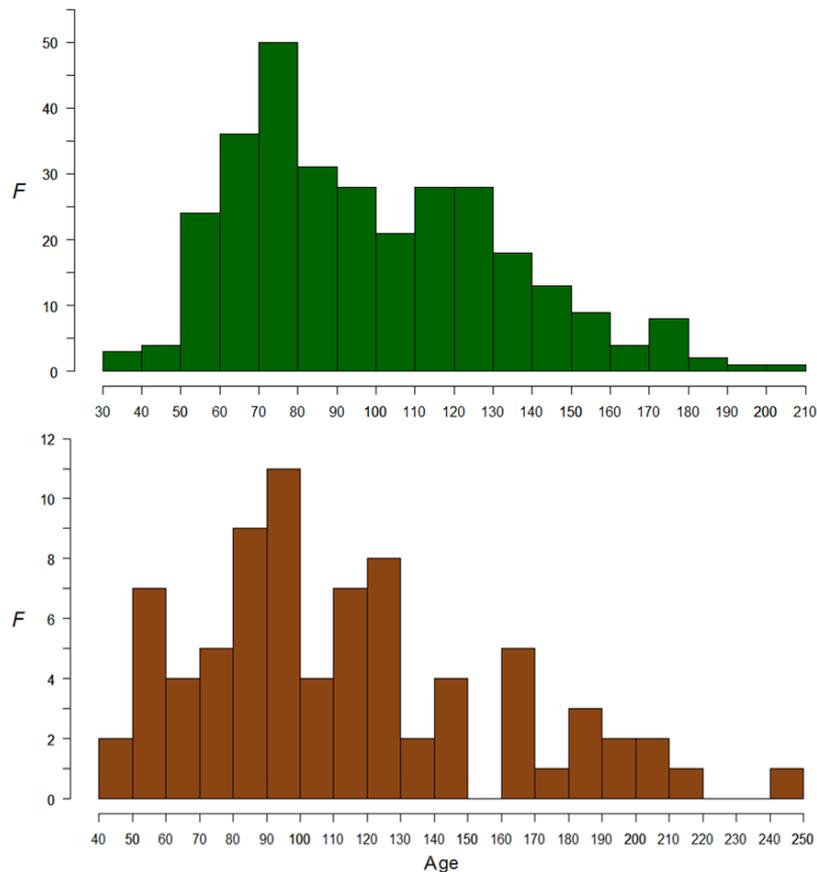


Figure 18. Histogram representing the frequency (F) of tree age in year for 317 beech trees (green) and 79 oaks (brown) in the Ciron valley.

is due to my sampling scheme. Indeed, here I studied and modelled the growth of adult trees in the Ciron valley.

Studying dead trees can give us some information on certain demographic aspects of the studied population, including the last part of the histogram of beech age and the possible cause of mortality of trees in this stand. I was able to detect eight recently dead adult trees whose wood was still analysable. These trees had an average C130 of 184 cm with a range between 125 and 250 cm and an average age of 141.4 years old with a range between 96 and 205 years old.

When placing these dead trees in the cloud of points of all sampled beech trees (**Figure 19**), we can notice that the dead individual are among the largest and above the curve, that is, older than the average for a given size. This indicates that they had grown more slowly than the average. In addition to this common point, I found no single general scheme for the dead trees. They are not necessarily the oldest or the biggest trees. We can also notice that death may occur at different ages. This is consistent with my results in (**Figure 18**) and the field observations (A. Ducouso, S. Irola and A. Quénu) as previously discussed. Furthermore, the date of climatic stressful years that seem to initiate a period of growth decline is not the same for all trees (**Table 2**): 1980, 1999, 2002, 2005, and 2011 (dry years) and 2013. Also, the duration of the growth decline before death varies from 1 to 27 years. Often, the decay of a tree extends over ten years.

Table 2. Table summarizing information on dead beech trees. Id = individual name, C130 = circumference at breast height in cm, tree age in year, stress date, last tree ring produced and die-off time in year. Reminder: date of coring = 2015.

Id	C130	Tree age	Stress date	Last tree ring	Die-off time
H484	96	125	2005	2008	4
H628	109	151	2002	2003	2
H622	124	208	2002	2014	13
H554	134	170	1980	2007	28
H071	150	179	2013	2013	1
H123	155	184	2011	2013	3
H620	158	250	1999	2008	10
H602	205	205	2002	2009	8

These various observations suggest a role of certain climatic stressors on the decay of these trees. However, the relationship is not very clear, especially because in some cases I observed some alive trees of similar size in close vicinity to the dead. Moreover, two of the dead trees were located next to the river, so droughts alone should not have affected them as much. It is possible that these individuals had an intrinsic potential that did not allow them to withstand stress, or that biotic factors (such as fungi) affected them more than their neighbours. On the other hand, a storm destroyed many large beech trees in 1999. Indeed, I also saw two windthrows during my field phase, one in autumn 2015, the other in spring 2016 in the upstream part of the population on a very steep slope. The common feature of these two trees is a weaker growth than the average. However, many living trees have the same characteristics as those who died and they are right next to them. In the end, tree mortality does not seem to follow a single pattern and various factors can intervene on standing beech trees such as pathogens, storm, drought etc.

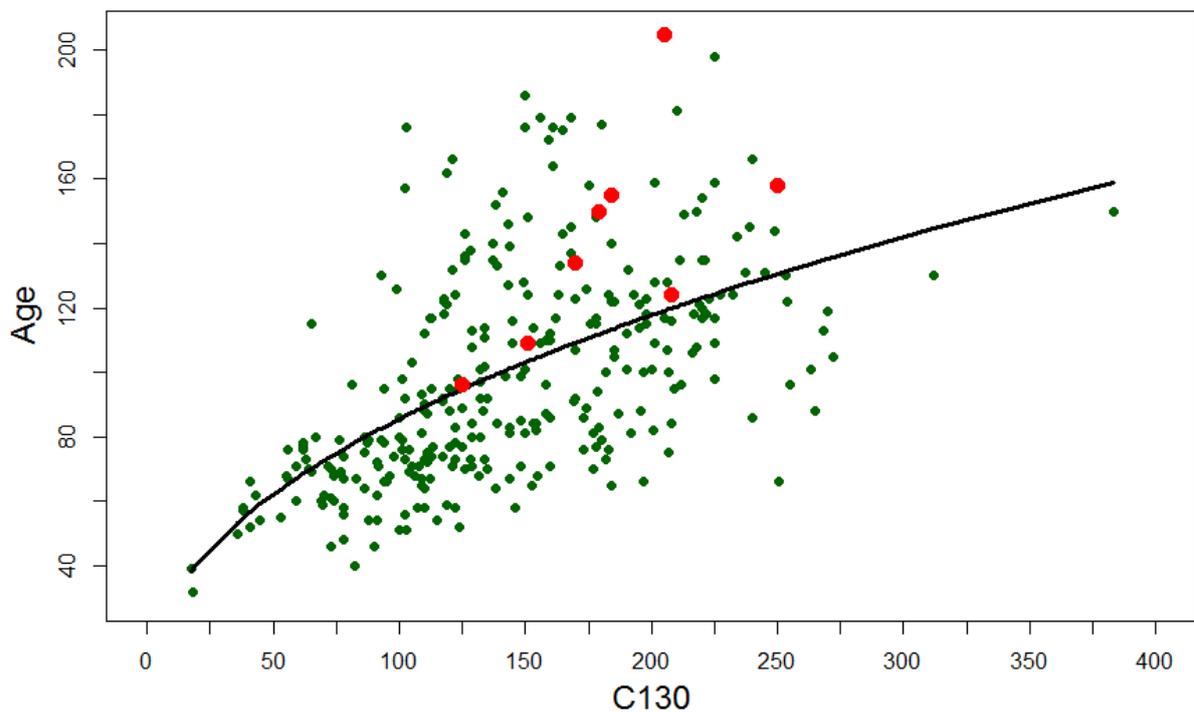


Figure 19. Tree age in year as function of circumference at breast height (C130) in cm of 317 beech trees (green dots). The black curve represent the adjustment of tree age as a function of C130 according to this equation $\text{Tree age} = 10.198 \times \text{dbh}^{0.462}$. Red dots indicate the eight sampled dead beech trees.

The relationship between the age of the tree and its C130 for beech trees (**Figure 19**) and oak (**Figure 20**) showed that the older a tree the bigger it is. However, a big tree is not necessarily old. For example at a tree age of 100 years, the C130 can range from 50 to 250 cm. Inversely a tree with an C130 of 200 cm can be between 60 and 200 years old. It will therefore be tricky to convert the inventory of circumference into an inventory of ages, for instance for my exhaustive genetic sampling (n = 932 beech trees). This is why I used cores as my only material to study the distribution of tree ages.

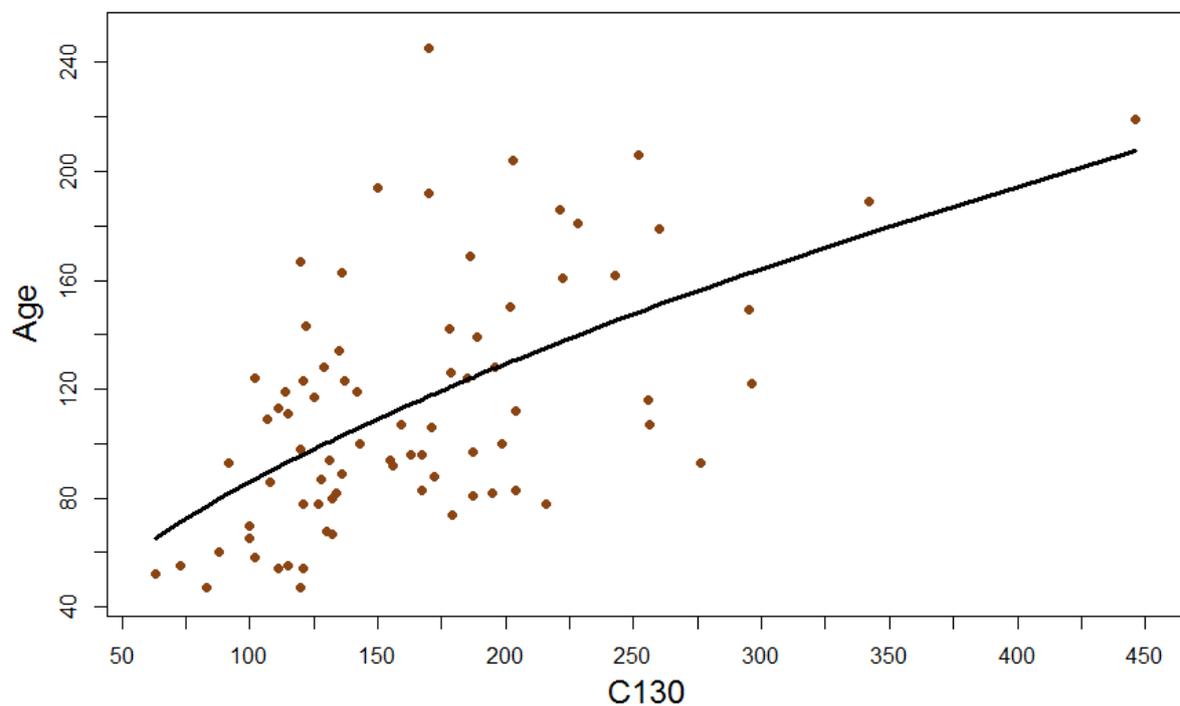


Figure 20. Tree age in year as function of circumference at breast height (C130) in cm of 79 oaks (brown dots). The black curve represent the adjustment of tree age as a function of C130 according to this equation $\text{Tree age} = 5.642 \times \text{dbh}^{0.591}$.

The average C130 (**Table 3**) of beech is equal to 144.5 cm with a range from 17.4 to 383.0 cm. This is consistent with the results of other studies on the same species (Lebourgeois et al., 2005: mean C130 of studied stands between 91.1 and 157.1; Jump et al., 2006: mean C130 of 177.8, 148.6 and 161.2 with a range from 88.6 to 370, from 81.1 to 208 and from 104 to 214 respectively; Weber et al., 2013: mean C130 of studied stands between 87.9 and 179.1). For oaks, the average C130 of sampled beech trees was equal to 165.5 cm with a range from 63.0 to 446.0 cm. Van der Werf et al. (2007) found a C130 for *Q. robur* between 44 and 116.2 cm for trees aged between 50 and 80 years old. This result is similar for that same class of age in

the Ciron valley (**Figure 20**). In all the studies found in literature they measured the diameter at breast height (dbh); therefore I converted the dbh to C130 according to this formula $C130 = \pi \times dbh$, to make the comparison.

3.6 Tree productivity

A subsample of 12 beech trees, with a range of age between 39 and 181 years old, was taken to get a raw estimate of the productivity level in this population. The height of these trees was measured in the field with LTI laser telemeter and linked to the production tables for beech (Teissier du Cros et al., 1981). The average height of the 12 beech trees was 18.8 m (**Table 3**). My sampling was not intended to measure the level of productivity as in the production tables. In this case, one should sample the $n \times 100$ biggest trees at breast height on a plot of $n \times 100 \text{m}^2$ units (Pardé, 1956). Indeed, within my sample the chosen trees were generally dominant but the number was not sufficient. Instead, I just wished to have a preliminary idea of the productivity of the Ciron beech population in compared to other regions of France (**Figure 21**).

The curve obtained with the sampled trees indicates that the height is about 19 m for 100-years-old trees (**Figure 21**). This level of productivity is included between class 3 (17.9 m to 100 years) and class 5 (24.4 m to 100 years) for northeastern France (Teissier du Cros et al., 1981). Thus, we have a class 4 with an average production of $4 \text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ (**Figure 21 A**). Similarly, if we take the tables for northwestern France, we find that the production level of beech in the Ciron valley corresponds to class 4 (height of 19.4 m at 100 years (Teissier du Cros et al., 1981) (**Figure 21 B**). However, there is no tables for southwestern France. Hence, the productivity of the Ciron beech population is very low compared to the northern parts of France. This result is not surprising given the climatic conditions and the heterogeneous landscape of the site.

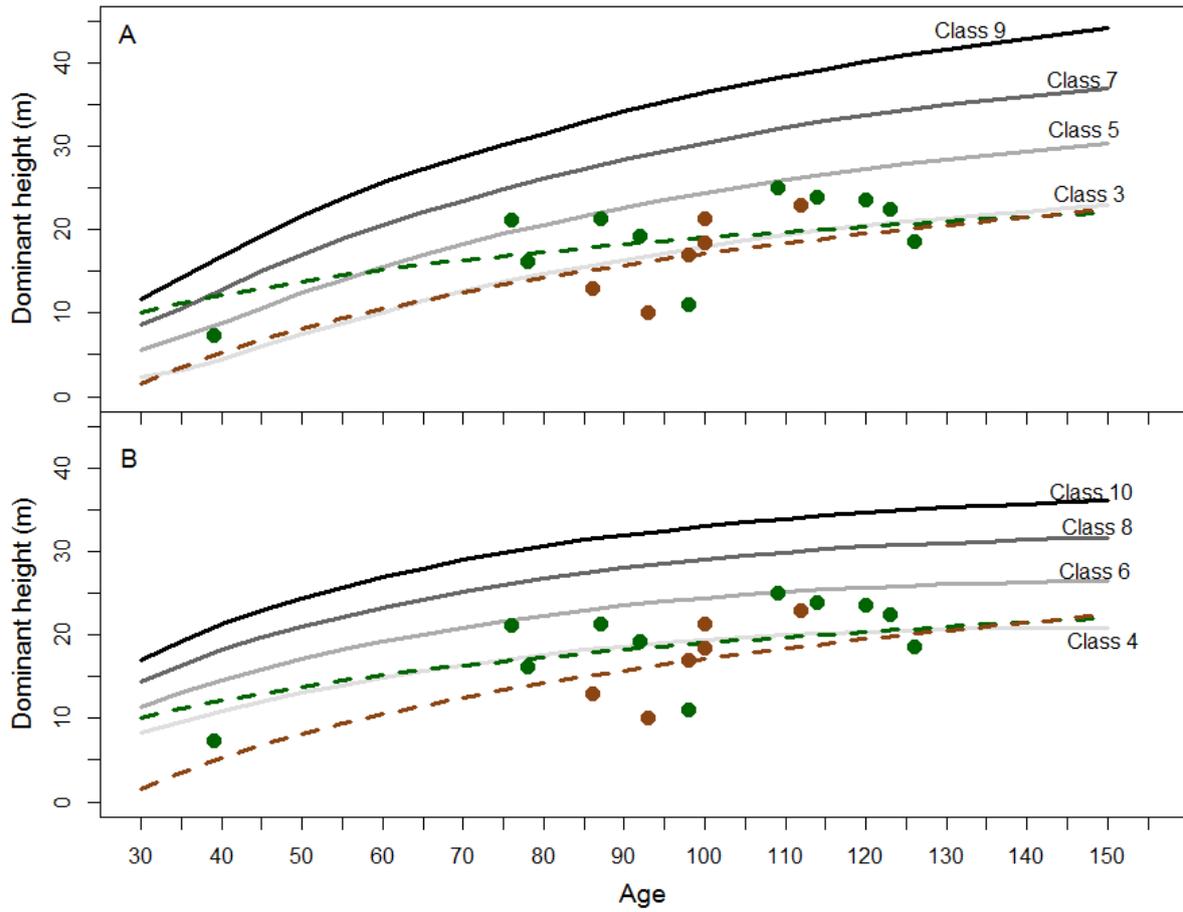


Figure 21. Dominant height (m) as function of the tree age (year) of different classes taken from Teissier du Cros et al., 1981. (A) for northeastern France and (B) for northwestern France. Each shade of grey indicate a class of productivity in both graph (A) and (B). Green dots indicate the height of 12 beech trees and the brown dots indicate the height of 8 oak trees sampled in the Ciron valley. The dashed curves represent an indicative nonlinear regression for the sampled beech trees (green) and for the sampled oak trees (brown).

4 STANDARDIZATION

One of the main objectives of dendrochronological standardization is to remove the progressive decline of ring width along a cross-sectional radius that is caused by the corresponding increase in stem size and tree age over time (biological trend; Biondi and Qeadan, 2008a). The problem of increase in stem size is overcome by transforming RW into BAI, and the evolution due to age can be solved by standardization methods.

Standardization methods are based on the elimination of signals considered as noise, in order to preserve and to filter out consistent signals of biological relevance. It is therefore essential to correctly target the frequency of the signal to be studied. Classically, the study of tree ring-climate relations focuses on high-frequency signals, which are supposed to reflect interannual climate variations. Low and medium frequency signals must be estimated and extracted from the raw series (Lebourgeois and Mérian, 2012).

Many techniques require the elimination of the biological trend by fitting a curve to the raw ring width measurements. Standardized indices are then computed as the ratios between the measurement and the fitted curve value. Estimation techniques are numerous and widely described in the literature. In my study, I adopted the Regional Curve Standardization method (Becker, 1989; Esper et al., 2003) to detrend the chronologies from the pith to the bark. This method computes the expected value of the tree-ring BAI as a function of cambial age (that is, years since pith formation), then to use the resulting growth curve to standardize the individual tree-ring series. For beech (**Figure 22**), I used a quartic polynomial function determined by a progressive multiple stepwise regression for the increasing part of the curve, where tree rings are younger than 77 years old.

Eq. (age ≤ 76)

$$BAI_{\text{adjusted}} = 2.40e^{-1}Age + 2.18e^{-2}Age^2 - 4.53e^{-4}Age^3 + 2.46e^{-6}Age^4$$

Also I used an exponential function to detrend the series for the decreasing part of the curve where tree-rings are 77 to 155 years old.

Eq. (76 < age ≤ 155)

$$BAI_{\text{adjusted}} = e^{6.73583 - 0.78752 \log(Age)}$$

The BAI curve as a function of age fluctuates markedly as soon as the number of rings falls below 15. This corresponds to a cambial age greater than 155 years old. For this purpose, an extrapolation of the same exponential equation above was used for the cambial ages greater than 155.

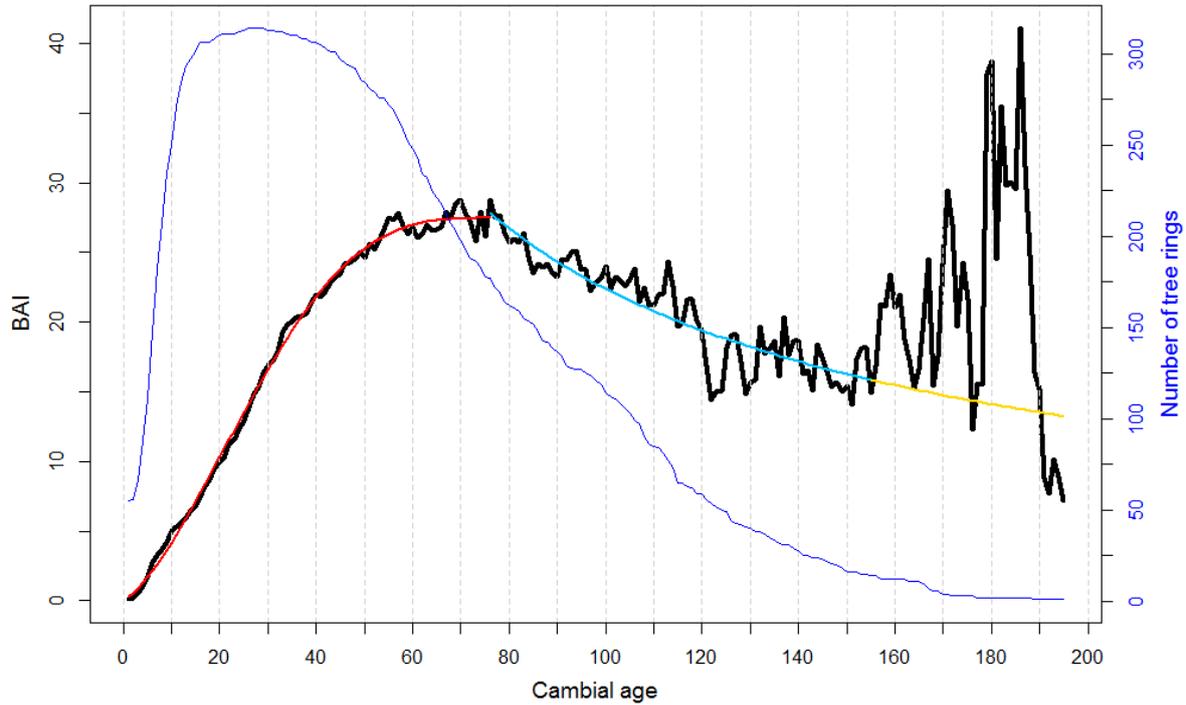


Figure 22. Average BAI (cm²) as function of cambial age (year) for the 317 beech trees sampled in the Ciron valley. The red line indicates the quartic polynomial function used for the cambial age between 0 and 77 years. The sky-blue line indicates the exponential function used for the cambial age between 77 and 155 years. The yellow line indicates the extrapolation of the same exponential function of cambial ages higher than 155 years. The blue curve indicates the number of tree rings for each cambial age.

The same procedure was applied to the oaks (**Figure 23**). The chronology was detrended using a square function for tree rings younger than 51 years old.

Eq. (age ≤ 50)

$$BAI_{\text{adjusted}} = 7.43e^{-1}Age + 7.16e^{-3}Age^2$$

I used an exponential function for tree rings between 51 and 83 years old.

Eq. (50 < age ≤ 82)

$$BAI_{\text{adjusted}} = e^{3.4057 - 0.1154 \log(Age)}$$

The same as beech, the BAI curve as a function of age fluctuates clearly after an age of 82 years with an unusual increase in the curve between 82 and 155 years. This may be related to the small sample number, and/or age imbalance (Lebourgeois and Mérian, 2012). For this

reason, an extrapolation of the same exponential equation above was used for the cambial ages > 82 years for the oak sample.

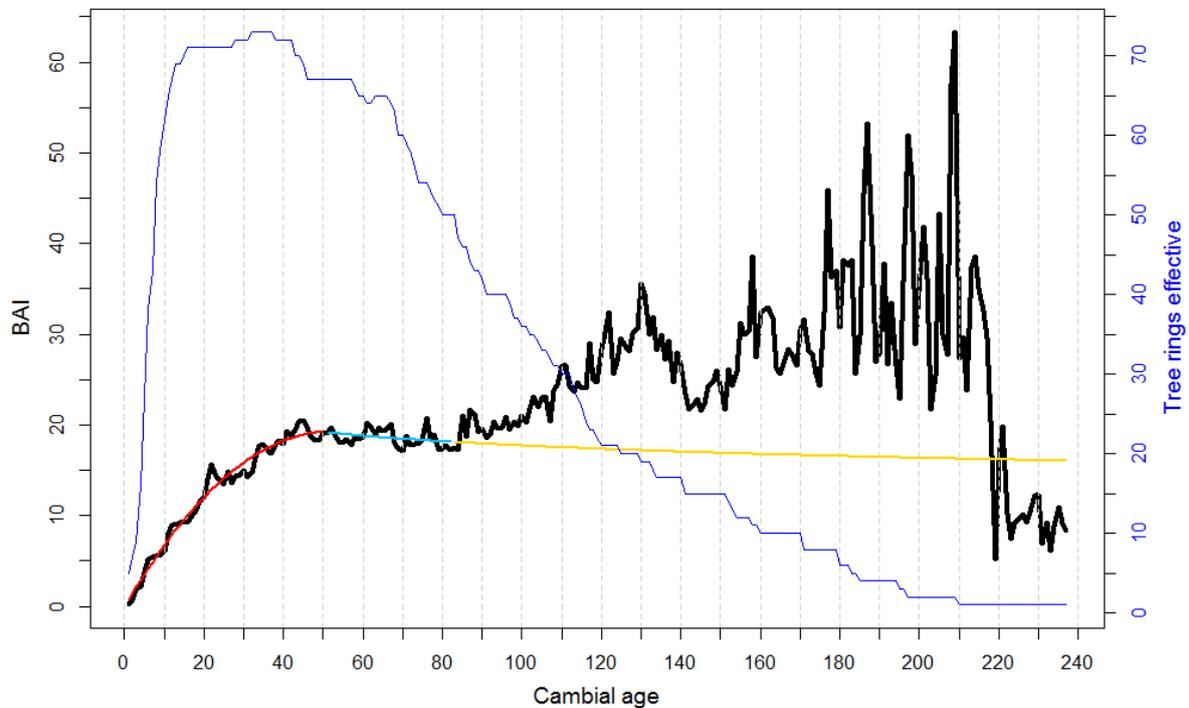


Figure 23. Average BAI (cm²) as function of cambial age (year) for the 79 oaks sampled in the Ciron valley. The red line indicates the square function used for the cambial age between 0 and 51 years. The sky-blue line indicates the exponential function used for the cambial age between 50 and 83 years. The yellow line indicates the extrapolation of the same exponential function of cambial ages higher than 82 years. The blue curve indicates the number of tree rings for each cambial age.

This step allows to remove long-term trends related to ageing and disturbances (Cook and Kairiukstis, 1990). These equations allow to convert BAI to a Growth Index (GI), which is typically measured in percent:

$$GI_t = 100 \frac{BAI_t}{BAI_{model}}$$

where t is one year and BAI_{model} is the value of the detrended curve for a defined ring cambial age.

Afterwards, the mean curve of GI was plotted as a function of date and called “master chronology”. Finally, I used a smooth curve to detect a trend in the curve according to date.

Table 3. Summary of statistical properties of the raw tree-ring series for beech and oak in the Ciron valley.

	Beech	Oak
Mean TRW (mm)	2.506	2.245
S.D. TRW (mm)	0.902	0.759
Mean BAI (cm²)	18.981	17.848
Mean Sensitivity (MS_i)	0.289	0.243
Crossdating coefficient SR (=MS_m/MS_i)	0.498	0.597
Mean r_{bt}	0.283	0.264
Expressed Population Signal (EPS)	0.981	0.938
Signal to Noise Ratio (SNR)	52.09	15.1
1st order Autocorrelation (AR)	0.248	0.213
Gini coefficient (G)	0.161	0.141
Number of trees	317	79
Mean age (years)	99.2	113.6
Age range of trees (years)	32-205	47-245
Mean C130 (cm)	144.5	165.5
Mean height (m)	18.8 ^a	19.2 ^b

^a subsample of 12 beech trees

^b subsample of 8 oaks

5 FACTORS STRUCTURING TREE GROWTH

Many factors, biotic or abiotic, can affect trees (Fritts, 1976; Cook, 1985) and their responses to the surrounding environment. These can be modulated by station-specific determinants in addition to determinants specific to each tree. My study site is a climate refugia with considerable small-scale heterogeneity in landscape and local conditions. Also, the evolutionary history of the studied beech population reflects a certain peculiarity at the genetic level (see chapter 1). It therefore is important to assess the direction and the amplitude of the local ecological conditions effects and some aspect of intrinsic effects on beech growth in the Ciron valley.

For this aim, I constructed two separate mixed effects models for each species with the mean growth index of the last twenty years (GI_{last 20 years}) and mean sensitivity (MS) as

dependent variables. $GI_{\text{last 20 years}}$ is the mean growth index for each sampled tree for the last twenty years of the studied chronology from 1996 to 2015. Given that the youngest trees that I sampled were 32 (beech) and 47 (oak) years old, my choice of the last 20 years of the chronology did not exclude any tree from the analysis. Moreover, GI is assumed to be free from age effects but not from date effects. The fact that the magnitude of climate change is relatively minor at 20 years of scale makes my choice of the mean GI for the last 20 years reasonable and could represent the growth rate of each individual tree. On the other hand, MS represents the mean sensitivity or the year-to-year variability (i.e. subchapter 3.3) calculated for each tree on the entire chronology of the corresponding tree core.

The altitude of the tree above the river, its topographical position and its location on the left or right riverbank (see subchapter 2) were included as fixed factors. Moreover, I calculated a variable from each tree's GPS coordinates position that represents its position, in one dimension, along the valley (see below). This variable was designated as "adjusted position" and was also included as fixed factor in the models. Finally, trees were included in the models as random factor. The linear shape of the population in the valley allowed me to calculate the "adjusted position" of each tree. Knowing the coordinates of one tree, I could calculate its coordinates on a line that adjusts the orientation of the valley as a regression line of latitude as a function of longitude:

$$\text{latitude} = a \times \text{longitude} + b,$$

with $a = 0.664$ and $b = 0.666$. Then, the new coordinates (X_2, Y_2) of a point (X_1, Y_1) projected on the line are of the form:

$$X_2 = X_1 - d \times \theta_x$$

$$Y_2 = Y_1 + d \times \theta_y$$

$$\text{with } d = |(a \times X_1 + b - Y_1) / (a^2 + 1^2)^{1/2}|$$

for θ_x and θ_y we must do 2 cases if the points are above the regression line,

$$\theta_x = \cos((3\pi/2) - \theta) \text{ and } \theta_y = \sin((3\pi/2) - \theta);$$

and if the points are below the regression line,

$$\theta_x = \cos((\pi/2) - \theta) \text{ and } \theta_y = \sin((\pi/2) - \theta)$$

$$\text{with } \theta = a \times \tan(a).$$

Moreover, I constructed two more mixed effects models for beech where I added the probability of each tree to belong to the orange genetic cluster ($P_{\text{original cluster}}$) identified in chapter 1 (which corresponds to the putative ancient population) as fixed factor and trees as random factor. These models accounted for interactions with the selected station-specific factors.

To sum up, I constructed a total of 6 models in order to assess the importance of the station-specific factors and of the genetic identity on tree growth and their response to their environment. An lmer (linear model with random effect), was applied by using the package lme4 (Bates et al., 2015) in R (R Development Core Team 2016), to define the factors that significantly influence tree growth and its mean sensitivity. Finally the tested models were:

- **For beech:**

M. 1: $GI_{\text{last 20 years}} \sim \text{Altitude} + \text{Topographical position} + \text{Riverbank} + \text{Adjusted position}$

M. 2: $MS \sim \text{Altitude} + \text{Topographical position} + \text{Riverbank} + \text{Adjusted position}$

M. 3: $GI_{\text{last 20 years}} \sim P_{\text{original cluster}} + P_{\text{original cluster}} \times \text{Altitude}$
 $+ P_{\text{original cluster}} \times \text{Topographical position} + P_{\text{original cluster}} \times \text{Riverbank}$
 $+ P_{\text{original cluster}} \times \text{Adjusted position}$

M. 4: $MS \sim P_{\text{original cluster}} + P_{\text{original cluster}} \times \text{Altitude} + P_{\text{original cluster}} \times \text{Topographical position}$
 $+ P_{\text{original cluster}} \times \text{Riverbank} + P_{\text{original cluster}} \times \text{Adjusted position}$

- **For oak:**

M. 5: $GI_{\text{last 20 years}} \sim \text{Altitude} + \text{Topographical position} + \text{Riverbank} + \text{Adjusted position}$

M. 6: $MS \sim \text{Altitude} + \text{Topographical position} + \text{Riverbank} + \text{Adjusted position}$

For each global model, the selection of the most explanatory submodel was made after following a stepwise model refinement using AIC. To do that I used the function “stepAIC” in the package MASS (Venables and Ripley, 2002) as implemented in R version 3.3.1 (R Development Core Team 2016). Then I used a type II ANOVA approach (Wald χ^2 test) to ensure that significance testing was unaffected by the order in which variables entered the final

submodel. The results are reported in a table under each most explanatory model, *** ($P < 0.001$); ** ($P < 0.01$); * ($P < 0.05$); ns ($P > 0.05$). Here are the most explanatory models:

- **For beech:**

M. 1:

	χ^2	d.f.	P
Altitude	0.06	1	ns
Topographical position	0.81	2	ns
Riverbank	5.72	1	*
Adjusted position	7.73	1	**

M. 2:

	χ^2	d.f.	P
Altitude	9.81	1	**
Topographical position	0.21	2	ns
Riverbank	0.08	1	ns
Adjusted position	15.29	1	***

M. 3:

	χ^2	d.f.	P
P _{original cluster}	0.8	1	ns
P _{original cluster} × Altitude	0.02	1	ns
P _{original cluster} × Topographical position	8.27	2	*
P _{original cluster} × Riverbank	1.69	1	ns
P _{original cluster} × Adjusted position	16.56	1	***

M. 4:

	χ^2	d.f.	P
P _{original cluster}	9.46	1	**
P _{original cluster} * Altitude	1.38	1	ns
P _{original cluster} × Topographical position	1.8	2	ns
P _{original cluster} × Riverbank	0.12	1	ns
P _{original cluster} * Adjusted position	3.52	1	ns

- For oak:

M. 5:

	χ^2	d.f.	P
Altitude	4.29	1	*
Topographical position	2.65	2	ns
Riverbank	0.59	1	ns
Adjusted position	0.002	1	ns

M. 6: No significant predictors were identified.

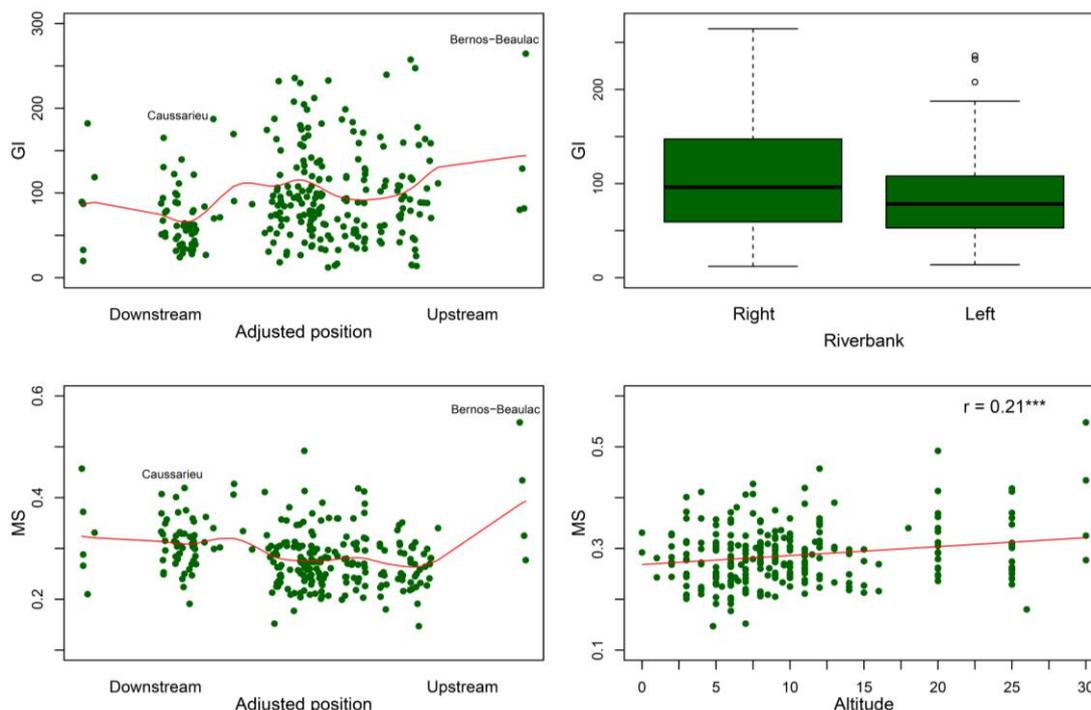


Figure 24. The representation in a plot graph of the significant factors that come out from the models M. 1 and M. 2 of *F. sylvatica*. First row: mean growth index (GI in %) of the last 20 years of the studied chronology (1996 -2015) as function of adjusted position and riverbank. Second row: mean sensitivity as function of adjusted position and altitude of trees above the river (m). In the figures on the left, Caussarieu and Bernos-Beaulac indicate two areas of the study site. ***, $P < 0.001$ according to Pearson's correlation test.

The GI of beech trees showed lower values in the downstream part of the river and on the right bank, while MS showed higher values in the downstream part and with increasing altitude of the tree above the river (**Figure 24**). The observed riverbank effect on GI could be due to the fact that trees on the right side of the Ciron river tend to receive more light owing

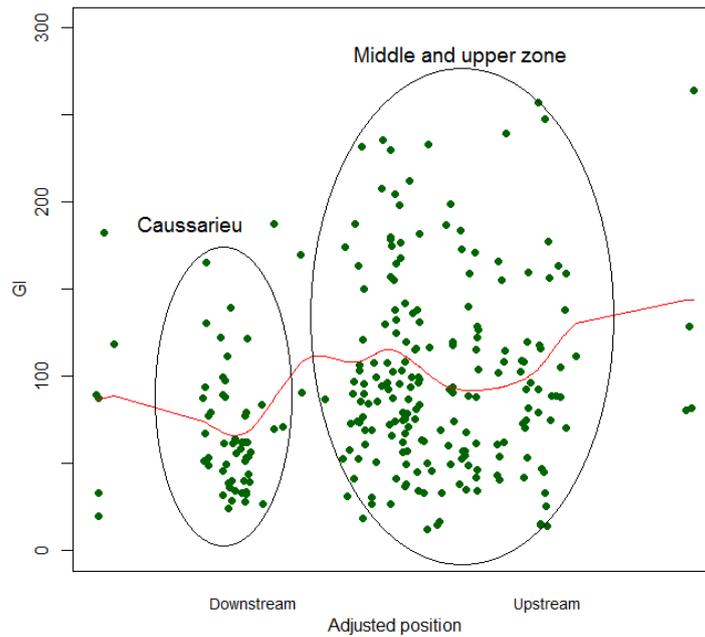


Figure 25. The same figure of the top left figure of (Figure 24) but with two well defined areas of the study site, “Caussarieu” and “Middle and upper zone”.

to their exposition towards the sun. The altitudinal effect on MS indicates that the distance of trees from the water table triggers their susceptibility to constraints in water supply.

The fact that both models (M. 1 and M. 2) were also driven by the adjusted position of the tree as a major effect reflects that the beech group in the Caussarieu zone tends behave differently than those of the other zones of the valley: its growth is weaker and its sensitivity is stronger (**Figure 25**). The difference is difficult to explain based on the available data, although it seems likely that it is triggered by some differences in small-scale environmental conditions between the upper and the lower reach of the Ciron. In addition, the observed trends actually are rather weak and loaded with statistical noise (**Table 4**).

Table 4. Adjusted position effect on $GI_{last\ 20\ years}$ and MS. *** ($P < 0.001$); ** ($P < 0.01$); ns ($P > 0.05$).

	$GI_{last\ 20\ years}$			MS		
	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>
Intercept	98.38	4.08	***	0	0.06	ns
Adjusted position	11.71	4.09	**	-0.18	0.06	***

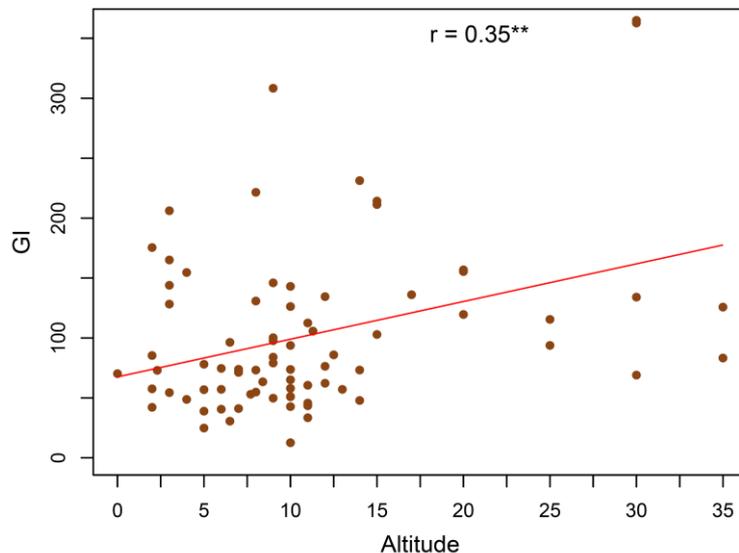


Figure 26. The representation in a plot graph of the significant factor that come out from the model M. 5 of *Q. robur*: mean growth index (GI in %) of the last 20 years of the studied chronology (1996 - 2015) as function the altitude of the trees above the river (m). **, $P < 0.01$ according to Pearson's correlation test.

The GI of oak trees (M. 5) could only be linked to their altitude from the river (**Figure 26**), while I found no single predictor of MS for this species (M. 6). The lack of significant relationships could be related with the lower sample size for this species.

Furthermore, the model for beech GI that included a genetic effect (M. 3) revealed no single driver but significant interactions between the variable genetic cluster with the two variables adjusted position and topographical position, respectively. The corresponding model for MS (M. 4) revealed, however, that trees with high probability to belong to the original genetic cluster tended to show a lower sensitivity. The observed interaction between the adjusted position and the genetic identity of the tree within the first model can be rather easily explained with the spatial distribution of the two genetic clusters that was described in chapter 1. The second interaction is much more difficult to interpret: Trees with a strong probability to belong to the original genetic cluster tended to grow slower than the average when in downslope position, whereas no differences existed in other tree positions (**Figure 27**). On the other hand, the lower sensitivity of trees from the original genetic cluster (**Figure 28**) could be interpreted as an eventual signal of local adaptation that would have rendered these trees less susceptible to drought stress (see Bosela et al., 2016, for a similar case with *Abies alba*). Such an interpretation would however be highly speculative. Further investigation

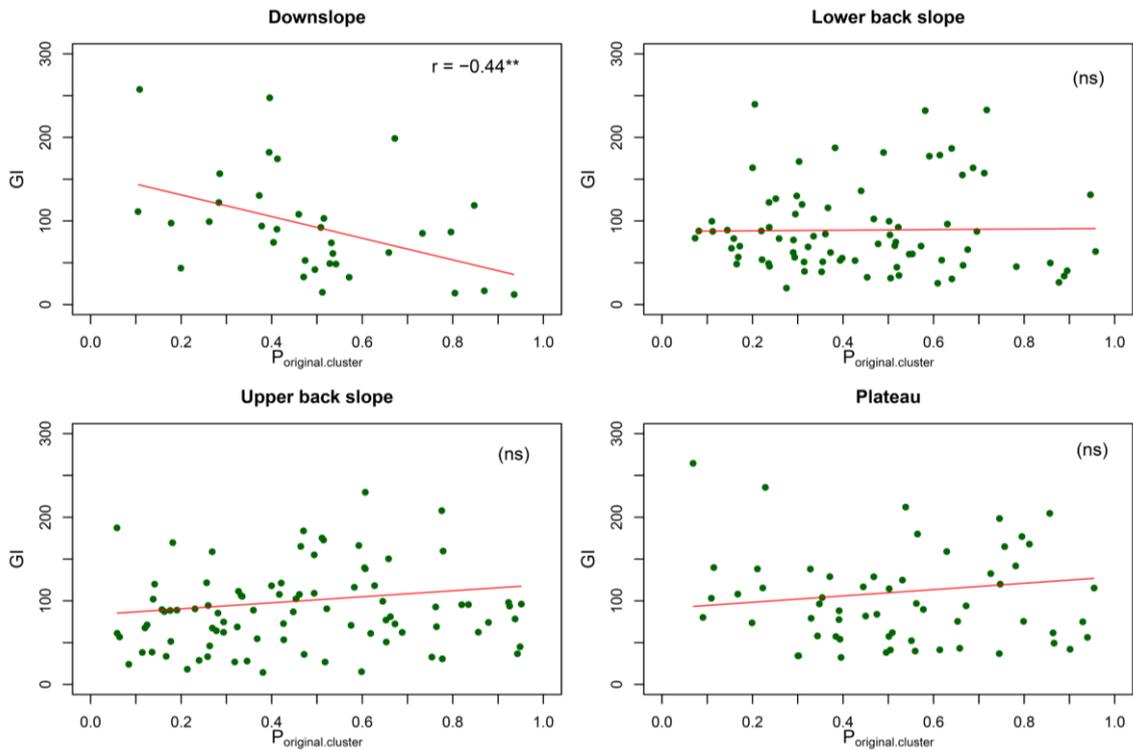


Figure 27. Scatterplots of the interactions between the topographical position of a given beech tree and its probability of belong to the putative original genetic cluster on GI of the last 20 years of the studied chronology (1996 -2015). **, $P < 0.01$; ns, $P > 0.05$ according to Pearson's correlation test.

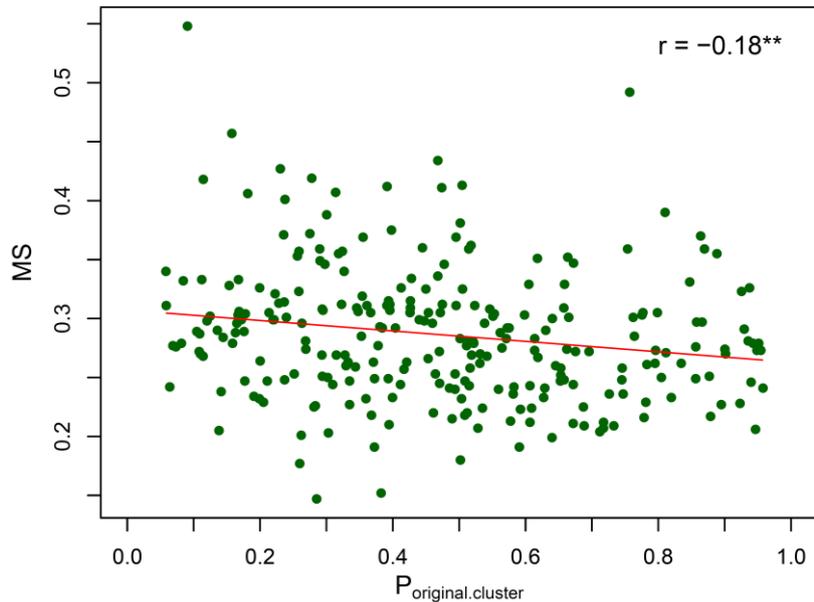


Figure 28. Effect of the probability of beech trees to belong to the putative original genetic cluster on mean sensitivity. **, $P < 0.01$ according to Pearson's correlation test.

need to be performed before we can infer real biological differences between the two genetic clusters of the Ciron beech population.

6 LONG-TERM EVOLUTION OF PAST GROWTH

6.1 Master chronology

I plotted the growth index (GI) of both species as a function of the date using the ggplot2 package (Wickham, 2009) in R version 3.3.1 (R Development Core Team 2016) in order to detect possible trends in the curve. Thus, I used the smooth function “stat_smooth” of that same package in order to eliminate the inert-annual fluctuations of the time series. (**Figure 29** and **Figure 30**). I also computed the standard error bounds using a t-based approximation method as implemented in ggplot2. Moving correlations between GI and the date were estimated over the period 1860-2015 over a 30-year moving window. Then, the correlation results were plotted as function of the first year of the window (**Figure 31**). This procedure allows to have a clearer idea of the significance of the observed growth trends through the study period.

The long-term trend of past growth for beech shows a strong increase in GI between 1860 and 1920 followed by a slight decrease till 1940s, then a modest increase that ceased later on (**Figure 29** and **Figure 31 A**). Then growth declined imperceptibly since the 1980s. This trend in beech growth is comparable to those found for marginal beech populations in the studies of Jump et al. (2006) in north-eastern Spain and Piovesan et al. (2008) in central Italy. These two studies showed a consistent decline of mature tree growth since the late 1970s. Our results is also consistent with Cavin and Jump (2016) where a slight decline started after 1990 in the southern range of the western European distribution of beech. Charru et al. (2010) also found the same trend in their study in north-eastern France since 1985. This finding suggests that the factors in question could act in the same way not only at the xeric range margin of the species but also in its temperate range edge, confirming a more general transition in beech growth in the mid-1980s (Bontemps et al., 2013). I observed a similar trend for oak (**Figure 30** and **Figure 31 B**) that was even more pronounced decline than in beech. Hence, the responsible factors seem to affect both species in the same way.

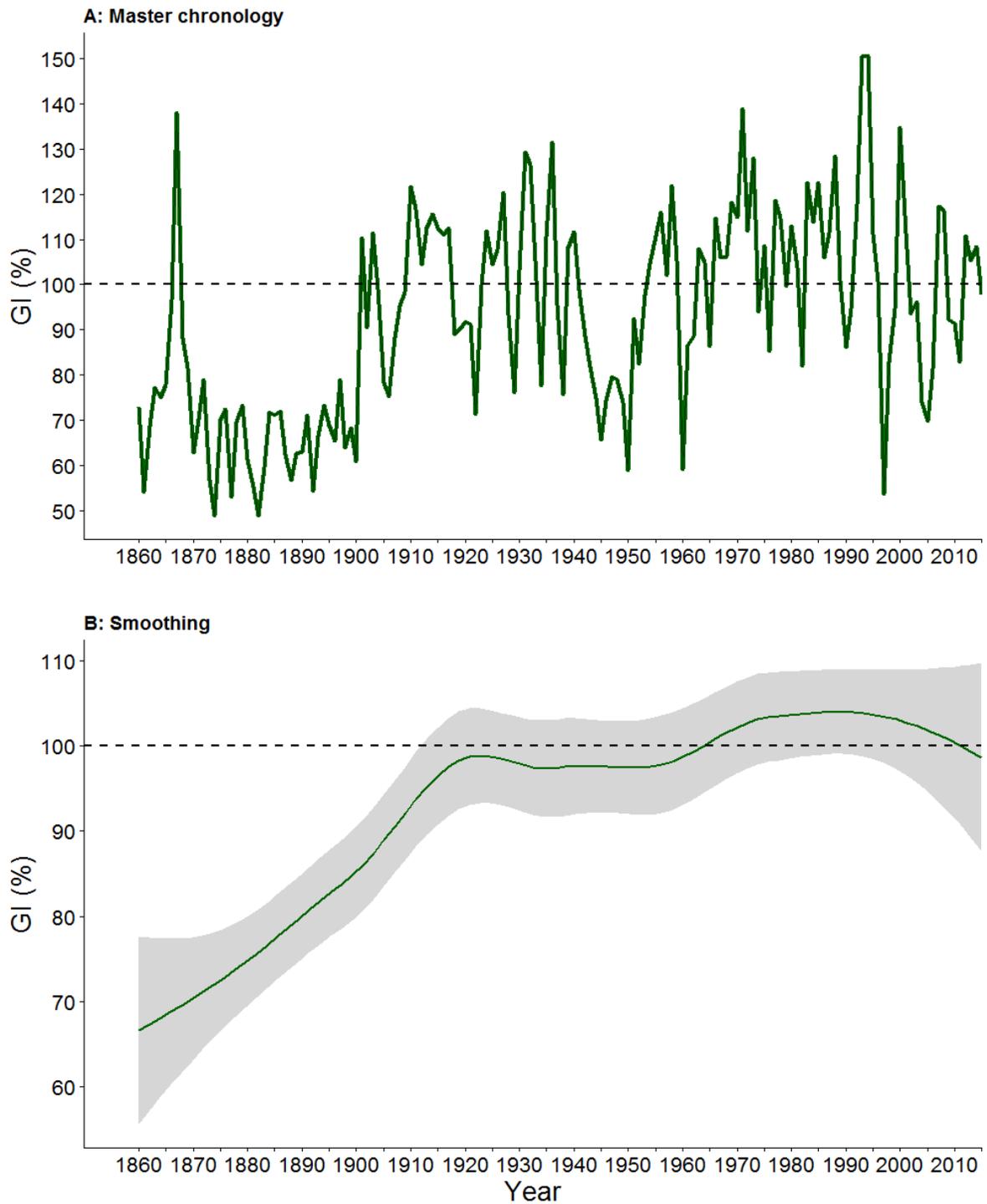


Figure 29. Beech master chronology with (A) GI in % as a function of the year for the 317 beech trees sampled in the Ciron valley and (B) the smoothing of the curve. The grey zone represents the standard error bounds.

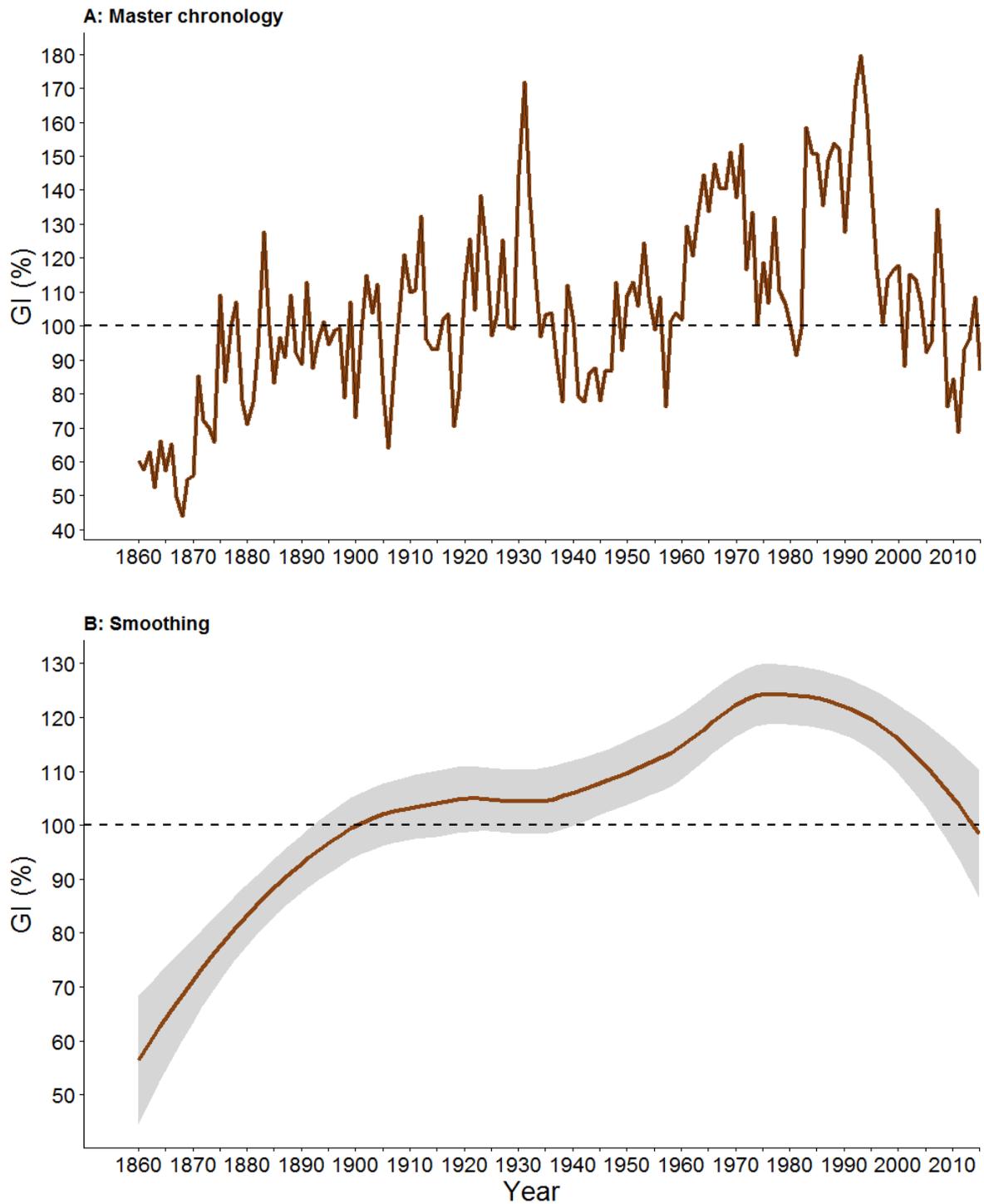


Figure 30. Oak master chronology with (A) GI in % as a function of the year for the 79 oaks sampled in the Ciron valley and (B) the smoothing of the curve. The grey zone represents the standard error bounds.

Beech has been shown to be sensitive to drought (Dittmar et al., 2003; Lebourgeois et al., 2005) and the decline of its growth is also consistent with the sensitivity of beech to water stress (Charru et al., 2010). Accordingly, Jump et al. (2006) and Piovesan et al. (2008) has attributed the decline of beech growth in the Mediterranean region to long-term drought stress. Similarly, Charru et al. (2012) concluded that “the higher sensitivity to drought in the southern range edge of beech may explain why the decline occurred earlier in this context and had a greater intensity” (Charru et al., 2010). Thus, my result is not surprising, as the beech population in the Ciron valley is located at the xeric range margin of the species distribution in Europe. However, the more pronounced decline in oak growth in the Ciron valley is somewhat unexpected, as the oak is known to be more tolerant to drought (Scharnweber et al., 2011).

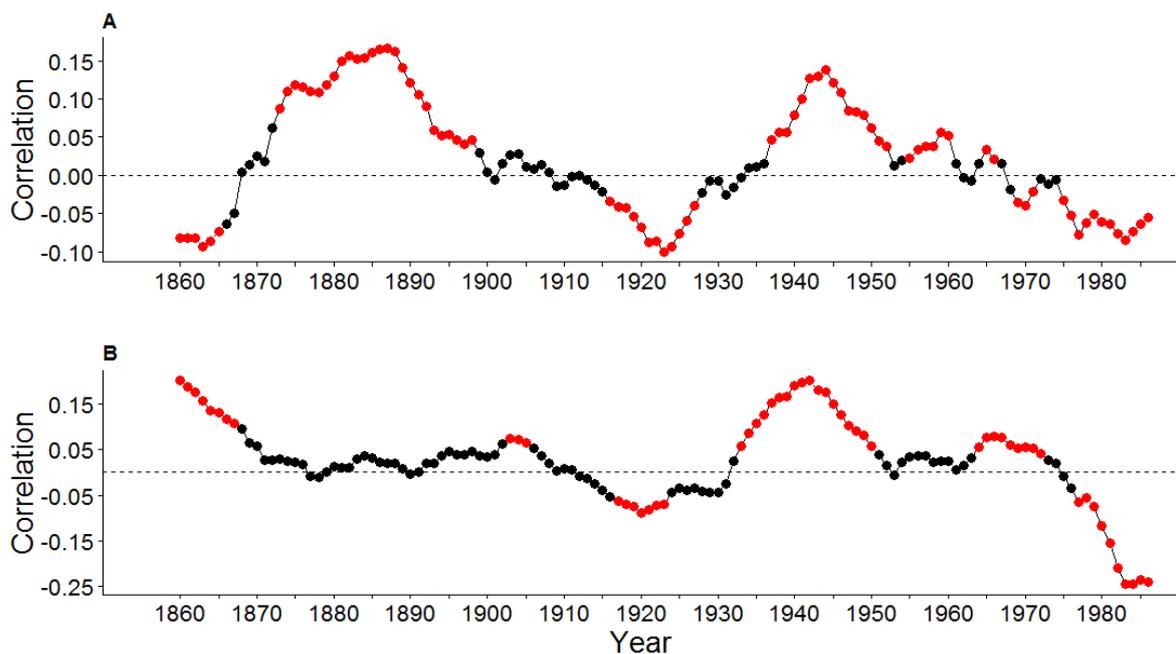


Figure 31. Moving correlation score as function of the year of a window of 30 years for beech (A) and oak (B). The x-axis represent the lower bound year of the window and the y-axis represent the result of Pearson correlation. Black dots means that the correlation between GI and the 30 selected years is not significant ($P > 0.05$). Red dots means that the correlation between GI and the 30 selected years is significant ($P < 0.05$).

6.2 Constant age method with BAI curve

On the other hand, I assessed long-term trends in beech and oak growth by testing the significance of BAI trends as a function of the year at a given cambial age. Then I estimated the correlation between the BAI and the date (once for the entire period and once before vs. after 1980). This allows to compare the results of this method with the previous one and to eventually confirm the observed trends. For some ages, I could not test the correlation between BAI and the chosen period because of the lack of number of tree rings having this age.

The results showed a general increase of BAI throughout the studied period for both species. The increase is significant before 1980 for all trees older than 40 years for beech (**Table 5**) and between 35 and 37 years old for oak (**Table 6**). However, the decrease of BAI after 1980 is not significant for beech but for oak trees aged between 38 and 43 years old. These results correspond roughly to those obtained with the previous method (see section 6.1), especially concerning the increasing trend before 1980. On the other hand, the weak decrease after 1980 was not evident, probably due to the small sample size.

Applying two different methods, I found a significant increase of BAI before 1980 and a slight decrease after 1980 for both species. These general trends can be explained by different factors that have acted before and after 1980, respectively, and effect known as divergence. D'Arrigo et al. (2008) argued that this divergence could result from a shift in the environmental limitation of trees that were previously constrained primarily by temperature and now by moisture. Thus, in order to disentangle the effect of temperature and drought on tree growth of beech and oak in the Ciron valley, isotopic and climatic analyses were performed.

Table 5. Pearson correlation score of BAI as function of date for a selected cambial age for *F. sylvatica* from 1860 to 2015; from 1860 to 1980; and from 1980 to 2015. The red numbers indicate a significant correlation with $P < 0.05$, the blue numbers indicate a marginally significant correlation with $P < 0.1$, and black numbers indicate an insignificant correlation.

Cambial age	Pearson correlation coefficient r		
	1860-2015	1860-1980	1980-2015
30	0.101	0.075	0.001
31	0.1	0.054	-0.010
32	0.106	0.04	0.01
33	0.102	0.043	0.008
34	0.106	0.059	0.054
35	0.107	0.089	0.077
36	0.107	0.116	0.076
37	0.11	0.124	0.041
38	0.102	0.1	0.016
39	0.1037	0.102	-0.026
40	0.103	0.111	-0.04
41	0.117	0.126	-0.043
42	0.119	0.129	-0.057
43	0.119	0.139	-0.082
44	0.105	0.147	-0.123
45	0.092	0.153	-0.119
46	0.08	0.156	-0.116
47	0.068	0.151	-0.099
48	0.065	0.172	-0.071
49	0.058	0.174	-0.073
50	0.046	0.159	-0.068
55	0.048	0.177	-0.079
60	0.052	0.246	-0.144
62	0.075	0.279	-0.144
65	0.067	0.259	-0.146
68	0.076	0.251	-0.08
70	0.126	0.292	0.057
72	0.147	0.296	0.097
74	0.132	0.258	0.108
75	0.124	0.209	0.096
80	0.058	0.259	-0.084
85	0.097	0.205	-0.248
90	0.104	0.115	-0.06

Table 6. Pearson correlation score of BAI as function of date for a selected cambial age for *Q. robur* from 1860 to 2015; from 1860 to 1980; and from 1980 to 2015. The red numbers indicate a significant correlation with $P < 0.05$, the blue numbers indicate a marginally significant correlation with $P < 0.01$, and black numbers indicate an insignificant correlation.

Cambial age	Pearson correlation coefficient r		
	1860-2015	1860-1980	1980-2015
30	0.172	0.201	-0.177
35	0.233	0.277	-0.138
36	0.198	0.224	-0.221
37	0.202	0.219	-0.331
38	0.218	0.211	-0.412
39	0.212	0.189	-0.503
40	0.209	0.188	-0.627
41	0.218	0.187	-0.453
42	0.251	0.231	-0.327
43	0.256	0.223	-0.28
44	0.267	0.202	-0.219
45	0.278	0.156	-0.135
50	0.238	0.103	-0.21

7 CARBON STABLE ISOTOPES

One of the main results that I obtained in this study is the long-term growth trend of beech and oak population within the climate refugium in the Ciron valley. Basically, the long-term trend of growth can be summarized by a strong increase between 1860 and 1920 that ceased later on and was followed by a slight decline since the 1980s. Physiological responses of trees such as photosynthesis, respiration and water-use efficiency have been linked to environmental changes through time, such as atmospheric CO₂ concentrations and climate. For growth and tree functioning, the flow of time has two components that need to be disentangled: the year (Hughes, 2000) and the current age (Bert et al., 1997). I applied an ecophysiological approach to detect the long-term trend of CO₂ assimilation rate, stomatal conductance for water vapour (g_s) and intrinsic water-use efficiency (iWUE), in order to compare them with growth trends in both species.

The intrinsic water-use efficiency (iWUE) is defined as the ratio between CO₂ assimilation and stomatal water conductance during photosynthesis. Its variations are recorded in the variation of the carbon isotope discrimination (Δ) of the annual tree-ring

cellulose that is formed during each growing season (e.g. Ehleringer et al., 1989; Bert et al., 1997; Duquesnay et al., 1998). Therefore, these annual records of carbon discrimination provide a mean to study past variations of the ecophysiology of trees with environmental changes (i.e. Dawson et al., 2002). Studies on a global scale and also in Europe (Bert et al., 1997; Duquesnay et al., 1998; Waterhouse et al., 2004) have shown that trees are able to increase their water-use efficiency as atmospheric CO₂ concentrations levels rise. One possible explanation of such response is that the $\delta^{13}\text{C}$ discrimination at the time the carbon was fixed in tree rings also responds highly to climatic variables such as growing season temperature, relative humidity and precipitation. Thus, changes in iWUE indicate a shift in the physiological balance between photosynthesis and stomatal conductance, and are often caused by changes in relative humidity and soil water status at dry sites (McCarroll and Loader, 2004).

Beech, tends to show a stronger iWUE response than other species such as oaks (Lebourgeois et al., 2005; Peñuelas et al., 2008). Thus, I aimed in this study to compare the trends in iWUE with that of GI in order to test if a possible increase of iWUE (driven by the continually increasing levels of CO₂ and/or drought), can compensate the decrease in GI. When dealing with long-term trends of tree physiological functioning, we must account for age effects in addition to other long-term effects. Thus, I also aimed to characterize the carbon isotope discrimination changes due to the age of the trees in the sampled beech population. Finally, the same was applied for oak so that to compare the response of beech.

7.1 Principle and method

In general, discrimination or biological isotope fractionation is defined as the partitioning of heavy and light isotopes between a source substrate and the product in a biological system. Many biochemical processes discriminate against the heavier isotope in a mixture. Thus, for example trees discriminate against ¹³CO₂ more than ¹²CO₂ during photosynthetic carbon fixation (Dawson and Brooks, 2001).

In the following, I will present the method that I applied to calculate carbon isotopic discrimination (Δ) and intrinsic water-use efficiency (iWUE). So that we can estimate the discrimination, in the first place, we need to calculate the isotopic composition of a carbon

compound ($\delta^{13}\text{C}$). $\delta^{13}\text{C}$ represent the proportional deviation of the $^{13}\text{C}/^{12}\text{C}$ ratio from the internationally accepted Peedee belemnite (PDB) carbonate standard (Craig, 1957):

$$\delta^{13}\text{C}(\text{‰}) = \left(\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{PDB}}} - 1 \right) \times 1000 \quad (\text{Eq. 1})$$

During carbon fixation, some fractionations associated with physical and enzymatic processes lead organic matter in plant to be ^{13}C depleted in comparison with the air. Indeed, the $\delta^{13}\text{C}$ of atmospheric CO_2 , δ_a , has a current value of about -8‰. However, within plant material δ_{plant} , ranges from -22‰ to -34‰. This carbon isotopic discrimination is expressed as

$$\Delta(\text{‰}) = \frac{(\delta_a - \delta_{\text{plant}})}{(1000 + \delta_{\text{plant}})} \times 1000 \quad (\text{Eq.2})$$

The relative rates of CO_2 diffusion, via stomata, into the leaf and its fixation by ribulose-1,5 bisphosphate carboxylase/oxygenase (RuBisCO) are the primary factors determining Δ . According to the model proposed by (Farquhar et al., 1982),

$$\Delta(\text{‰}) = a + (b - a)(C_i/C_a) - d \quad (\text{Eq. 3})$$

where a is the discrimination against $^{13}\text{CO}_2$ during CO_2 diffusion through the stomata ($a = 4.4\text{‰}$, O'Leary, 1981), b is the discrimination associated with carboxylation by RuBisCO ($b = 27\text{‰}$, Farquhar and Richards, 1984), d is a term related to a variety of factors (respiration, liquid-phase diffusion, etc.), often taken as a constant of 1‰ , and C_i and C_a are intercellular and ambient CO_2 concentrations.

Given Fick's law:

$$A = g_{\text{CO}_2}(C_a - C_i) \quad (\text{Eq. 4})$$

where A net photosynthesis measured as CO_2 uptake, and g_{CO_2} leaf conductance to CO_2 , are linked by Fick's law, and given that $g_{\text{H}_2\text{O}}$, the leaf conductance to water vapour is $1.6g_{\text{CO}_2}$, Δ can be related to the ratio $A/g_{\text{H}_2\text{O}}$ by

$$\Delta(\text{‰}) = a - d + (b - a)\left(1 - \frac{1.6}{c_a} \frac{A}{g_{H_2O}}\right) \quad (\text{Eq. 5})$$

A/g_{H_2O} is called intrinsic Water Use Efficiency (iWUE) (Ehleringer et al., 1993), which is a component of plant transpiration efficiency, the long-term expression of biomass gain with respect to water loss at the level of the whole plant.

Finally, according to the last formula the instantaneous iWUE is expressed as the following,

$$\frac{A}{g_{H_2O}} = \frac{c_a}{1.6} \left(1 - \frac{\Delta - a + d}{b - a}\right) \quad (\text{Eq. 6})$$

7.2 Sampling design

An appropriate sampling is key for meeting the goal to test for effects of long-term trends (age and date), genetic cluster and station-specific factors on the evolution of $\delta^{13}\text{C}$. The tree-ring sampling procedure for isotopic analyses is summarized in three steps: tree-ring choice between individuals; sample preparation (tree rings cutting and grinding); and finally cellulose purification followed by cellulose yields analysis and cellulose weighing. The requirements of the isotope analysis define the basic sampling unit: a group of 5 successive tree rings (pentad) from a given tree at a given age and date. Then the average year or the average age corresponding to these 5 tree rings is noted. For example, a pentad of 30 years corresponding to the year 2000 and from a given tree, means that I sampled from this same tree the five rings aged from 28 to 32 years old and that were formed respectively from the years 1998 to 2002.

Tree-ring sampling: age effect

To study the age effect on $\delta^{13}\text{C}$ evolution in beech, I sampled many tree rings from several trees and for different dates. To illustrate the effect of age, it is necessary that each age must be sampled on several dates to average the date-effect. Also, each age must be sampled from several trees to average the individual effect. On the other hand, growth in BAI is strongly evolving between 1 and 155 years, then the curve shows random variations beyond 155 years (**Figure 22**). Furthermore, the number of rings for beech is low for a cambial age less

than 15 years old and over 155 years old. For this reasons, I chose to study trees between 15 and 155 years old. According to the available number of tree rings, 97 beech trees were selected for isotopic analyses (**Figure 32 A**). I chose trees aged with an interval of 15 years and an interval of 20 years over 90 years old (**Table 7**). The greater number of trees sampled at 45 years old is because I planned to use only this age to study the effect of date if the age-effect was demonstrated. Once the tree-rings were chosen, the average BAI of each pentad was plotted on the BAI curve as a function of age to check that the points do not deviate too much from the curve and that they follow the same trend of BAI (**Figure 33**).

Table 7. Number of beech (N_{beech}) and oak (N_{oak}) trees sampled for each age. For beech, the same tree can be sampled at different ages.

Age	15	30	45	60	75	90	110	130	150
N_{beech}	23	27	78	16	15	16	13	11	8
N_{oak}			68						

For oaks, unlike for beech, the smaller number of trees only allowed to study the date effect based on a sample of 45 year-old trees. All individuals with a 45-year-old ring were taken, which allowed me to sample 68 oaks (**Figure 32 B**).

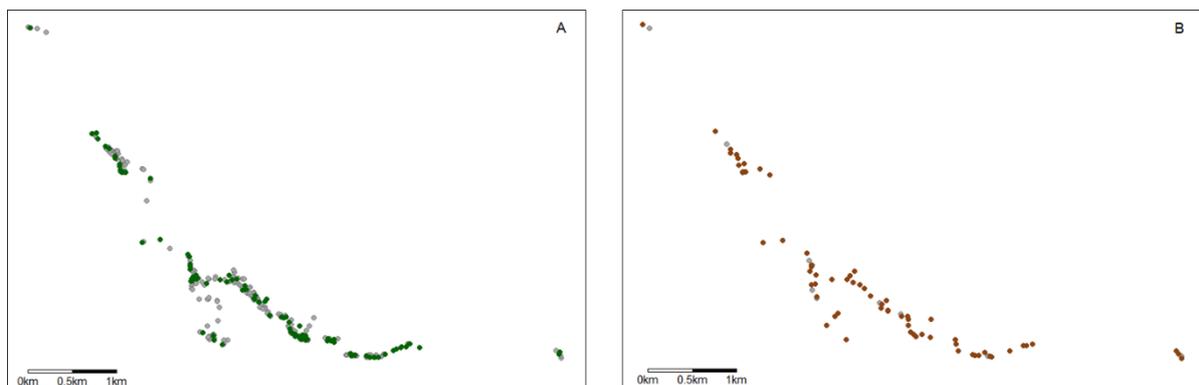


Figure 32. Spatial distribution of selected trees for the isotopic analysis in the Ciron valley. Grey dots of the graph (**A**) represent all the beech trees sampled and the green dots of the same graph represent the 97 beech trees selected for isotopic analysis. Grey dots of the graph (**B**) represent all the oaks sampled and the brown dots of the same graph represent the 68 oaks selected for isotopic analysis.

Finally, I added 20 standard control samples of *Pinus pinaster* wood to control for eventual variation arising from the cellulose purification protocol and the isotopic measurement (see Appendix S3 for more details).

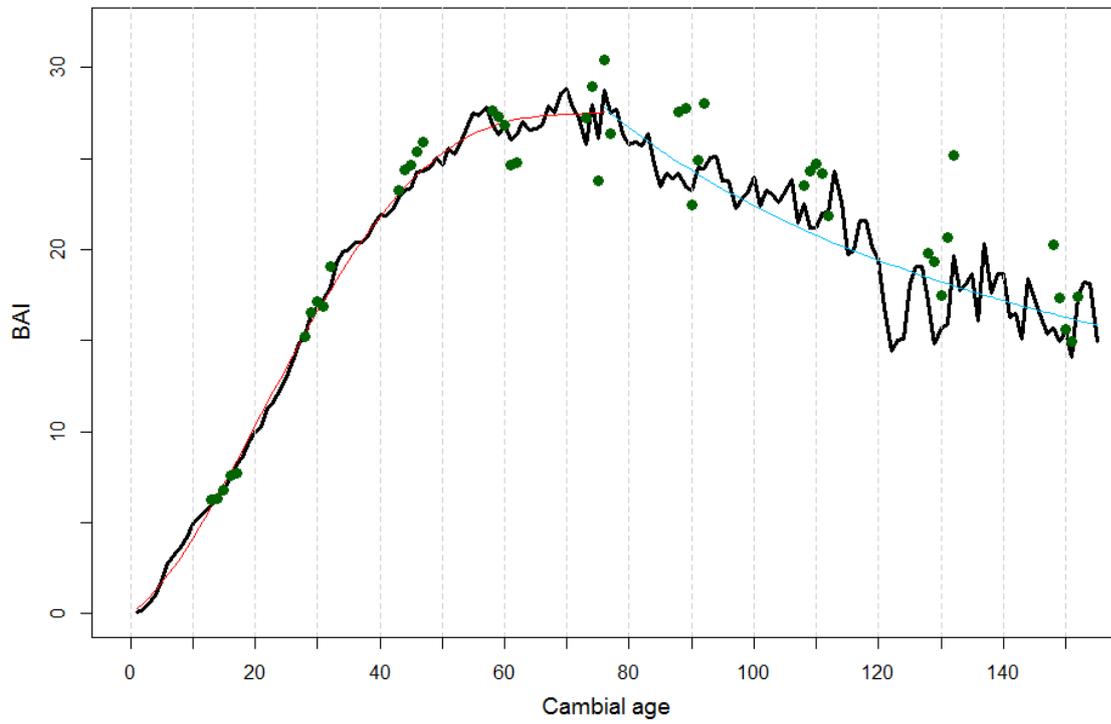


Figure 33. Mean BAI of pentads selected for isotopic analysis along the BAI-cambial age curve for beech (green dots). The black curve represents BAI as a function of cambial age with the adjustment curves (red and blue) as represented previously in Figure 22 and a cut off at 155 years.

Tree-ring sampling: date effect

If the age effect would have been demonstrated, it would be theoretically possible to take it into account while studying the effect of date. This is possible by applying a standardization method for $\delta^{13}\text{C}$ measurements as I did for BAI with the RCS method to obtain the growth indices. However, this approach would be very consuming in isotopic data and tricky to implement. For this reason, I chose to apply a constant age method where the effect of age is eliminated by analysing only tree ring at a given age. One limitation of this method is that this chosen age must have been reached on all the dates for the period between 1880 and 2015. Thus, I retained 78 beech trees and 68 oaks at age 45 years to study the effect of date since 1880 (**Table 8**).

In general, an age around 50 years is a commonly suggested cut-off to remove age effects (i.e. McCarroll and Loader, 2004; Waterhouse et al., 2004).

Table 8. Number of beech (N_{beech}) and oak (N_{oak}) trees sampled for each period. For beech, the same tree can be sampled at different dates.

Period	1870	1880	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
N_{beech}	1	4	4	6	6	3	7	7	7	7	4	5	6	8	3
N_{oak}	2	2	2	3	0	4	3	8	6	4	11	9	6	4	4

Tree-ring sampling: Interaction between genetic and topographical position effects

For testing the effect of the interaction between the $P_{\text{original cluster}}$ and the topographical position on the evolution of $\delta^{13}\text{C}$ for beech, I had to choose tree rings (pentads) where I could clearly see the effect. Thus, I selected 20 trees in downslope position and 20 trees in plateau position spread throughout the study site (**Figure 34**). Afterwards, I selected three periods, between 1980 and 2015, for each of the 40 selected trees, to have a total sample of 120 pentads. These periods were chosen after performing a correlation analysis between GI and the probability of belonging to a genetic cluster, in each year of the selected period. Thus, I chose the pentads that showed a significant correlation for at least one of the two topographic positions. This allowed me to compare the effect of the interaction between the $P_{\text{original cluster}}$ and the topographical position on $\delta^{13}\text{C}$ in three different periods between 1980 and 2015 selected on the same trees: 1988 – 1991 (quadra and not pentad), 1995 – 1999 and 2004 – 2008 (**Figure 35**). These periods show no obvious difference in climate variables.

Samples preparation

After the tree-rings were selected, I identified the tree rings to be analysed with a monitor displaying the image of the core with dates on the tree rings using WinDENDRO. A second monitor showed the real core under a binocular in order to identify the tree rings to be cut. The pencil tickmarks at the end of tree rings were removed with a scalpel, and the selected pentad was cut with a scalpel. The pentads were put in a flask, roughly cut with a cutting pliers and ground with a robot prototype Labman called “INRA Wood Grinding & Dispensing System” built by Labman Automation Ltd, Seamer Hill, Seamer, UK. In some cases, the small amount of

wood within samples required to put them in an Eppendorf vial and grind them with a Genogrinder 2010.

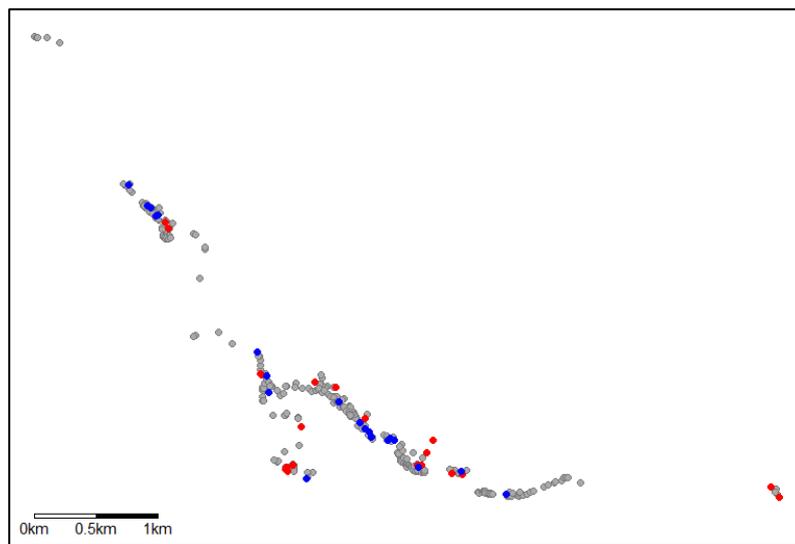


Figure 34. Distribution of beech trees sampled for the isotopic analysis to study the effect of belonging to a genetic cluster and the effect of the topographical position. Red dots correspond to the trees on the plateau and blue dots correspond to the trees in downslope position.

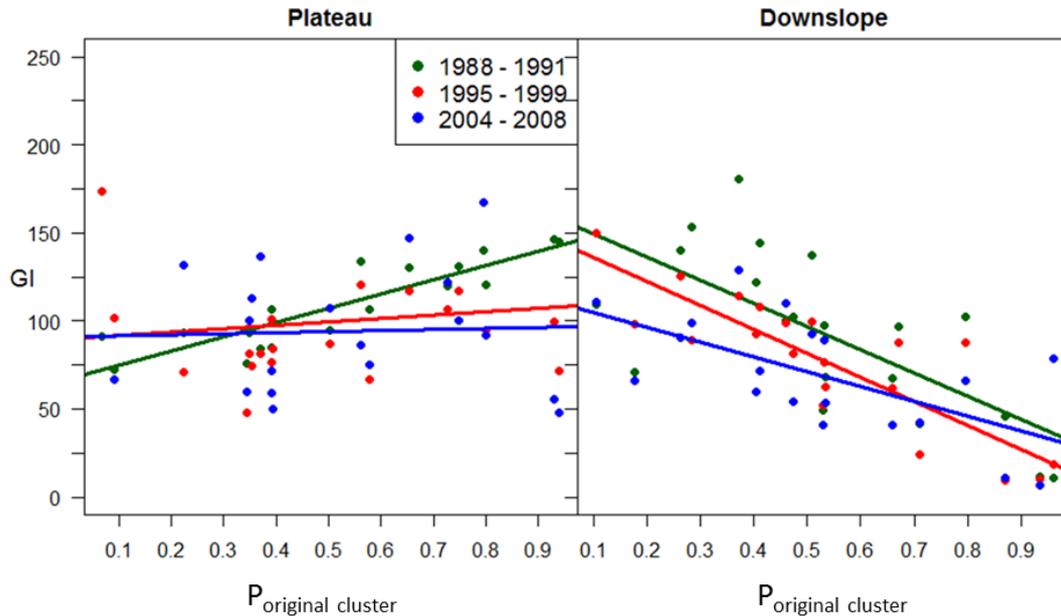


Figure 35. Mean growth index (GI in %) of 20 beech trees sampled in plateau position and for 20 beech trees sampled in downslope position, for the three chosen periods (1988 – 1991, 1995 – 1999 and 2004 – 2008).

Then, the powder was transferred into a PTFE Membrane Filters (or Teflon membrane) and weighed dry with an analytical balance. To have the exact dry weight of the powder, I weighed each empty PTFE Membrane Filters after 24 hours in an oven at 65 °C (P1), then I repeated the same procedure after putting the powder inside the filters (P2). Once weighed, I proceeded with the cellulose purification.

Cellulose purification

Wood is a heterogeneous structure made of several materials: mainly cellulose, hemicelluloses, lignin, and extractives which all have different isotopic signatures. Most isotopic analyses have been carried out on cellulose, which is a standard for dendro-isotopic studies due to its molecular homogeneity and structure, where the strong carbon bonds allow it to retain its original isotopic composition the wood formation. In addition, cellulose is laid down during the growing season so that the cellulose of a given tree-ring of a given year is produced during that same year (Leavitt and Danzer, 1993; Loader et al., 1997; Au and Tardif, 2009). The cellulose purification process for carbon isotopic analyses was carried out following the procedure described in Richard et al. (2014). The procedure aims to remove the extractives, lignin, pectin and hemicelluloses from the wood sample, without degrading the cellulose polymer (Richard et al., 2014) (i.e. Appendix S3 for detailed protocol for the cellulose extraction). Once the cellulose extraction was done, I estimated the percentage yield of cellulose as the ratio of cellulose mass to wood mass (**Figure 36**).

For beech, the average cellulose yield was 84.4% (**Table 9**), close to previous studies on beech (Avat, 1993: 82%; Thiebaud, 1995: 83%; Godin et al., 2010: 73.3%). For oak, the average cellulose yield was 69.2%, also close to previous studies (Hamada et al., 2016: 74%). Finally, for the pine standards I found an average of 79.3%.

Table 9. Mean yield, its standard deviation (SD) and the sample size (N) of beech, oak and pine.

	Beech	Oak	Pine
N	317	68	20
Mean	84.4	69.2	79.3
SD	4.4	4.2	4.6

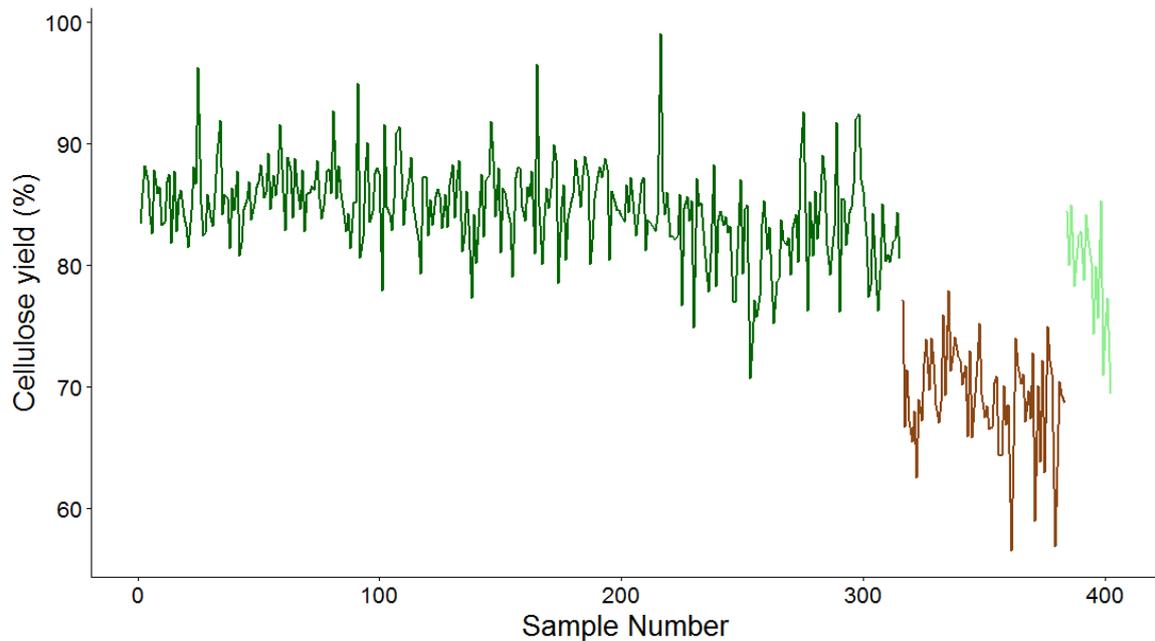


Figure 36. Cellulose yield in % of sampled beech (dark green), sampled oak (brown) and sampled pine (light green).

After the verification of the cellulose yields, an amount of about 1mg was taken from each sample and put in a tin pellet. The exact cellulose weight was noted for each sample. Finally, all the samples were placed in an Elisa plate and sent to the “Plateforme technique d’écologie fonctionnelle (PTEF)” in INRA Champenoux where $\delta^{13}\text{C}$ levels were measured for each sample with an elemental analyser (vario ISOTOPE cube, Elementar, Hanau, Germany), interfaced in line with a gas isotope ratio mass spectrometer (IsoPrime 100, Isoprime Ltd, Cheadle, UK) with 0.2 ‰ accuracy.

7.3 Age effect

As stated above, my tree ring sampling was adapted to test the effect of age on the evolution of $\delta^{13}\text{C}$ for beech. The significance of observed age trends was tested by simple linear regression fitted to the observed values of $\delta^{13}\text{C}$ as a function of age. The results showed that there is no significant age effect on $\delta^{13}\text{C}$ (**Table 10** and **Figure 37**).

Table 10. Effect of age on $\delta^{13}\text{C}$ for beech in the Ciron valley. *** ($P < 0.001$); ns ($P > 0.05$).

	$\delta^{13}\text{C}$		
	Estimate	SE	P
Intercept	-26.617	0.147	***
Age	0.003	0.002	ns

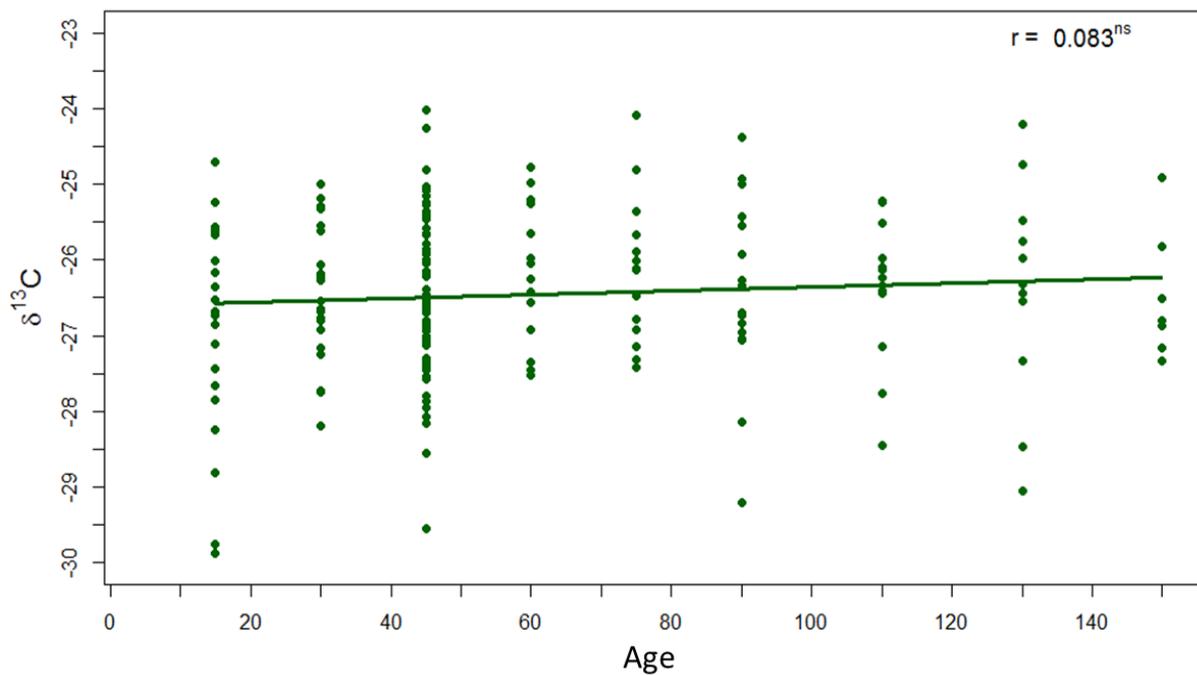


Figure 37. Carbon isotope composition of beech tree rings ($\delta^{13}\text{C}$ in ‰) as function of cambial age (year) in the Ciron valley.

Many studies (e.g. Bert et al., 1997; Duquesnay et al., 1998; and Brienen et al., 2017) have discussed the importance of taking into account the effect of tree age in long-term $\delta^{13}\text{C}$ studies. In some studies the effect was found during the first decades of growth (Bert et al., 1997; Duquesnay et al., 1998) and in others the effect was found throughout the entire life of the tree (Brienen et al., 2017). In all cases, taking into account the effect of age on the evolution of $\delta^{13}\text{C}$ allows to disentangle the effects related to the age of the tree from effects due to environmental changes (Bert et al., 1997). Changes in micro-environmental variables with stand maturation and physiological changes linked to tree structural development may

be potential causes affecting the evolution of $\delta^{13}\text{C}$ with age (Peñuelas et al., 2008). Increasing tree height, the uptake of soil-respired CO_2 when growing under the canopy and changing crown illumination over a trees' life are major drivers that can affect $\delta^{13}\text{C}$ trends (Brienen et al., 2017). However, the absence of this effect in beech in the Ciron valley is consistent with the result of Peñuelas et al. (2008) on beech in northern Spain. The absence of an age effect was shown only within a stand in a dry and warm conditions at the lower limit of their study site (around 1000 m of altitude). Since my study site is also located at the southern range edge of beech (in lowlands), this absence of an age effect could be related to dry and warm conditions. Such conditions may lead to less negative $\delta^{13}\text{C}$ values in the first decades of growth, ultimately resulting the overall absence of trends (Peñuelas et al., 2008). In addition, the linear and narrow structure of my stand and the way the trees are arranged in the shallow gorge (like floors) can easily mask effects of luminosity, tree height and uptake of soil-respired CO_2 , resulting in a lower effect of these important drivers. This highlights the fundamental importance of local features of the site for understand the functioning of tree populations.

7.4 Date effect

I also studied the long-term evolution of $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, C_i and $i\text{WUE}$ as a function of date in beech and oak. Given the absence of an age effect on $\delta^{13}\text{C}$ for the studied beech stand, I chose to use all the 97 sampled beech trees with different ages. However, the selected oak tree-rings for isotopic analysis were all 45 years old, a precaution that has been taken in case the age-related effect would existed for the studied oaks.

Since industrialisation, anthropogenic increases in the concentration of CO_2 in the atmosphere (C_a) have resulted in a lowering of the aerial carbon isotope composition ($\delta^{13}\text{C}_a$) by about 1.5% (McCarroll and Loader, 2004). Consequently, this trend may appear in tree ring $\delta^{13}\text{C}$ series. Thus, the evolution of the $\delta^{13}\text{C}$ values in tree rings must be corrected for the evolution of $\delta^{13}\text{C}_a$. A well-known approach to removing the atmospheric decline in $\delta^{13}\text{C}$ in environmental physiology is to express the $^{13}\text{C}/^{12}\text{C}$ ratio in terms of discrimination against ^{13}C using $\Delta^{13}\text{C}$ (i.e. Eq.2 in section 7.1). Afterward, $\Delta^{13}\text{C}$ will be used to calculate WUE from the atmospheric CO_2 content (C_a). The two variables $\delta^{13}\text{C}_a$ and C_a must therefore be available throughout my study period from 1880 to 2015 (**Figure 38**). $\delta^{13}\text{C}_a$ data were recovered (Friedli et al., 1986; Keeling et al., 1989; Leavitt and Long 1989; Marino and McElroy 1991; White et

al., 2015), compiled and smoothed as indicated in Bert et al. (1997) to get the appropriate long-term chronologies. I adjusted the evolution of $\delta^{13}\text{C}_a$ over time with a spline, before using the model to obtain the annual estimates from 1800 to 2015. For the variation C_a , a spline fit was calculated and uploaded from the Scripps CO₂ Program (Keeling et al., 2005), where atmospheric CO₂ data are recorded from ice core data before 1958 (Ethridge et. al., 1996; MacFarling Meure et al., 2006) and yearly averages of direct observations from Mauna Loa and the South Pole since 1958.

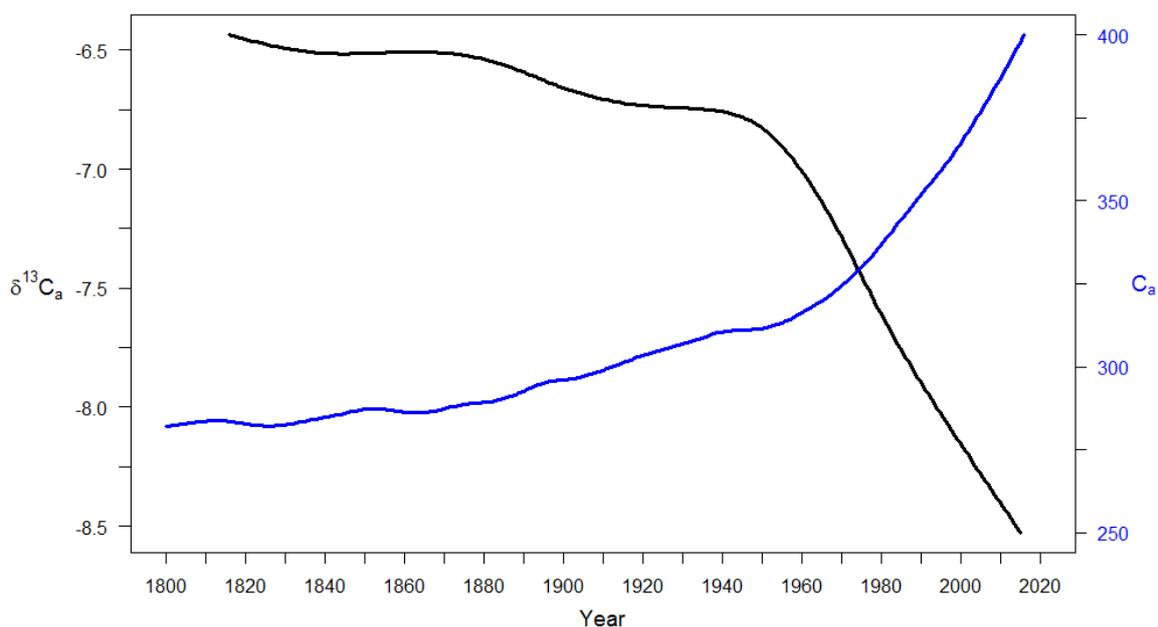


Figure 38. The black line represents the adjusted carbon isotope composition of atmospheric air $\delta^{13}\text{C}_a$ in ‰ VPDB. The adjustment was done by a smooth.spline function as implemented in R, with a smoothing parameter (spar = 0.9). The blue line represents the variation in atmospheric CO₂ content (C_a in $\mu\text{mol mol}^{-1}$). The spline fit was calculated and uploaded from the Scripps CO₂ Program. The online site provides annual CO₂ values from the year 0 to 2016:

http://scrippsco2.ucsd.edu/data/atmospheric_co2/icecore_merged_products

The result (**Figure 39**) shows that, for beech, the long-term trend of tree $\delta^{13}\text{C}$ was not the same than of atmospheric air $\delta^{13}\text{C}_a$. The decrease of $\delta^{13}\text{C}_a$ after 1950 is not reflected in plant fractionation for beech. However, for oak the trends are almost parallel throughout the study period. This trend of $\delta^{13}\text{C}_a$ due to anthropogenic increases in the concentration of CO₂ is more obvious in the studied oaks than in beech trees, implying that plant fractionation was less affected by long-term environmental changes (Bert et al., 1997) in beech than in oak. For

a better interpretation of fractionation trends, the trees $\delta^{13}\text{C}$ was corrected for $\delta^{13}\text{C}_a$ with the formula of carbon isotope discrimination ($\Delta^{13}\text{C}$).

The results in **Figure 39** also showed a lower discrimination $\Delta^{13}\text{C}$ with time for beech and consequently a significant increase of the intrinsic water-use efficiency (iWUE) along the studied period. A correlation test between iWUE and the date, for the period between 1860 and 1950, showed a significant increase until 1950 ($r = 0.29$, $P = 0.0086$). This increase is more remarkable over the second half of the 20th century ($r = 0.41$, $P = 2.7e^{-06}$) after performing a correlation test between 1950 and 2015.

The trend for iWUE in beech is in agreement with other studies on beech (Duquesnay et al., 1998; Waterhouse et al., 2004; Peñuelas et al., 2008; Brienen et al., 2017) and other species (Bonal et al., 2011). Moreover, Peñuelas et al. (2011) have pointed out in their meta-analysis, that iWUE increased by an average of 20.5% over the second half of the 20th (between 1960 and 2000). The increased by 19.3% that I observed during the same period between 1960 and 2000 fit quite well with this average.

A higher increase in iWUE between 1920 and 2003 was reported in the study of Peñuelas et al. (2008) for beech in the warmest and driest site. The reported increase of iWUE was 10%, against 6% or absence of increase within the wetter and cooler sites of the same study. This result was associated to a warming not accompanied by increased precipitation. Thus, the fact of having a higher increase (19.3%) for the same period in my beech stand can be expected. Furthermore, I were fortunate in my study to have a long chronology that allowed me to estimate the increase of iWUE since 1860. The result in **Figure 39** shows an increase of iWUE level of 66.7%. This means that since 1860 the level of iWUE has increased no less than two thirds.

The trend was not the same for oak. An absence of trend of iWUE was observed from 1872 to 1950 ($P = 0.75$), followed by an increase in discrimination up to the year 1990 ($r = 0.44$, $P = 0.01$) that ceased later on ($P = 0.27$). In addition, the increase in the level of iWUE was 15.4% between 1872 and 2015 and 9.4% between 1960 and 2000 (against 20.5% for the same period in Peñuelas et al. (2011)). The observed trend is not in agreement with previous

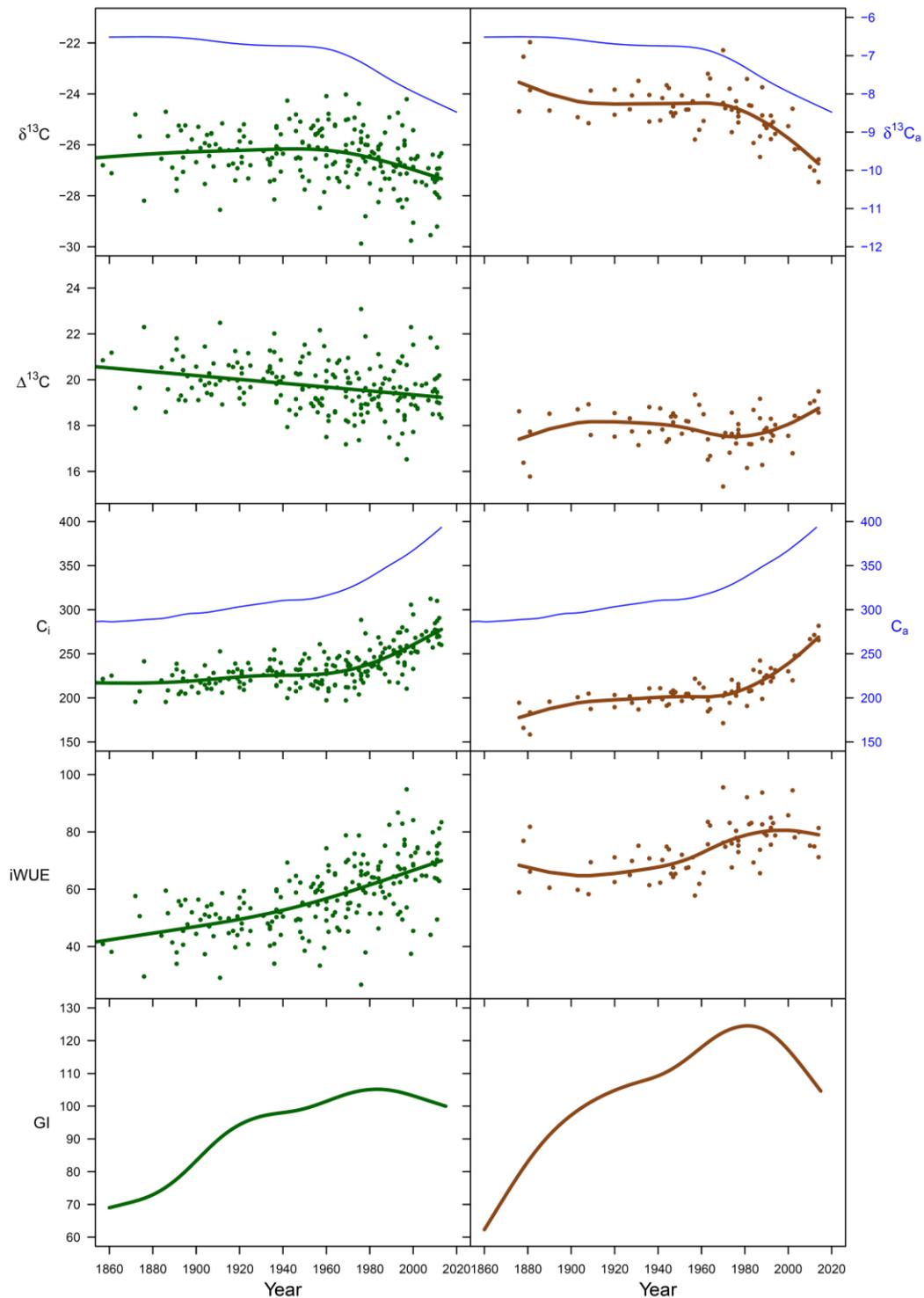


Figure 39. Mean $\delta^{13}C$ (‰), $\Delta^{13}C$ (‰), C_i ($\mu\text{mol mol}^{-1}$) and intrinsic water use efficiency (iwUE in $\mu\text{mol mol}^{-1}$) for *F. sylvatica* (green) during the period 1860-2015 and for *Q. robur* (brown) during the period 1872-2015. Growth index (GI in %) presented in this graph is the master chronology of all the sampled beech trees (317) and oaks (79) as in (Figure 29.B) and (Figure 30.B), respectively. Blue curves show the adjusted carbon isotope composition of atmospheric air ($\delta^{13}C_a$ in ‰) and the variation in atmospheric CO_2 content (C_a in $\mu\text{mol mol}^{-1}$) as represented in the (Figure 38).

studies on the same species where the authors found a continuous increase of iWUE until now (Waterhouse et al., 2004; Loader et al., 2008; Brienen et al., 2017). Such trend may be related to the coupled effect of age and date. Indeed, Brienen et al. (2017) found that time trends in iWUE for beech and oak are much weaker than the increase in iWUE with tree age. This result led them to ask for more investigation into the influence of historical stand development, like the role of competition, light availability and height gains on iWUE trends. Unfortunately, my data set for oak does not allow to test the magnitude of each effect. However, for beech I observed quite the opposite of Brienen et al. (2017) results on the magnitude of age and date effect as I found a strong effect of date and no age effect. After all, beech shows a stronger iWUE response in trend and amplitude than oak, which may be a result of the particularly drought sensitive nature of this species (Lebourgeois et al., 2005). Thus, these findings can lead the conclusion that the beech in the Ciron valley is more likely to be affected by environmental factors than oaks.

The graph of iWUE plotted against atmospheric CO₂ concentrations (C_a) (**Figure 40**) highlights that iWUE increases further for beech than for oak with the increase in CO₂ concentration ($P = 2.2e^{-16}$). This result indicates an increasing sensitivity to increasing levels of CO₂ for beech with more arid conditions. My results are in accordance with (Peñuelas et al., 2008) who showed that the rate of increase in iWUE is higher in the dry and warm site. However, for oaks the levels of iWUE are higher than for beech but iWUE increase was observed up to a threshold of 350 $\mu\text{mol mol}^{-1}$ ($P = 1.18e^{-05}$) that ceased later on ($P = 0.25$). This lessening in the sensitivity of oak to increasing C_a has also been shown in the study of Waterhouse et al. (2004). Thus, my results confirm the saturation effect with increasing C_a discussed in their study.

Furthermore, given that atmospheric CO₂ is the only atmospheric substrate for photosynthesis in terrestrial plants species and that rising CO₂ affects stomatal regulation of leaf gas exchange, it is important to study the level of adjustment of the interior concentration of CO₂ (C_i) to the atmospheric CO₂ (C_a) (Marshall and Monserud, 1996; Brienen et al., 2017). This enables to study the homeostatic maintenance of C_i by calculating the air-to-leaf CO₂ difference ($C_a - C_i$) for each year. The results in (**Figure 41**) shows an absence of homeostasis for beech because $C_a - C_i$ level increases with time (0.31 [SE = 0.03], $P = 2.2e^{-16}$). The same is

observed for oak (0.23 [SE = 0.04], $P = 4.64e^{-07}$). However, for oak the level of $C_a - C_i$ remained constant before 1950 (0.03 [SE = 0.08], $P = 0.75$) and after 1990 (-0.41 [SE = 0.3], $P = 0.22$), and increased significantly (0.53 [SE = 0.19], $P = 0.01$) between 1950 and 1990, indicating therefore a homeostasis only before 1950 and after 1990. It has been shown that there is a difference of gas regulation strategy over the life of trees, and there is also a difference among species (Brienen et al., 2017).

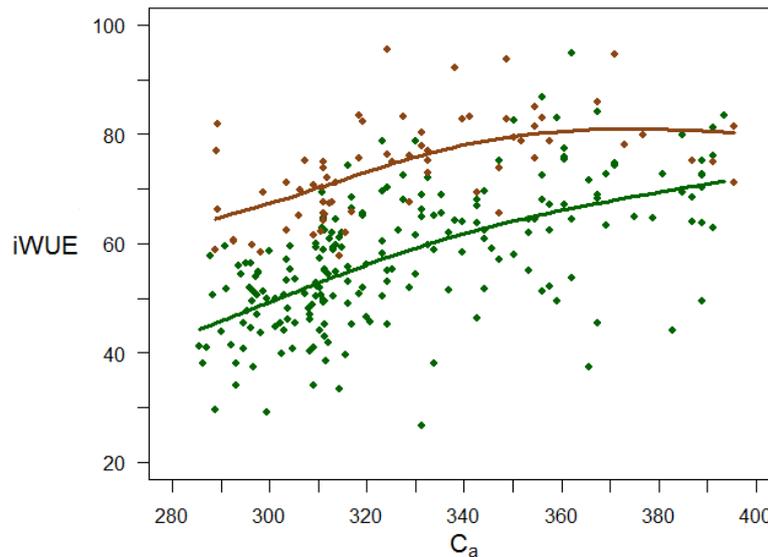


Figure 40. Mean intrinsic water use efficiency (iWUE in $\mu\text{mol mol}^{-1}$) with atmospheric CO_2 concentrations (C_a in $\mu\text{mol mol}^{-1}$) changing since the 1860th century for beech (green) and oak (brown). The adjustment was done by a smooth.spline function as implemented in R with a 3 degrees of freedom.

In all cases, my results imply that the response of beech appeared to be more related to the availability of CO_2 in the air (fertilization effect) than to any stomatal response while the opposite is true for oak (Bonafant et al., 2011). Indeed, for oak the absence of an increase of iWUE as function of date, during all the studied period except for the period 1950 – 1990, may be due to their homeostatic physiological response by increasing stomatal conductance (g_s) with no associated change, or even an increase, in net photosynthesis (A), but smaller than the increase in g_s . This reaction could explain the increase of GI and the trend of iWUE in the studied oak trees.

At last, the GI of the master chronology (see subchapter 6) indicates an overall increase of growth trends since 1860 for both species (**Figure 29** and **Figure 30**), followed by a decrease

since the 1980s. On the other hand, a significant increase of GI, of the chosen trees for the isotopic analysis, with the increase of iWUE has been shown in oak but not in beech (**Figure 42**). As a matter of fact, the increase of iWUE in beech since the 1950s (that should result in an increase in photosynthetic rates) was not sufficient to be accompanied by the same amplitude of increase in GI. However, for oak even if the level of iWUE did not increase with date except for the period between 1950 and 1990, the increase in internal CO₂ concentration resulting from the rise of atmospheric CO₂ concentration might be translated into increasing growth until the year 1980 followed by a decrease.

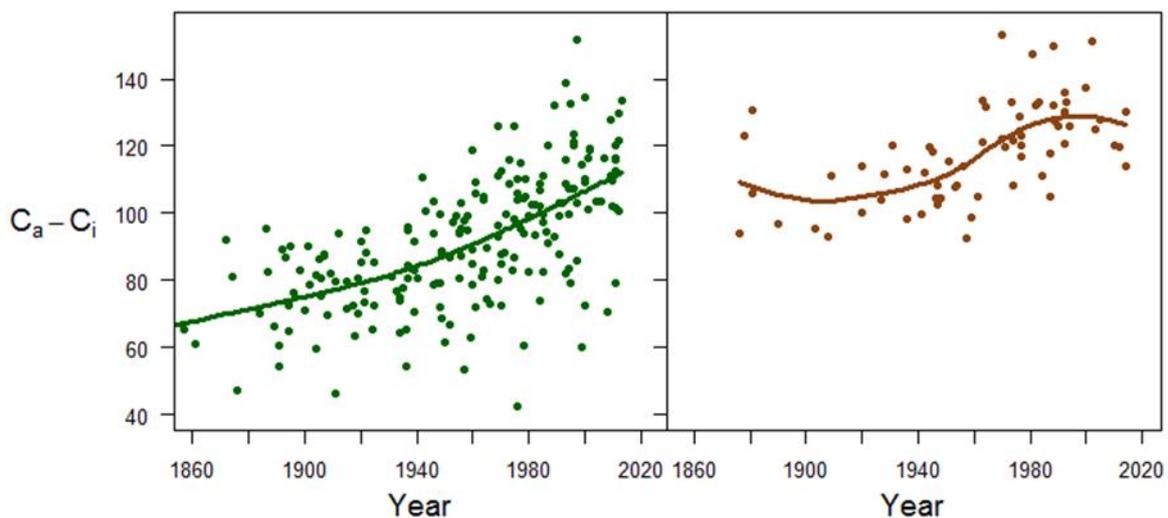


Figure 41. Air-to-leaf CO₂ difference ($C_a - C_i$ in $\mu\text{mol mol}^{-1}$) plotted against the date (year) for beech (green) and oak (brown) samples of isotopic analyses.

Many studies have shown that an increase in iWUE does not necessarily translate into an increase in plant growth (Silva et al., 2010; Peñuelas et al., 2011 and references therein). This was also the case in the Ciron beech population, whose the observed growth trend might be related to climate conditions such as warming and drought (Jump et al., 2006; Jump et al., 2007; Peñuelas et al., 2008; Piovesan et al., 2008) rather than to the fertilization effect of CO₂. On the contrary, even if that was not the case for oak we can notice that there is a kind of saturation after 1980 that can be explained by other factors such as nutrient limitation. Indeed, the lack of nutrients may limit the CO₂ fertilization effect on plant growth and may drive the saturation of the plant CO₂ response (Norby et al., 2010). Besides, the combined effect of climate conditions and other factors such as nutrient limitation could also explain the

lack of growth responses to increased CO₂ for both beech and oak. The effect of CO₂ fertilization clearly must be studied more finely.

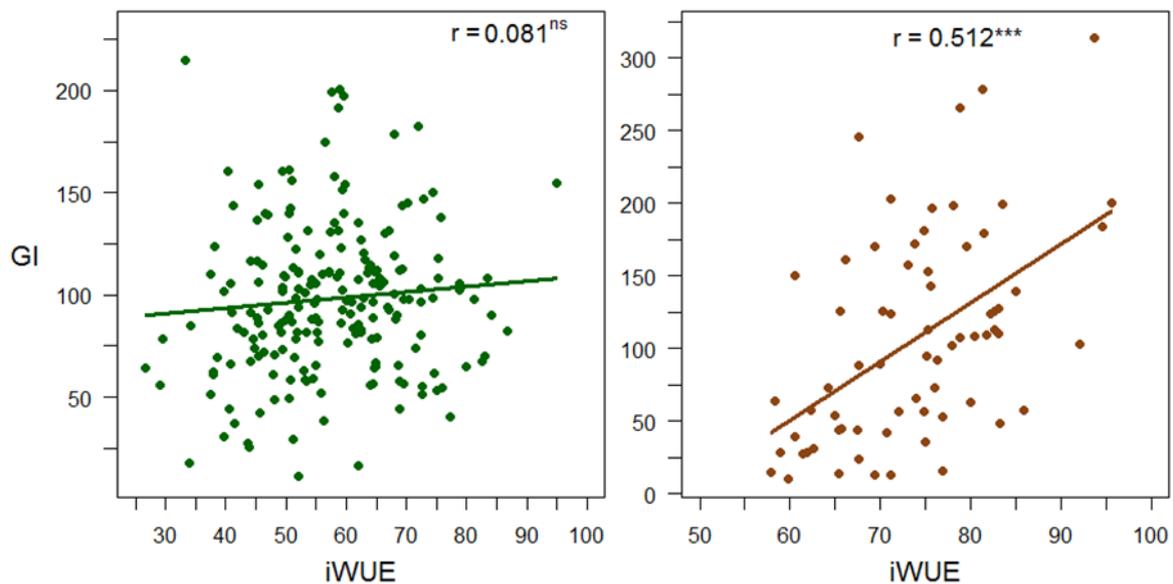


Figure 42. Relationship between growth index (GI in %) and intrinsic water use efficiency (iWUE in $\mu\text{mol mol}^{-1}$) for beech (green) and oak (brown).

7.5 Interaction between genetic and topographical position effects

Previously, I had shown that the interaction between the genetic identity of trees and their topographical position within the valley had an (indirect) effect on GI (see subsection 5, model M. 3; **Figure 27**). To test whether certain genotypes are disproportionately tolerant to excess of water, an adapted sampling of tree rings for isotopic analysis was done for three periods (1988-1991; 1995-1999 and 2004-2008). The corresponding mean GI and iWUE of these periods were plotted against the probability of trees to belong to the putatively original genetic cluster (**Figure 43**). Given that the effect was small in my previous analysis, I chose to consider only trees from the two extreme topographical positions: plateau and downslope. Then I tested whether beech trees with a higher probability (assignment rate >0.5) to belong to the putative original population tended to have a higher iWUE than their counterparts (<0.5).

The GI of trees on the plateau showed a significant increase as function of genetic cluster only for the period 1988-1991 (**Figure 43**; $r = 0.884$; $P = 2.23e^{-07}$). In downslope position, the GI decreased significantly for the three periods 1988-1991 ($r = -0.682$; $P = 0.001$), 1995-1999

($r = -0.849$; $P = 2.13e^{-06}$) and 2004-2008 ($r = -0.627$; $P = 0.004$). This last result is not surprising, because it is similar to the one observed for all the trees without any selection on the period (**Figure 27**). On the other hand, no significant correlation was observed between iWUE and the belonging to a genetic cluster on any of the selected period and topographical position. The same was observed after applying a linear model with random effect (trees as random factor), to define if the interaction between the belonging of trees to a genetic cluster with the topographical position of the trees influenced significantly the iWUE,

$$iWUE \sim P_{\text{original cluster}} \times \text{Topographical position}$$

This model showed that this interaction had no significant effect on iWUE ($F = 1.64$, $P = 0.22$).

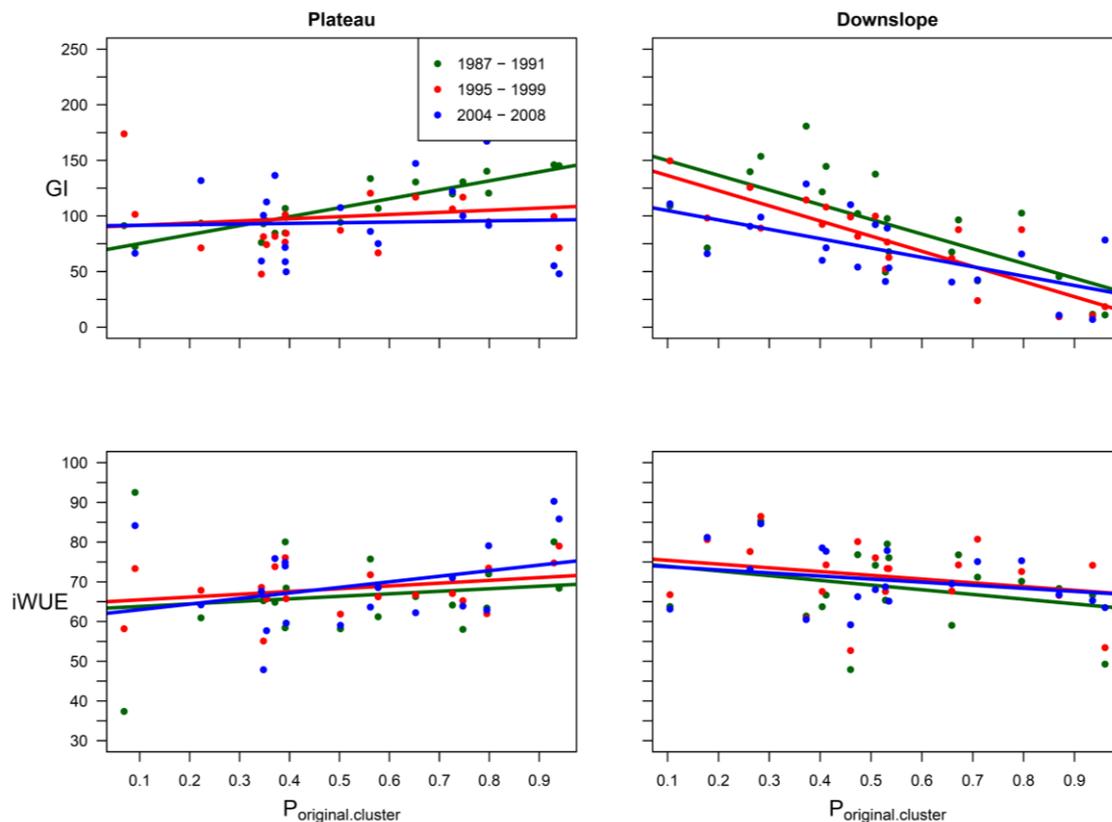


Figure 43. Mean growth index (GI in %) and water use efficiency (iWUE in $\mu\text{mol mol}^{-1}$) of 20 beech trees sampled in plateau position and for 20 beech trees sampled in downslope position, for the three chosen periods (1988 – 1991, 1995 – 1999 and 2004 – 2008).

These results seem to indicate that the genetic cluster has no strong effect on iWUE, although conclusions must be drawn with care given the high variability of $\delta^{13}\text{C}$ measures and the low number of samples (20 trees in each topographical position). Indeed, **Figure 43** shows consistently higher iWUE for trees with Genetic cluster values >0.5 on the plateau and lower

iWUE for that same group in downslope position, in all the chosen periods. Overall, my findings suggest in any case that the Ciron beech population could be a very suited study system that future in-depth investigations on genetic effects on trees growth and vitality.

8 DENDROCLIMATOLOGY

Climate models predict that the magnitude and intensity of drought events is increasing with modern climate change. This phenomenon has already affected various biomes during recent decades (Parmesan, 2006; Allen et al., 2010). On the other hand, variation in annual growth associated with annual changes in climate is likely to depend on the geographical position of the stand.

In some cases, climate seems to affect more the tree growth at interannual than at longer time scales (Fekedulegn et al., 2003), especially for tree populations located in xeric sites because they are more sensitive to climatic variation (Fritts, 1976). In this part of the study, I evaluated the climate-growth relationship of beech in the Ciron valley and compared it to oak in the same site. The purpose of this study is to identify the climatic drivers of tree growth and to predict how they will affect long-term trends under future climate change.

8.1 Climate – growth analysis

The study of the high frequency signal of the interannual climate variations in both species (so-called climate signal) requires extracting in first place the medium frequency signal from the studied chronologies. This is feasible by removing the impulses linked to local endogenous and exogenous perturbations within the study stands. To do so, extracting the year-to-year climatic signal by standardization with cubic splines (Cook, 1985; Cook and Peters, 1981) is necessary in order to obtain stationary series in which only the high frequency signal is conserved (Fritts 1976; Schweingruber, 1990). This procedure was performed using the *climate* function of the dplR package (Mérián, 2012a) in R version 3.3.1 (R Development Core Team 2016), resulting in a detrend series that enables to examine climate-tree growth relationships.

Daily climate data were obtained from a weather station of Météo-France located in Sauternes (44°32'39"N, 0°19'45"W), ca 17 km north of the study site in the Ciron valley. Climate data at this station have been manually collected since 1st January 1897 and automatically since 1st January 1991. The climate variables obtained from this weather station were minimal and maximal temperature per day (in °C) and rain rate per day (RR, in millimetres). From these data, I derived the following variables over the period 1897 – 2015 for my analyses:

Annual mean temperature; monthly mean temperature; total annual precipitation; monthly precipitation; annual and monthly potential evapotranspiration (PET) (using the Thornthwaite method; Thornthwaite, 1948); and monthly and annual water balance (WB).

$$PET = 16 \times D \left(\frac{10 \times T_m}{I} \right)^a$$

with PET: the monthly potential evapotranspiration in mm,

T_m : is the mean monthly temperature (°C),

I: sum over 12 months of $\left(\frac{T_m}{5} \right)^{1.514}$,

$$a = (492390 + (17920 \times I) - (77.1 \times I)^2 + (0.675 \times I)^3) \times 10^{-6}$$

D: coefficient that represents the mean possible duration of sunlight that differs from month to month and according to latitude (Thornthwaite, 1948). In the Ciron valley (44° N), D equals: Jan = 0.81; Feb = 0.82; Mar = 1.02; Apr = 1.13; May = 1.27; Jun = 1.29; Jul = 1.3; Aug = 1.2; Sep = 1.04; Oct = 0.95; Nov = 0.8 and Dec = 0.76.

$$WB = P - PET$$

with WB: monthly water balance in mm,

P: mean monthly precipitation in mm,

PET: monthly potential evapotranspiration in mm.

I performed the analysis with a climate data series of 119 years (from 1897 to 2015) and the chronologies of all 317 beech trees and 79 oaks. Previous results showed a strong noise reduction, where the expressed population signal (EPS) is near to 1 (i.e. subchapter 3.3 and **Table 3**). Thus, I chose to run my analysis with 0 iteration among the chronologies and 1000

bootstrap repetitions among the 119 years (default in the *climate* function in dplR). This was methodologically correct because my master-chronologies for beech and oak possesses a low noise level with many degrees of freedom within the linear adjustments. In addition, the high number of years (119 years) allowed to reduce the weight of extreme events that sometimes pollute the estimation of average tree responses to climate factors. The climate variables used in this analysis were the water balance of each month separately and the combination of several months spread over the growing season. There were 28 possible combinations between March and September (**Figure 44**). However, for the precipitation level I tested the effect of each month separately and of the winter from October to December of the previous year and January and February of the same year, plus the combination of these 5 months. Thus, I finally obtained 15 variables to test the effect of water reserve (**Figure 45**).

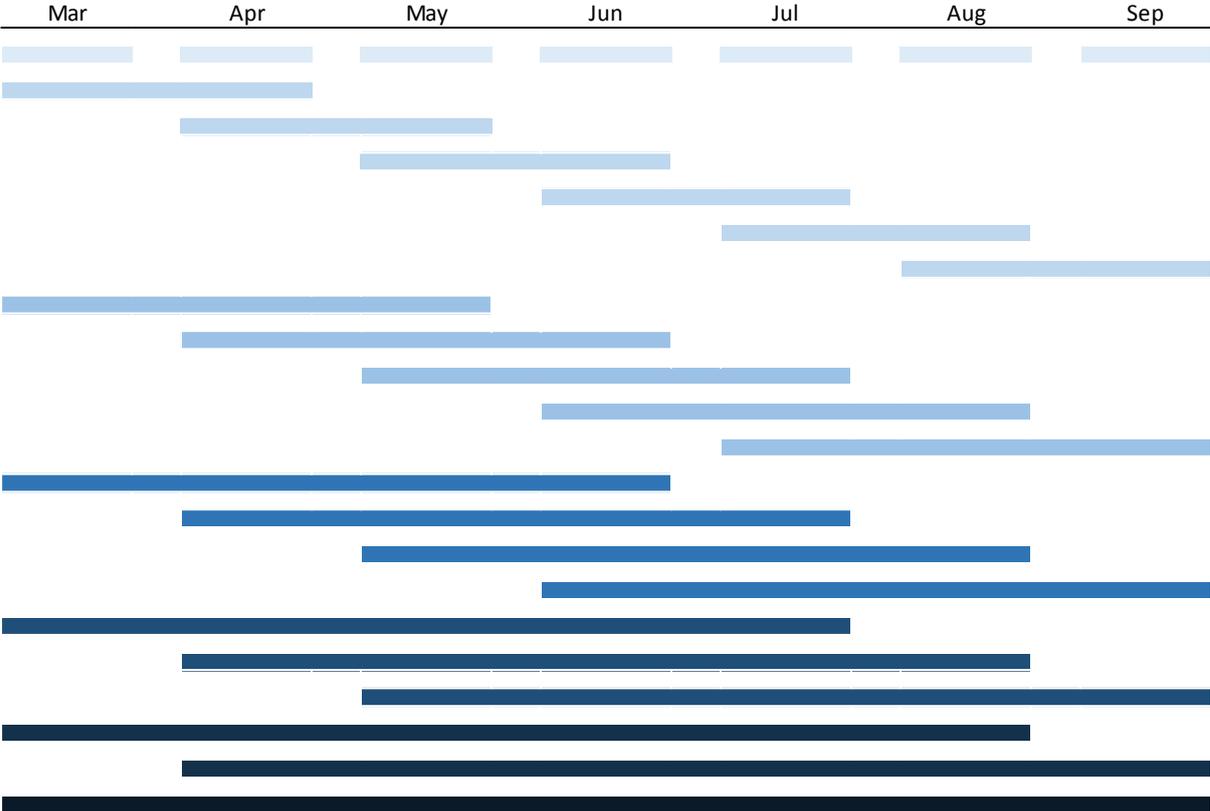


Figure 44. Combined months of water balance used for bootstrap correlation with the double detrended growth index for beech and oak. Each bar indicates one of the possible combinations of months of the growing season from March to September.

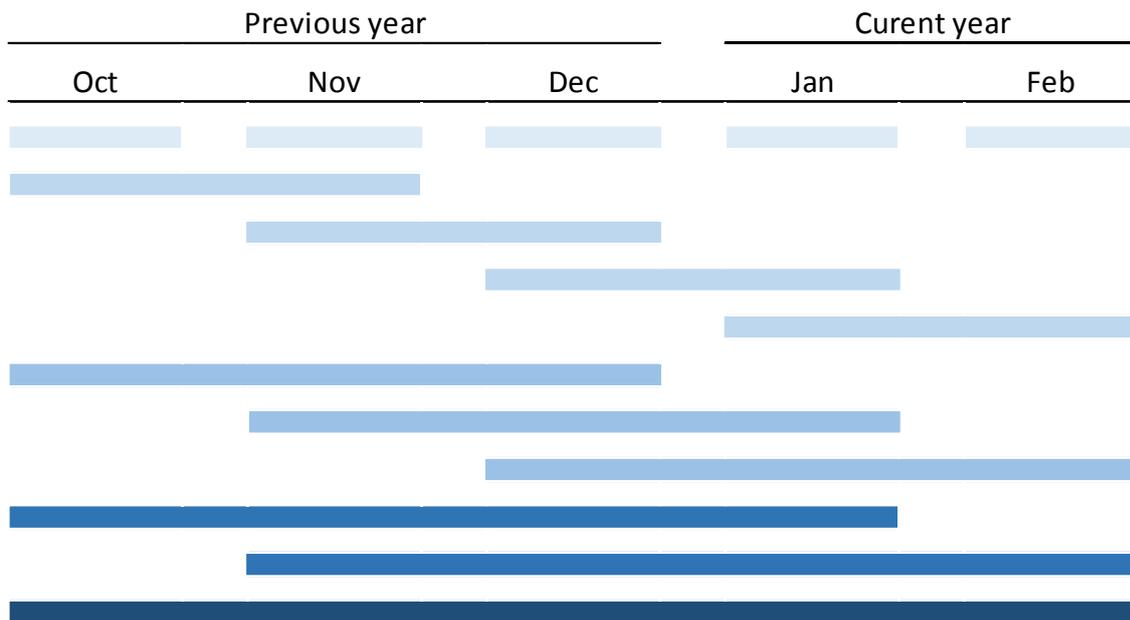


Figure 45. Combined months of precipitation used for bootstrap correlation with the double detrended growth index for beech and oak. Each bar indicates one of the possible combinations of months of the winter from October of the year to previous wood formation till February of the year of wood formation.

8.2 Results and model selection

The results in **Figure 46** showed that, for beech, both the precipitation rate and the water balance in February, June and July had a significant positive effect on tree growth. Thus, a low evapotranspiration coupled with high precipitation in June and July promoted beech growth in the Ciron valley. This is consistent with a study in Spain showing that beech growth is limited by high growing-season temperature and favoured by high precipitation during the growing season (Peñuelas et al., 2008). Similarly, Dittmar et al. (2003) showed that, for a population of beech at low altitude in central Europe, low temperature and high precipitation support the formation of wide tree rings during the vegetation period, especially in June and July. My results points towards the same conclusion: that interannual variations in tree growth depend more on the water regime than on the direct effect of temperature (Lebourgeois and Mérian, 2011). Notwithstanding, the observed significant effect in February is not common; it is out of the growing season but it might play a role in water storage within the site.

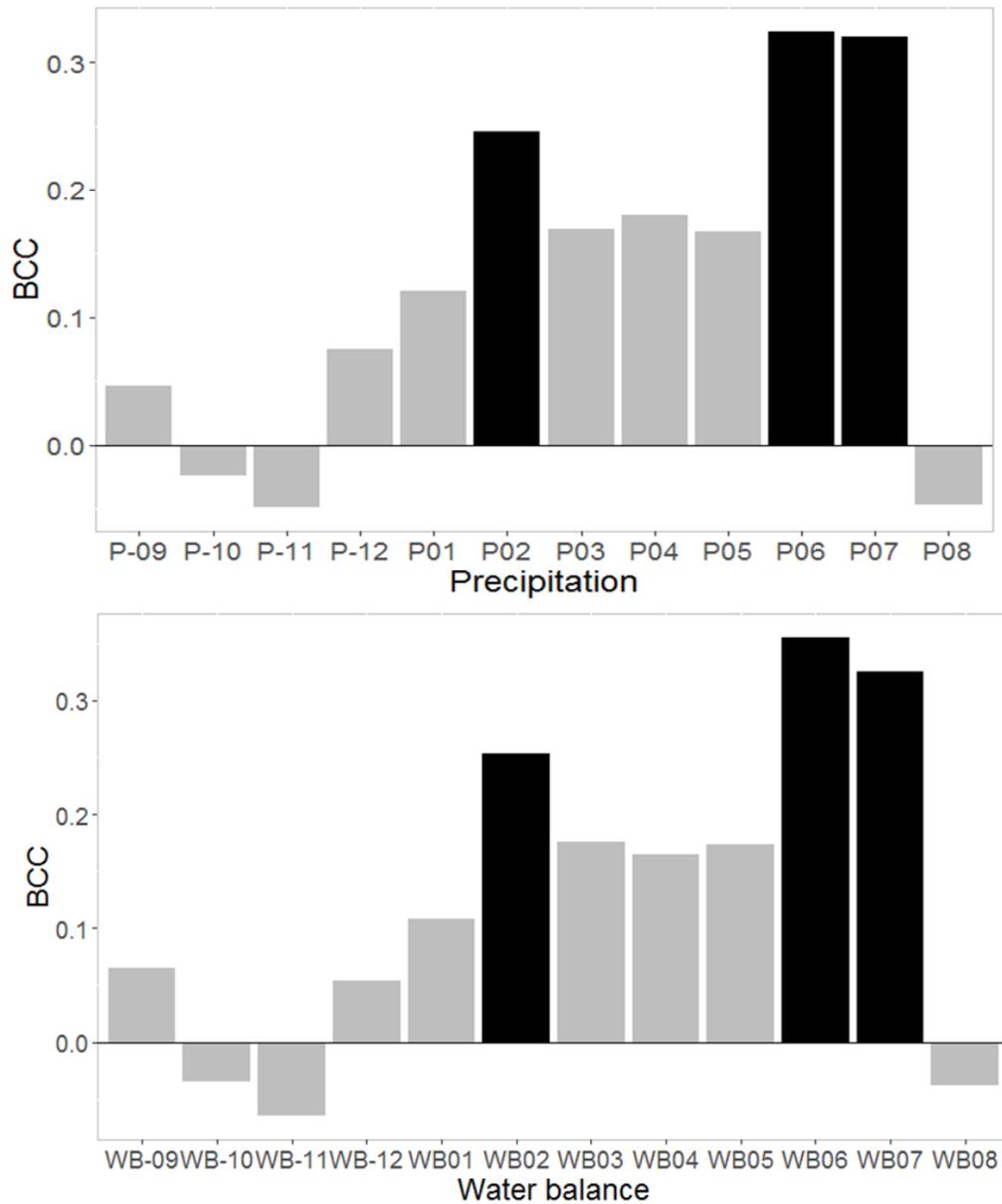


Figure 46. Bootstrap coefficient correlation (BCC) for beech between double detrended growth index, and mean monthly precipitation (P) in mm and water balance (WB) in mm for the period 1897 – 2015. Grey bar indicate a non-significant correlation and black bars indicate a significant correlation (at $P < 0.05$). Labels on the x-axes indicate the number of the month of the year. A negative sign before the month number indicates the month of the year previous to the wood formation and the absence of the negative sign indicates the month of the same year of wood formation, for example P-9 indicate the mean precipitation in September of the year previous to wood formation.

For oak, as for beech, the precipitation and water balance of June and July had a positive significant effect on tree growth (**Figure 47**). The interannual variability is much more difficult to appreciate for oak because tree growth appears to depend not only on the climatic

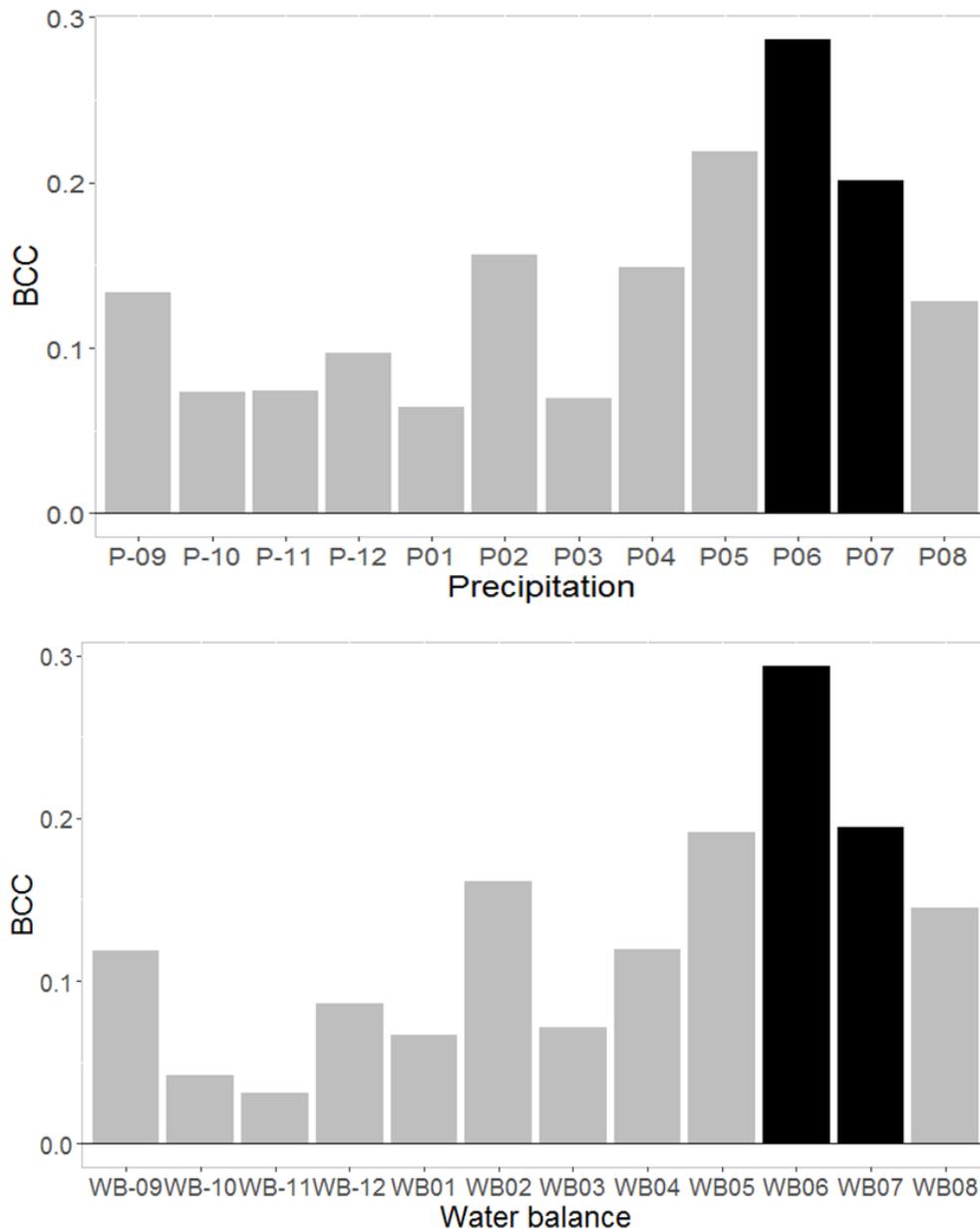


Figure 47. Bootstrap coefficient correlation (BCC) for oak between double detrended growth index, and monthly precipitation (P) in mm and water balance (WB) in mm for the period 1897 – 2015. Grey bars indicate a non-significant correlation and black bars indicate a significant correlation ($P < 0.05$).

conditions of the growing season, but also on the autumn and winter conditions preceding the ring formation (Lebourgeois and Mérian, 2011). However, both species tend to show a remarkable susceptibility to summer drought within my study site. Such an increase in susceptibility to spring or summer drought has been observed lately for many stands, especially under hot and dry conditions (Lebourgeois and Mérian, 2011).

On the other hand, the results of all the combined months of water balance and precipitation described previously (**Figure 44** and **Figure 45**), revealed that the variables that explain beech growth the most are the precipitation in February and the water balance from March to July (P02 and WB37 respectively, **Figure 48**). The water balance from March to July was more significant than water balance of June and July separately and even more significant than the water balance of June and July together. However, for oak, the most significant factor was the water balance from April to August (WB48, **Figure 49**).

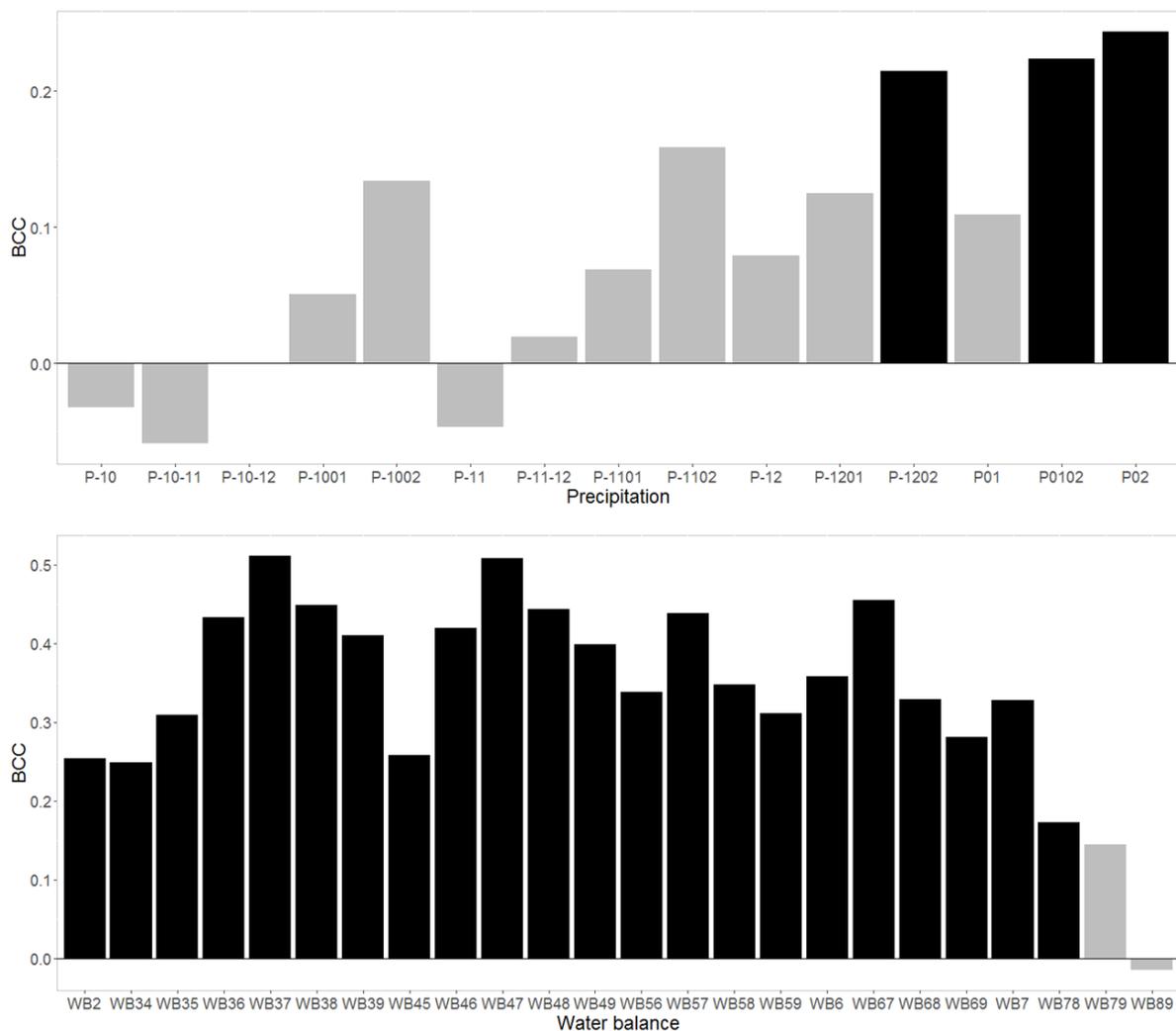


Figure 48. Bootstrap coefficient correlation (BCC) for beech between double detrended growth index, and the combined months of the winter of precipitation (P) in mm and the combined months of the growing season of water balance (WB) in mm, the monthly water balance (WB2, WB6 and WB7) that were significant previously were also added. Grey bar indicate a non-significant correlation and black bars indicate a significant correlation ($P < 0.05$).

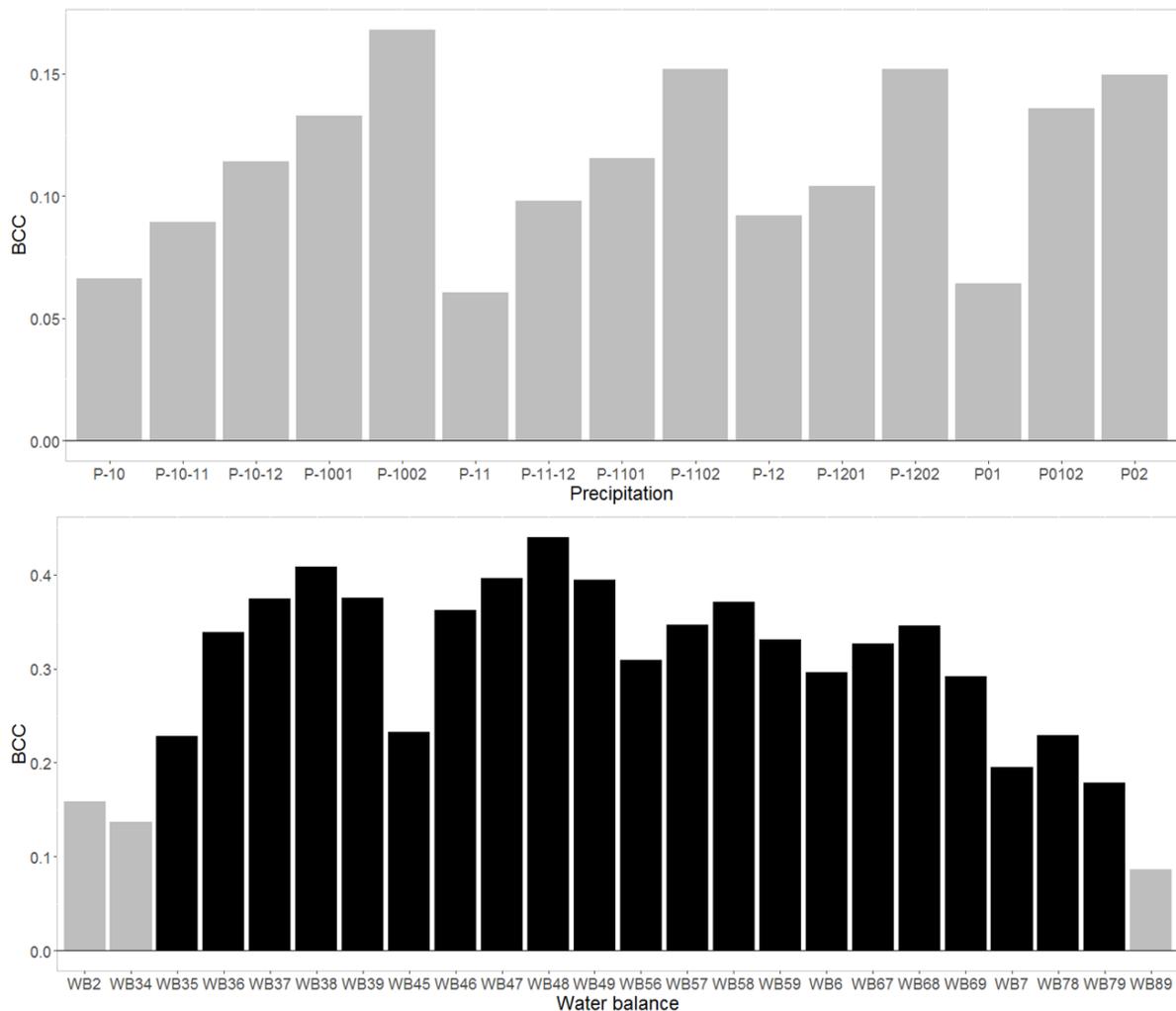


Figure 49. Bootstrap coefficient correlation (BCC) for oak between double detrended growth index, and the combined months of the winter of precipitation (P) in mm and the combined months of the growing season of water balance (WB) in mm, the monthly water balance (WB2, WB6 and WB7) that were significant previously were also added. Grey bar indicate a non-significant correlation and black bars indicate a significant correlation ($P < 0.05$).

The previous analyses were performed on the double detrended tree ring series and the combination of variables that I had calculated previously. However, many of these variables are correlated or nested. So, to choose the one that explain best the GI, I used the *climate* function of the *dplR* package (Mérian, 2012a) in R version 3.3.1 (R Development Core Team 2016) on each of these variables separately. After performing these analysis, I conclude that, for beech, the variables that explain best the GI were the water balance of all the growing season from March to July combined (bootstrap correlation coefficient = 0.511, lower CI = 0.336, upper CI = 0.637) and the precipitation of February of the same year (bootstrap correlation coefficient = 0.243, lower CI = 0.082, upper CI = 0.398). For oak, the variable that

explains best the GI is the water balance of all the growing season from April to August combined (bootstrap correlation coefficient = 0.440, lower CI = 0.249, upper CI = 0.589). Therefore, based on these latter results I selected one final model for each species with the following climate variables:

- **For beech:**

$$M. 7 : \quad GI_{\text{beech}} \sim WB_{\text{March-July}} + P_{\text{February}}$$

- **For oak:**

$$M. 8 : \quad GI_{\text{oak}} \sim WB_{\text{April-August}}$$

Finally, to validate the models I run a regression Bootstrap by using the function “bootStepAIC” of MASS package (Venables and Ripley, 2002) as implemented in R version 3.3.1 (R Development Core Team 2016). This function implements a bootstrap procedure to investigate the variability of model selection under a stepwise algorithm. The results were coherent with the correlation analysis and showed for the model M. 7 that both variables were significant, with a statistical significance of $WB_{\text{March-July}}$ equal to 100% and a statistical significance of P_{February} equal to 73.59%. In both variables, the regression coefficients were always positive (100% for both variables). The same was shown for model M. 8: $WB_{\text{April-August}}$ was significant a statistic significance of 99.9% and a regression coefficient always positive (100%). Thus, the regression parameters of both models were calculated:

- **For beech:**

$$Eq. 1_{(\text{beech})}: \quad GI_{\text{beech}} = 0.0008 \times WB_{\text{March-July}} + 0.0006 \times P_{\text{February}} + 1.029$$

- **For oak:**

$$Eq. 2_{(\text{oak})}: \quad GI_{\text{oak}} = 0.0007 \times WB_{\text{April-August}} + 1.11$$

Afterwards, I plotted the curve of double detrended GI and the GI calculated from these models, Eq. 1_(beech) and Eq. 2_(oak), against the years from 1897 to 2015 for beech (**Figure 50**) and for oak (**Figure 51**). Then I calculated the absolute deviation between the two curves in

each year, I , in order to estimate the mean deviation between the two curves. For beech, the deviation was 9.14% and for oak it was 9.51%. This result allowed me to make predictions of the tree growth as a function of the climatic variables that were most influential for tree growth in this site.

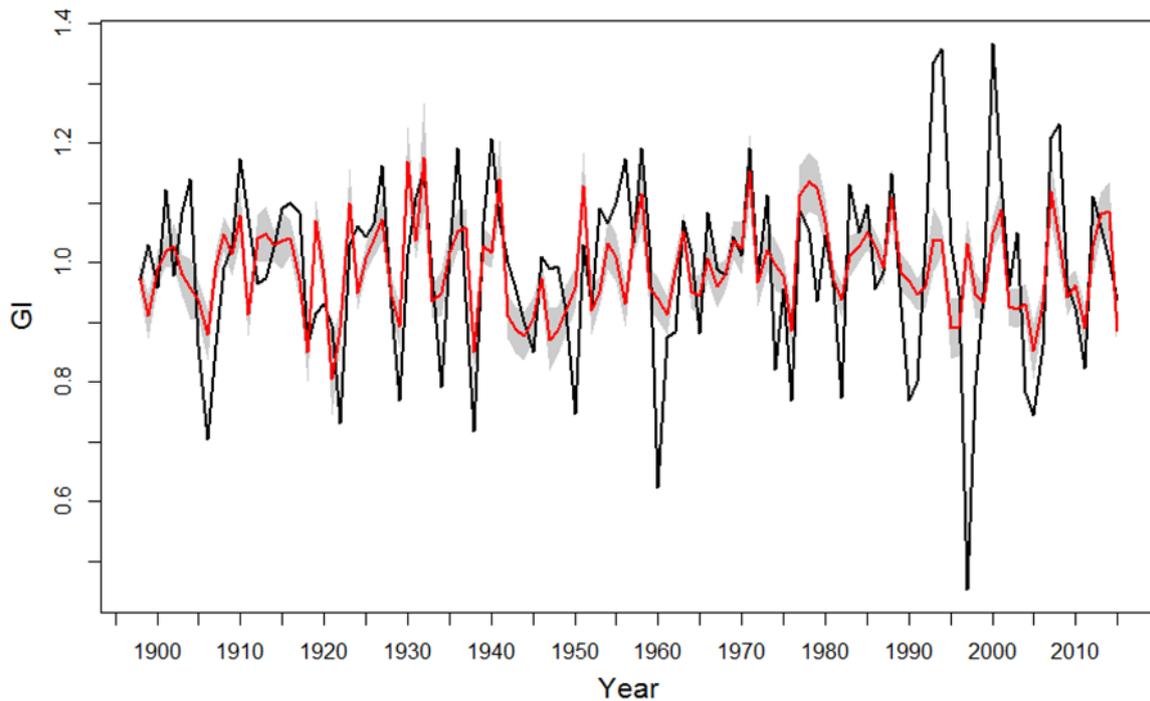


Figure 50. The black curve indicates the double detrended GI of the master chronology of beech as function of the date (Year). The red curve indicates the GI calculated after the equation Eq. 1_(beech) as a function of the date and the grey zone indicate the 95% confidence interval of the selected model.

Both climate variables, water balance of the growing season and precipitation, seem to explain well the year-to-year growth rate for both species in the Ciron valley. However, in some years like 1960 and 1997, beech growth was particularly small and could not be explained by the water balance or the precipitation level of that year. Indeed, **Figure 50** had shown that in these two years the growth estimated from the model Eq. 1_(beech) was quite the opposite of that observed in my chronology series. Instead, the meteorological data suggest that late frosts probably affected the Ciron in 1960 and 1997 (see Appendix S4, Figure IV). These frosts could have affected beech more than oak, which is flushing later in the study area. In addition, I observed that some beech trees had experienced much stronger growth declines in the two years in question than others.

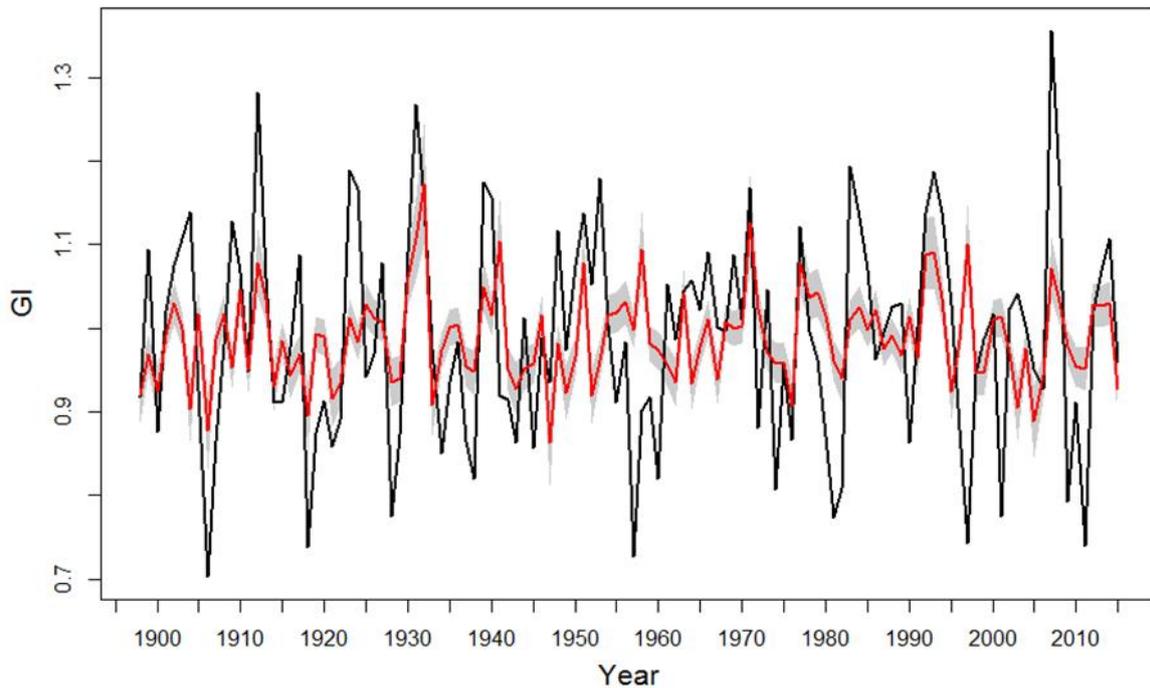


Figure 51. The black curve indicates the double detrended GI of the master chronology of oak as a function of the date (Year). The red curve indicates the GI calculated after the equation Eq. 2_(oak) as a function of the date and the grey zone indicates the 95% confidence interval of the selected model.

8.3 Phenological approach

Principle

Foliar development is a key factor in the study of the primary productivity of forest ecosystems (Lebourgeois et al., 2006). A destruction of the foliage by frost is a considerable loss for the tree. It has been shown that negative pointer years (increment minima) in the tree ring series of beech could be related to late frosts (Dittmar et al., 2006; Cailleret and Davi, 2011). Therefore, to find out the reasons for this different tree growth behaviour during 1960 and 1997 at interspecific (between beech and oak) and intraspecific (between beech trees) level in the Ciron valley, I monitored the phenology of bud break. It has been reported that, within a tree stand, the same trees flush earlier or later from year to year (Comps et al., 1987). This allowed me to test my hypothesis on the effect of late frost on trees growth in the Ciron valley.

Afterwards, I proceeded to the tree sampling. Thus, to select trees that showed low or strong growth in 1960 and 1997, I calculated the relative deviation in percentage of the tree ring width of the target years with the year before according to the formula,

$$100 \times (RW_t - RW_{t-1}) / RW_t,$$

where RW_t is tree-ring width for a year t and RW_{t-1} for the previous year $t-1$.

A visual study of the relative differences in 1960 and 1997, ranging from -100 to +243 for 1960 (243 series) and -100 to +327 for 1997 (327 series), allowed me to define a threshold that identifies the trees that marked the lowest level of growth, with a relative difference less than -65% in 1960 and in 1997. Then the trees that experienced both stresses were identified. Similarly, trees that benefited from an improved growth in 1960 or 1997 were identified with a relative difference higher than 30% in 1960 and 1997. In this last case, no trees fulfilled this condition during both years, 1960 and 1997, so a set of beech trees fulfilling the condition during one of each of these two years were identified. At the end, 23 beech were sampled with a low growth level in 1960 and 1997, 18 beech trees with a high growth level in 1960 and 1997, 19 oaks inside the valley and 20 oaks outside the valley for comparison. This sampling design allowed to make a phenological classification of the trees in 2017 and to test the following hypotheses:

H1: beech trees with small tree rings in 1960 or 1997 flushed earlier than the others

H2: oaks flushed later than the beech trees

H3: the microclimate within the valley has an effect on the date of flushing in oaks

Method

Four scores were defined for the bud break (**Figure 52**):

- Stage 0: closed bud
- Stage 1: swollen bud
- Stage 2: leaves outlet

• Stage 3: spread leaves



Figure 52. Stage of leaf phenology of beech and oak that I adopted in my sampling.

These scores can be attributed to a fraction of the crown because several stages can be present on the branches at a given date. A tree was then characterized by 1 to 3 codes in percentage of the crown concerned. For example, stage 1 can be attributed to 40% of the crown buds, stage 2 to 50% and stage 3 to 10% of the crown buds. Then, the score of the bud break of this tree was calculated as:

$$\text{Score} = 1 \times 0.4 + 2 \times 0.5 + 3 \times 0.1 = 1.7 \text{ for the tree at the day of notation.}$$

Each category (high-growth beech trees, low-growth beech trees, oaks inside the valley and oaks outside the valley) was composed of trees that do not have truly synchronized reactions. This variability required the calculation of an average for each of the 4 categories and then a comparison of averages by date within a species. The type of scoring applied is robust and efficient to demonstrate the hypotheses tested in this experiment.

The observations were carried out between 6 March (first strikes noted on 14 March) and 15 May 2017 by the same observers (D. Bert, Y. Mellerin and R. Segura). It took two months between the beginning of the bud burst of the earliest beech and the full spreading of the leaves of the latest beech.

Results and discussion

Figure 53 shows that the dynamics of bud burst of the oaks was sigmoidal whereas that of the beech trees marked two levelings: a first that led to stage 1 (swollen buds), followed by a latent phase before the leaf unfolding.

In the beech samples, the two groups classified according to their growth in 1960 and 1997 exhibit two clearly distinct curves. These curves were significantly different in spite of the large variability within the groups and the relatively small number of samples (18 and 23). Beech trees that experienced low growth in 1960 and 1997 flushed earlier (red curve) than beech trees that did not show growth reductions (blue curve). Thus, H1 could be confirmed. The delay was about 7 days during bud swelling, followed by about 4 days during leaf release and up to 9 days during leaf unfolding. These few days can be decisive when a freest event occurs.

I also had the chance during the sampling campaign to observe directly the effect of a late (but more moderate) frost that occurred during the nights of 20 to 22 April 2017 and especially of 27 to 29 April. Data from two meteorological stations installed in the central part of the population indicated temperatures of -2.4°C on April 21 and towards -2.2 on April 27 and 28 (Marion Walbott, Biogeco, personal communication). The damage was noted after observing the tree crown with a relative scale ranging from 0 to 4, with 4 for the most affected trees. (Note that even trees that were rated 4 were generally little affected by frost). The 12 oaks that we could sample in the Ciron valley had an average damage of 2.58, the 12 beech trees with low growth rate in 1960 and 1997 had an average damage of 1.75, and the 9 beech trees with high growth rate in 1960 and 1997 had only 0.89. The oaks and the 12 beech trees with low growth rate in 1960 and 1997 were basically the earliest and were most affected. I also observed that very early individuals had less damage because their leaves were probably more mature, whereas the leaves in stage 2 (leaves outlet) were more degraded. Oak trees generally had damage on the entire foliage, whereas beech trees were only affected on some leaves, often at the top of the crown. These observations allowed me to better identify the most sensitive stage after a moderate frost. However, a more intense frost would certainly

have had an impact on other stages. It appears from these observations that the most critical period is after stage 1.

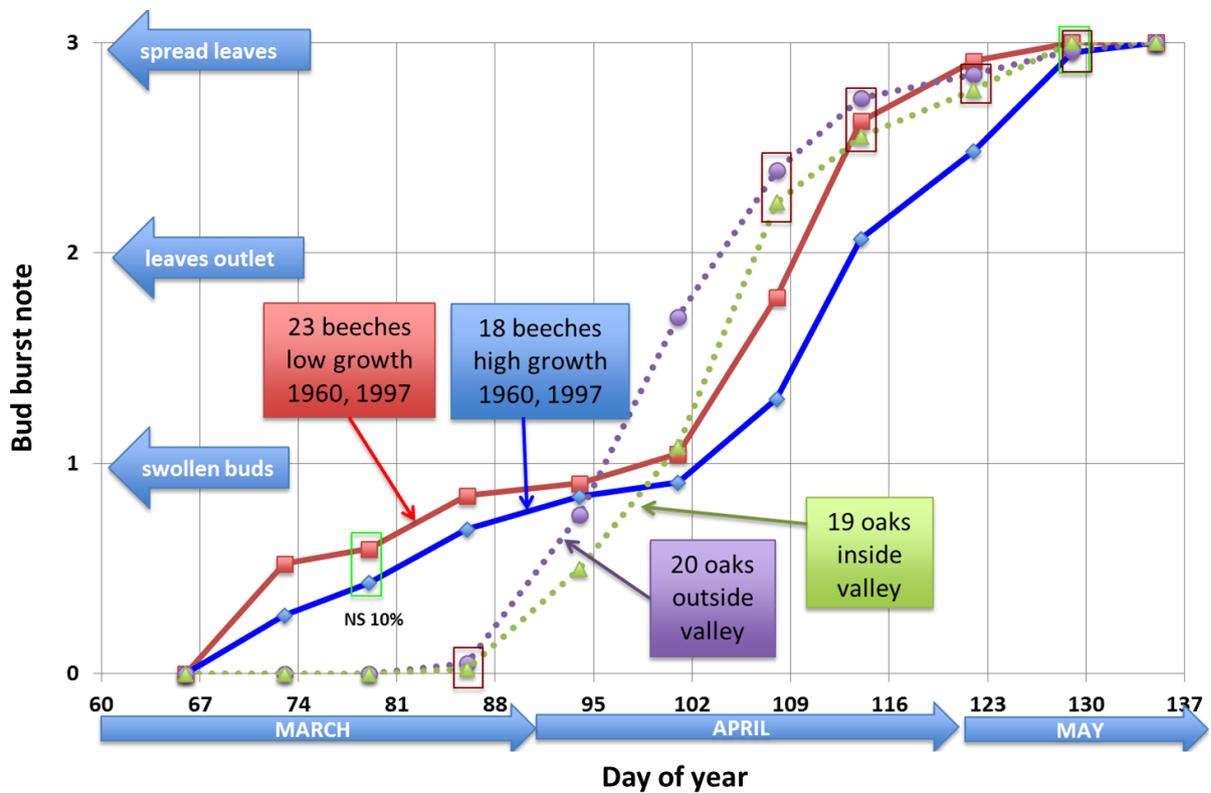


Figure 53. Bud burst note as function of the date (day of year) of the sampled beech and oak. The red curve represents the beech trees that showed a low growth in 1960 and 1997. The blue curve represent the beech trees that showed a high growth in 1960 and 1997. The green dotted curve represents the sampled oaks inside the valley. The purple dotted curve represents the oaks outside the valley. The red rectangle around the points that represent a bud burst notes for a given day means a non-significant student test. The green rectangle around the points that represent a bud burst notes for a given day means a non-significant student test at 10%.

The oak inside the valley start flushing later than beech until stage 1 (swollen bud). However, the curve showed that oak trees flush earlier than beech trees with low growth rate in 1960 and 1997 after the stage 1, then it became the same after stage 2 (leaves outlet). Thus, the hypothesis H2 is not validated. On the other hand, the comparison of the dynamics of oak trees outside and inside the valley showed that these two populations do not have the same timing of bud burst, since the oaks inside the valley tend to flush later. The delay reached about 5 days on average at the end of stage 1 towards April 4th. This may indicate a possible protection of oaks in the valley from a late frost event that may be linked to the microclimate of this particular site, thus validating the hypothesis H3.

In any event, common beech is known to be more sensitive to spring frost damage compared to other major European forest tree species (Dittmar et al., 2006). Frost below -4°C can kill the developing new shoots, reduce growth, and cause misshapen branching out. Overall, these results show that the effect of late frost on tree growth may even touch rear-edge populations, a little regarded phenomenon that deserves future investigation, even if the frequency of frost events is decreasing (Scheifinger et al., 2003; see also , Appendix S4 Figure V, for the study site).

8.4 Possible evolution of future tree growth

In the previous part of the study, I developed two simple models (Eq. 1_(beech) and Eq. 2_(oak)) that summarized the effect of climatic factors on the trees growth in the Ciron valley. These two models can be used to predict growth trends as a function of the future evolution of the climatic drivers in question, providing a preliminary and simplified idea of the possible evolution of beech and oak growth in this particular refugial site. In the following, I will describe the method that I followed in order to obtain these future growth trends.

General circulation models (GCMs), are numerical models that simulate the global climate system (IPCC, 2013). These models are used for modelling the state of the climate system and for simulating its response to changes in atmospheric concentrations of greenhouse gases and aerosols. On the other hand, regional climate models (RCMs) have been used to assess more detailed regional climate trends. These latter can provide information on the climate in higher spatial resolution (horizontal resolution between 2 and 50 km) than GCMs. In any event, projections of changes in the climate system are made using many models ranging from simple climate models, to models of intermediate complexity, to comprehensive climate models, and Earth System Models. Thus, a new set of scenarios, the Representative Concentration Pathways (RCPs), was used for the new climate model simulation. In all RCPs, atmospheric CO_2 concentrations are higher in 2100 relative to present day as a result of a further increase of cumulative emissions of CO_2 to the atmosphere during the 21st century (IPCC, 2013).

Météo-France researchers have produced fine-scale projections across France (resolutions ranging from 8 to 50 km). The most recent results are available to the public and

the scientific community on the portal "DRIAS, les futurs des climats". The latter offers access to about thirty climatic parameters and indicators, on a grid of 8 km resolution, simulated by several models and for different greenhouse gas emission scenarios, including the RCP scenarios used in the last Intergovernmental Panel on Climate Change report (IPCC, 2014). DRIAS provides regionalised climate projections carried out in the French climate modelling laboratories (IPSL: Institut Pierre-Simon-Laplace, CERFACS: Centre Européen de Recherche et de Formation Avancée en Calcul Scientifique, CNRM-GAME: Centre National de Recherches Météorologiques). It furnishes all the information provided by the climate models, for the scenarios (RCP) presented in the latest IPCC report.

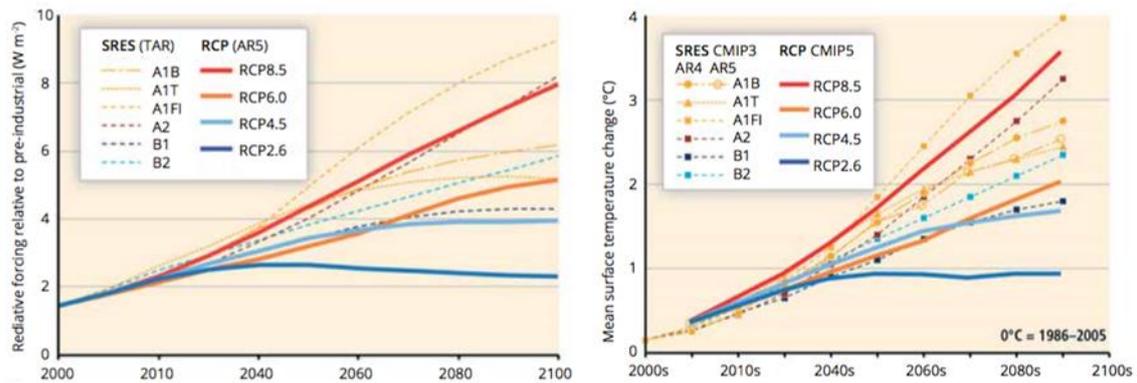
One approach that can be used in my study context is the multi-scenario model which consists in fixing a given index and a model experience. I can use this multi-scenario model, where the indices are monthly temperature and precipitation, in order to calculate the water balance, and the model experience is the study site within the Ciron valley.

The RCP scenarios (IPCC, 2014) represent radiative forcing evolution profiles, and are used as input parameters for climate models. A radiative forcing is a change in radiative balance due to a change in one of the factors of climate change such as the concentration of greenhouse gases. Four scenarios were developed according to the radiative forcing range for the year 2100 compared to 1750, of which three are available on the DRIAS site: RCP2.6, RCP4.5 and RCP8.5. Where RCP2.6 is the most optimistic scenario and RCP8.5 is the most pessimistic scenario (**Figure 54**).

- RCP2.6 (also known as RCP3PD) is a 'peak-and-decline' scenario that leads to very low greenhouse gas concentration levels. Its radiative forcing level first reaches a value of around 3 W/m² by mid-century, and returns to approximately 2.6 W/m² by 2100. In order to reach such radiative forcing levels, greenhouse gas emissions (and, indirectly, emissions of air pollutants) are reduced substantially, leading to net negative carbon dioxide emissions at the end of the 21st century.
- RCP4.5 is a stabilisation scenario in which total radiative forcing is stabilised at approximately 4.5 W/m² shortly after 2100, without overshooting the long-run radiative forcing target level.

- RCP8.5 is a high-emissions scenario in which total radiative forcing reaches approximately 8.5 watts per square metre (W/m^2) in 2100 and continues to increase afterwards. Its extension, ECP8.5, stabilises at approximately $12 \text{ W}/\text{m}^2$ in 2250.

Figure 1.1 Relationship between SRES scenarios and RCPs



Note: Projected radiative forcing (left) and global mean surface temperature change (right) over the 21st century using the Special Report on Emissions Scenarios (SRES) and representative concentration pathway (RCP) scenarios.

Source: IPCC, 2014a (Figure 1.4). © 2014 Intergovernmental Panel on Climate Change. Reproduced with permission.

Figure 54. The four Representative Concentration Pathways (RCP) scenarios as presented in IPCC (2014). Figure taken from European Environment Agency (2017).

I downloaded the daily temperature (minimum, maximum and average temperature) and precipitation data for the period 2006 – 2100 according to two scenarios, RCP4.5 and RCP8.5. Note that the general trends are supposed to be more realistic predictions than the global RCP scenarios. Afterwards, the mean monthly temperature and precipitation were calculated for the chosen period and the data set was formatted and adapted for subsequent analyses. Appendix S5 gives the mean monthly temperature, precipitation, evapotranspiration and water balance in the region for the past and the future climate scenarios RCP4.5 and RCP8.5.

Evapotranspiration (Thornthwaite, 1948) and water balance were calculated over the period 2006 and 2100 after adjusting the temperature and precipitation data of both scenarios (RCP4.5 and RCP8.5) by using the common period (2006 - 2015) between the climatic scenarios and my current meteorological data. For a given month and a given climatic variable, I calculated the difference between the mean level of the actual data of the variable and those of the future climatic models from 2006 to 2015. Then I added this difference to the predicted

values from 2006 to 2100, in order to shift all the future climate data. The (**Figure 55**) represents an example of one month (June) before and after adjusting the temperature and precipitation data to the past data. I did the same for all months of the year, then I calculated the evapotranspiration and the water balance from the adjusted climate data of the two models (RCP4.5 and RCP8.5).

Afterwards, I calculated the combined monthly variables of water balance that affected the most trees growth in the past, which are $WB_{\text{March-July}}$ for beech and $WB_{\text{April-August}}$ for oak. We, finally, applied the two equations (Eq. 1_(beech) and Eq. 2_(oak)) by using the predicted climate factors in order to obtain the possible evolution of growth rate of beech and oak in the Ciron valley. After plotting the results of the modelled GI against year for beech (**Figure 56**) and oak (**Figure 57**), I noticed a slight decrease of tree growth until 2100, for both species and in both future climate scenarios (RCP4.5 and RCP8.5). However, the correlation test between the modelled GI of the climate scenario RCP4.5 and the date was not significant either for beech (RCP4.5: $r = -0.085$, $P = 0.414$, RCP8.5: $r = -0.095$, $P = 0.316$) or for oak (RCP4.5: $r = -0.016$, $P = 0.881$, RCP8.5: $r = -0.089$, $P = 0.386$).

I also tested for each species whether there were any significant differences for the prediction of the evolution of tree growth between the two climate scenarios by comparing their respective regression slopes (**Figure 56** and **Figure 57**). No significant difference was observed.

The results of this study part indicate that, even under strong climate warming as simulated in the climate scenario RCP8.5 (Appendix S5 fig. VIII), both beech and oak may manage to persist without experiencing significant reduction in growth. This observation affirms the character of the Ciron valley as an effective climate refugium. It would be of interest to assess whether similar conclusions can be drawn for other lowland beech populations in the region.

Indeed, the chosen models in my study are simple linear model based on previous collected data. In addition, the selected models do not take into account the impulse linked to local endogenous (silviculture, senescence of dominant trees etc.) or exogenous perturbation to the stand (fires, storms, pathogens, insect outbreaks, late frost etc.). Also, in

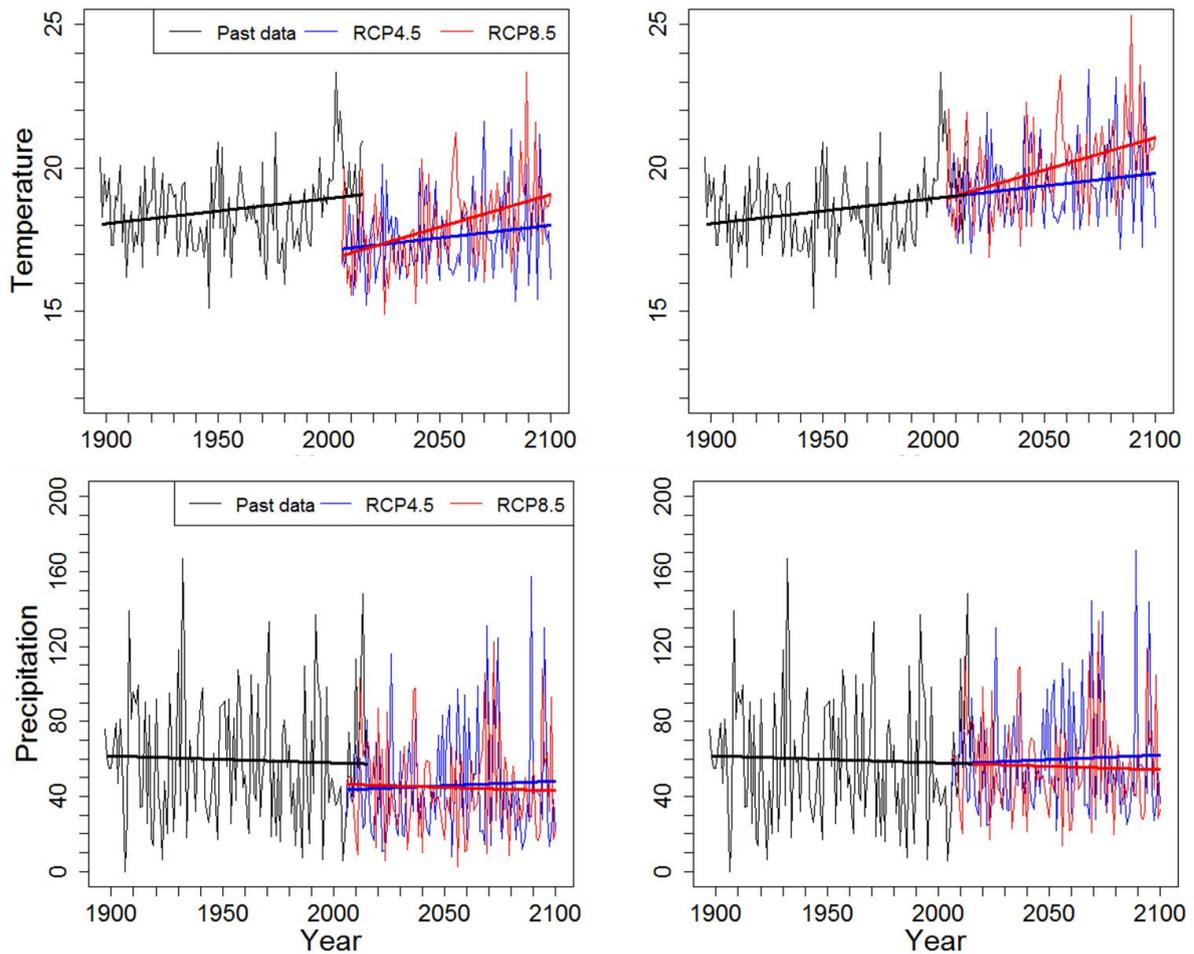


Figure 55. Mean temperature in °C (top graphics) and mean precipitation in mm (bottom graphics) in June, from 1897 to 2015 (past climate data) and from 2006 to 2100 (future climate data) before and after adjustment of the future climate data to the past data. Graphics on left represent the curves of the future data of both scenarios RCP4.5 (blue) and RCP8.5 (red) before the adjustment and the graphics on the right represent the same data after adjustment.

these models I adopted the uniformity principle and I assumed that the same factors that have been limiting tree growth in the past will be limiting trees growth in the future. This neglects the divergence principle (D'Arrigo et al., 2008; Lebourgeois and Mérian, 2011). However, the procedure adopted to select the models can be considered as reliable at local scale and may give a general idea about the future growth trends of the Ciron beech population.

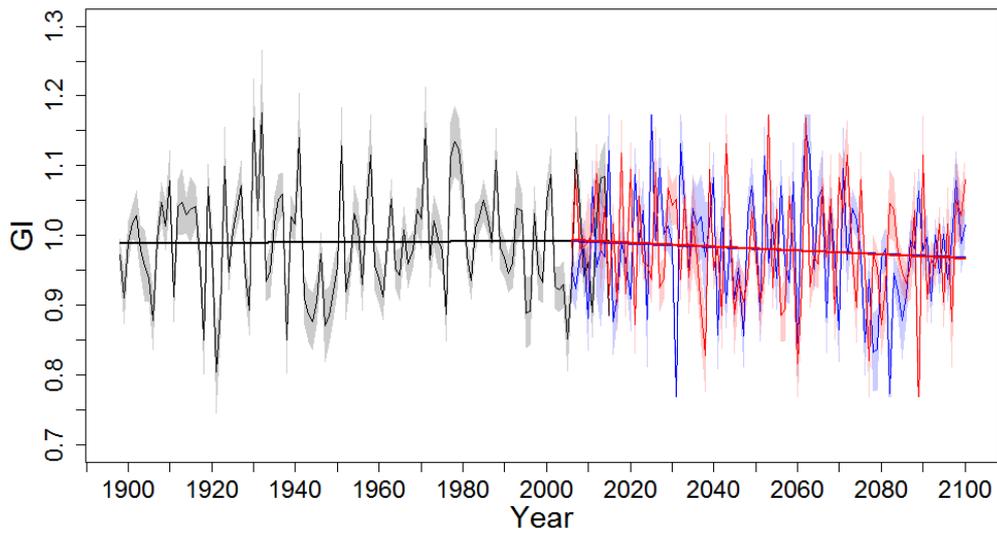


Figure 56. The black curve (the same as the red curve in Figure 50) indicates the GI for beech calculated after the equation Eq. $1_{(\text{beech})}$ as a function of the date (1897 – 2015) with its 95% confidence interval. Blue and red curves indicate the GI for beech calculated after the equation Eq. $1_{(\text{beech})}$ as a function of the date (2006 – 2100), respectively for the climate scenario RCP4.5 and RCP8.5 with their 95% confidence interval. The blue and red lines represent the linear regression of the calculated growth index as a function of the date (2006 – 2100), respectively, for RCP4.5 (slope = -1.492 [-0.254, -2.731]) and RCP8.5 (slope = -1.553 [-0.315, -2.791]).

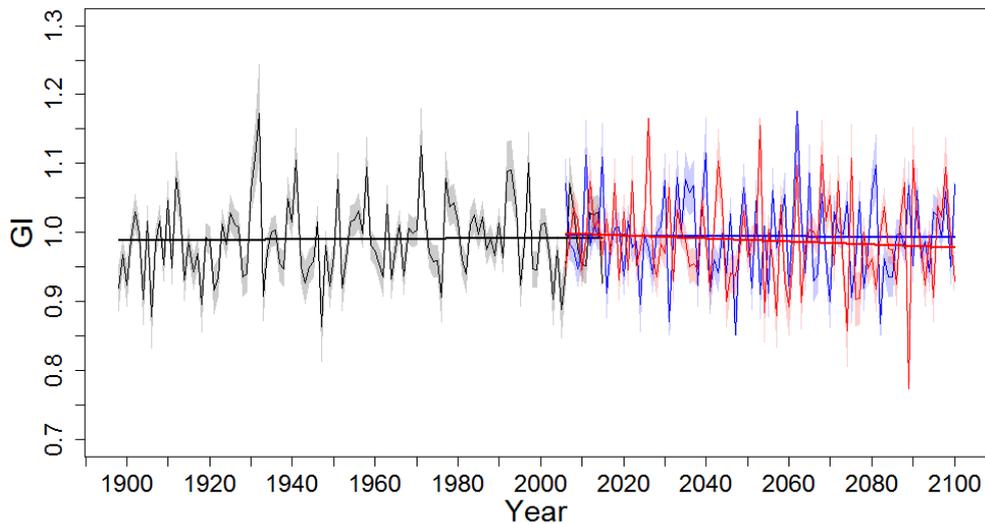


Figure 57. The black curve (the same as the red curve in Figure 51) indicates the GI for oak calculated after the equation Eq. $2_{(\text{oak})}$ as a function of the date (1987 – 2015) with 95% confidence interval. Blue and red curves indicate the GI for oak calculated after the equation Eq. $2_{(\text{oak})}$ as a function of the date (2006 – 2100), respectively for the climate scenario RCP4.5 and RCP8.5 with 95% confidence interval. The blue and red line represent the linear regression of the calculated growth index as function of the date (2006 – 2100), respectively for RCP4.5 (slope = -1.067 [-0.117, -2.016]) and RCP8.5 (slope = -1.429 [-0.425, -2.433]).

Overall, the dendroclimatological part of this study has clearly highlighted the importance of precipitation on beech growth in the Ciron area. Furthermore, this study has shown that exogenous factors such as late frost may also affect the more dominant long-term trends. This underlines the inherent complexity of climate-tree growth relationships, especially in particular context such as the Ciron study system.

9 DISCUSSION

In this section, the results and discussions of all the preceding sections are briefly recapitulated to achieve a better understanding of this dendroecological study as a whole. I discuss the relationship between beech growth and its changing environment, within a climate refugium, on the basis of our knowledge on the ecology of forest tree populations.

Growth and iWUE trends of beech and oak in the Ciron valley

The dendroecological analyses and modelling clearly show that growth trends of beech and oak have changed over time. An increase of growth in both species before 1980 was followed by a slight decrease since then. Interestingly, oak was more affected by this decline than beech, although the latter species is usually assumed to be more susceptible to drought (Leuschner et al., 2001; Dittmar et al., 2003; Lebourgeois et al., 2005; Scharnweber et al., 2011). On the other hand, beech showed a slightly higher mean sensitivity than oak and a very low productivity compared to beech forests in northern France (Charru et al., 2010), two trends that point to the existence of exogenous (and probably climate related) constraints on tree growth in the Ciron population. Cavin and Jump (2016) pointed out in their study a lower sensitivity and approximately stable growth of beech populations at the south range edge in Western Europe. Populations at the southern range edge of the species are mostly restricted to climate refugia, sites at which local conditions buffer them from regional climate (Hampe and Jump, 2011). Thus, the slight growth declines of beech in the Ciron valley could be due to generally elevated drought events at local-scale rather than at regional-scale (Cavin and Jump, 2016).

On the other hand, a marked increase of the intrinsic water use efficiency (iWUE) of beech has occurred since the 1860s. Such a trend has been associated to an increasing warming that is not accompanied by increased precipitation (Peñuelas et al., 2008). Beech shows a stronger iWUE response, both in trend and amplitude, than oak. The results of my study also suggest that the positive iWUE trend of beech is probably more strongly related to a fertilization effect (mediated by the increase of atmospheric CO₂) than that of oak. However, the increase in iWUE since the 1950s was not sufficient to catalyse a similar increase in beech growth rate. Overall, my study sheds light on the complexity and multiplicity of factors and their interactions, such as CO₂ and nutrient limitation (Norby et al., 2010), on trees' intrinsic water use efficiency and its response to climate change. In addition, my analyses showed that diverse station-specific effects such as the topographical position of trees and their position along the Ciron river, as well as intrinsic determinants such as their genotype, may further complicate and confound trends in tree growth and iWUE. Such confounding factors can render the isolation of single drivers an extremely challenging exercise. However, individual-level analyses of fine-scale drivers of tree growth certainly are a powerful way to achieve a better mechanistic understanding of tree growth-climate relationships. Perhaps the most intriguing example for this was the observed effect of the tree genotype on growth and its sensitivity (see also Bosela et al., 2016). Albeit not very strong, this effect certainly deserves further investigations to inform an adaptive conservation management of forest tree populations under a warming climate (Aitken et al., 2008; Alberto et al., 2013; Metz et al., 2016).

Growth-climate relationships of beech and oak in the Ciron valley

The tree growth-climate analyses revealed that the water balance of the whole growing season had a major effect on the interannual growth rate of both beech and oak and no signals of possible effect of climate of the years preceding wood formation (Dittmar et al, 2003; Lebourgeois, 2005; Peñuelas et al, 2008; Lebourgeois and Mérian, 2011). However, beech growth was particularly small in certain years (1960 and 1997), probably due to late frost events (Dittmar et al., 2006; Cailleret and Davi, 2011) whose consequences overwhelmed the long-term effect of water balance and affected especially early-flushing trees. Although relatively anecdotal, and probably of diminishing relevance in a warming climate, this

observation nicely pinpoints the possible role of climatic extreme events (Lebourgeois et al., 2010) and, more generally, the complexity of climate-growth relationships in long-lived organisms such as forest trees.

On the other hand, tree growth predictions for both species based on models that include only climate variables (mainly water balance) showed that trees can manage to persist within the Ciron area even under the most pessimist climate scenario. Based on the observed and projected growth trends, this study does not anticipate an imminent strong decline in the vitality of the Ciron beech population. This notion is further supported by the fact the stand appears to regenerate well, judging from the abundance of young trees and small recruits in the study area (personal observations). Yet regeneration would be expected to decline and disappear first in ecologically marginal, remnant populations (Jackson et al., 2009). However, the decline of tree populations is a long-term process (Gillner et al., 2013) as individuals can take decades to centuries to be replaced (Trumbore et al., 2015). On the contrary, future climate warming is expected to occur so rapidly that significantly different environmental conditions will prevail within one or two tree generations (IPCC, 2014). Furthermore, extreme climate events are likely to become globally more frequent and intense in the near future (IPCC, 2014), and even refugial beech populations will probably be exposed to climatic stresses of an intensity never yet encountered. Thus, more detailed investigations are needed on the inner functioning of climate refugia in order to better understand at what point landscape management can help to use and sustain favourable microclimates (Dobrowski, 2011; Hampe and Jump, 2011). The beech population of the Ciron has successfully persisted under very different climates from the Last Glacial Maximum until the present day (de Lafontaine et al., 2014). This study will hopefully be of help with the design of conservation and management strategies that can ensure the survival of this singular forest through the coming trees generations.

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11 APPENDIX

11.1 Appendix S1

Table I. Output results of pointer years for the studied beech stand (dplR package in R). Year: considered year (t), % positive: percentage of series displaying a significant positive radial growth variation, % negative: percentage of series displaying a significant negative radial growth variation, to indicate whether the year is a positive pointer year (blue) or negative pointer year (red), Mean_{RGV}: mean radial growth variations in percentage between Year-1 and Year.

Year	% positive	% negative	Nature	Mean _{RGV}
1923	86.7	6.2	positive	54.2
1930	77.2	5.7	positive	43.5
1937	11	77.4	negative	-19.2
1939	78.4	9.2	positive	66.9
1951	83.5	8.8	positive	56.9
1974	6.8	79.7	negative	-24.6
1983	87.9	3.3	positive	60.6
1995	7.6	78.9	negative	-22.3
1997	6.3	86.1	negative	-48.9
1998	81.9	9.5	positive	Inf
2000	78.5	5.4	positive	Inf
2007	78.7	9.2	positive	48.9
2012	79.5	6.4	positive	43.2

Table II. Output results of pointer years for the studied oak stand.

Year	% positive	% negative	Nature	Mean _{RGV}
1918	6.1	78.8	negative	-27.5
1923	75	11.1	positive	42.2
1928	10.3	82.1	negative	-24.5
1939	82.6	4.3	positive	45.6
1957	6.1	81.8	negative	-25.4
1972	4.2	83.3	negative	-24.8
1974	9.7	80.6	negative	-22.8
1983	88.6	2.5	positive	57.7
2001	6.3	82.3	negative	-24.9
2007	85.9	1.3	positive	46.9
2009	2.6	89.7	negative	-31.4
2012	78.2	10.3	positive	40.1

11.2 Appendix S2

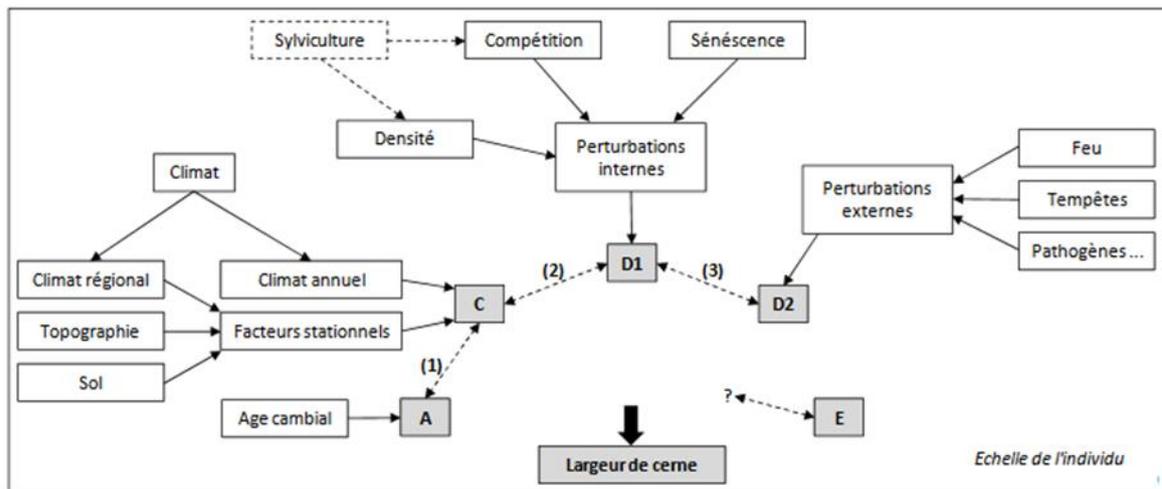


Figure I. Synthesis of factors that influence the variability of tree-ring width (Mérian, 2012b). Notes (1) to (3) illustrate some possible interactions between the five categories of signal (A to D2) of the conceptual model of Cook (1985). Figure taken from (Lebourgeois and Mérian, 2012).

11.3 Appendix S3

In the following I describe in detail the protocol that I applied to purify the cellulose and measure $\delta^{13}\text{C}$.

Sample preparation

Here are the steps I followed for the preparation of the samples and cutting tree rings.

- Check the quality of the core wood.
- Identify the areas or the pentads (group of 5 units) to be analysed. Then display the sought-after tree rings with a TIF file by using WinDENDRO; display the same area of the actual core under a binocular magnifier linked to a camera; finally mark the boundaries of the area to be cut with a pencil.
- Cut the identified area with a scalpel.
- Cut the pentad into smaller pieces with a chisel then put the pieces in a flask.
- Separate two batches of samples: abundant samples that will pass to the mill robot and samples with little wood that will be grinded into Eppendorf tube.
- Two machines were used to grind samples: Genobois robot and Genogrinder 2010. The big samples were grinded with Genobois robot and the little ones were grinded with Genogrinder 2010 after putting them in Eppendorf tube.
- Recover the powder.

In parallel, the PTFE Membrane Filters or Teflon membrane must be prepared as the following,

- Use PTFE membranes: Sodipro (ref. QDR6230417) of 47 mm of diameter and with pores of 10 μm .
- Cut with a chisel 40 cm of Teflon wire, then pass the end of the wire through the pore of PTFE membrane and make a double knot.
- Put the number of the samples on a Teflon label and pass the other end of the wire through the label.
- Place all the prepared PTFE membrane in an oven at 65 ° C for 24 hours.
- Cool in the PTFE membrane in a desiccator for 2 hours.
- Weigh PTFE membrane + wire + Teflon label with an analytical balance on a scale of 1/10th mg.
- Put the powder of the grinded wood in the PTFE membrane
- Put the samples in an oven at 65 ° C for 24h
- Use the same analytical balance to weight the PTEF membrane with the samples inside. The difference of the two weights gives the dry weight of the samples.
- Note the dry weight of each sample to calculate the cellulose yield.

Cellulose purification protocol

The cellulose purification protocol that I applied follows the one describe in Richard et al. (2014). In summary, their protocol is as follows,

- Soxhlet: 500 ml toluene + 250 ml ethanol (5 cycles)
- Soxhlet: 750 ml toluene (3 cycles)
- Beaker (2l): water at 100 ° C for 3 hours
- Beaker (2l): 800 ml water at 70 ° C + 21 g NaClO₂ + acetic acid, for 14 h
- Rinsing with cold water

If stalled on working days, this gives the following protocol,

Day 1:

- 8:30 start of heating at 100 ° C the soxhlet with 500 ml toluene + 250 ml ethanol
- 16:00 start of cooling, then throwing the used solvent into a container
- 16:30 start of heating at 100 ° C the soxhlet with 750 ml of ethanol

Day 2:

- 8:30 start of cooling, then throwing the used solvent into a container
- 9: 00 put into a 2 l beaker with 1.4 l of water at 100 ° C. with stirring and lid
- 13:00 throw the water away, then add 800 ml of clean water, 7 g of NaClO₂, 1 ml of glacial acetic acid and heat at 70 ° C with stirring and cover.
- 17:00 add 7 g of NaClO₂ and 1 ml of glacial acetic acid

Day 3:

- 8:30 add 7 g of NaClO₂ and 1 ml of glacial acetic acid
- 13:00 start of cooling, then throwing the used solvent into a container
- 13:30 make 4 rinsing with cold water
- 16:00 put the samples in an oven at 65 ° C

Weighing cellulose for $\delta^{13}\text{C}$ analysis

We have planned to measure 429 samples (413 samples 4 of which will be measured 5 times, i.e. isotopic measure effect). I decided to distribute the samples randomly in the 6 plates Elisa with 96 wells. According to the information provided by the Champenoux measuring platform "Plateforme technique d'écologie fonctionnelle (PTEF)", the wells A1 A2 A3 and the 8 wells in column 12 are reserved for the internal standards of Champenoux. Thus, I can place only 85 samples per plate. Finally, I realized a random drawing with excel of a series of 429 numbers that I place in front of the 85 wells x 6 plates Elisa.

To make the micro-weighed, I fill the first plate, well after well. For that I did the following:

- On the paper sheet, read the sample number that corresponds to the first usable well

- Pour the cellulose powder contained in the Teflon membrane into an Eppendorf tube
- Weigh 1 mg of cellulose taken from the Eppendorf tube
- Note the weighing on the sheet
- Close the tin ball by using two clamps
- Close the Eppendorf tube and put it back in its box
- Repeat the same steps for the net well etc.

When an Elisa plate is complete, lay a paper to close the wells, put the lid, and then put tape around the plate. Leave the filled plates in a desiccator until they are sent. Finally, once the $\delta^{13}\text{C}$ measurements were made at Champenoux, I received a file that I organized for my data analyses.

Quality of measurements

The standards used by the PTEF that intended to test the repeatability of the measurements along the range of the samples, have a fixed values of nitrogen, of carbon and of $\delta^{13}\text{C}$. However, in my protocol each sample will only be measured once to optimise the workload and budget. However, I need to have guidance on the variability associated with the measurement step. To do this, I will have two approaches:

- We have purified 20 standard pine woods which will be the subject of analysed $\delta^{13}\text{C}$ each. This variability will be the result of purification and isotopic measurement.
- We will carry out 5 weighing of the same cellulose sample to have the part tied only to the $\delta^{13}\text{C}$ measure. This will be repeated on 2 different samples. Criteria for choosing these samples:
 - a. Both species of the study: beech and oak
 - b. Sufficient amount of cellulose: more than 100 mg of wood powder
 - c. Tree rings of the same age: 45 years old
 - d. Tree rings should be developed at two different times to cover the range of years studied
 These criteria made it possible to choose two beech trees for the period 1892-1896 and the period 2008-2012, and two oaks for the period 1879-1883 for the period 2000-2004.

Thus, the use of maritime pine standards enabled me to be sure about the quality of my data by verifying the measurement from two different labs. The repetition of a measurement in different labs is called "Reproducibility" in certain field as in metrology. The results showed that there is no trend $\delta^{13}\text{C}$ as a function of the location in the series of measurements with a

variation of only 0.867 ‰ (from -26.156 ‰ to -25.289‰). The same results was shown for nitrogen and carbon with a variation respectively equal to 0.095 ‰ and 8.985 ‰. The values of my standards of maritime pine are therefore completely within the range of expected values. There is no trend related to the position in the series of measures.

On the other hand, the 5 weighing of the same cellulose sample allowed me to repeat the measurement under the same conditions, this is known as repeatability. According to the graph below (**Figure II**) the variation between the different measures is minim, showing therefore the good quality of my samples.

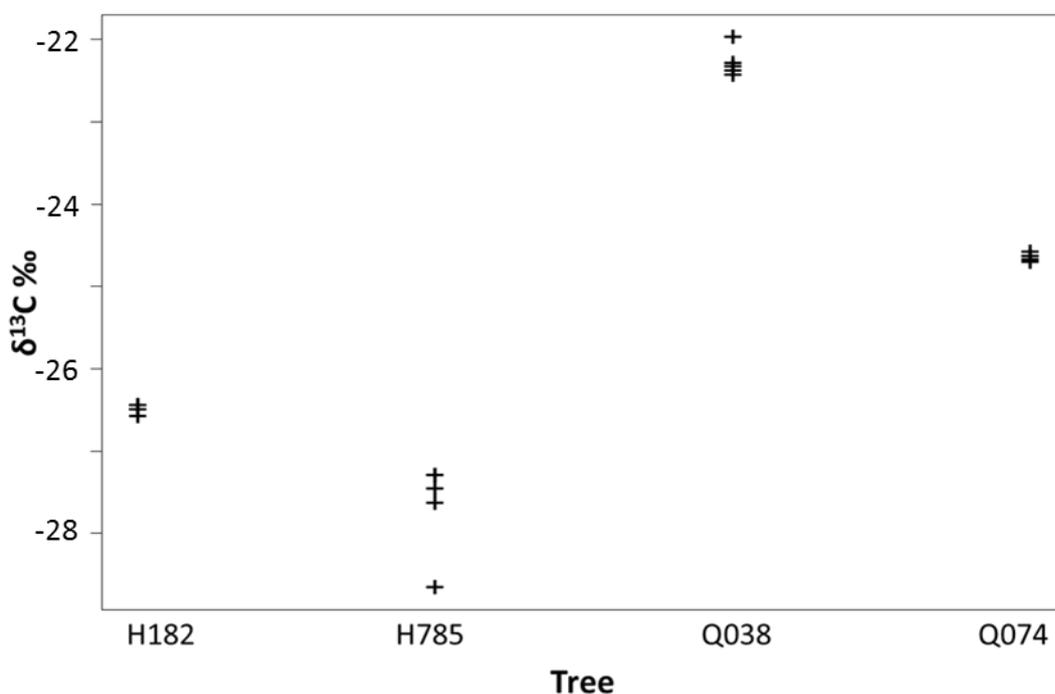


Figure II. The $\delta^{13}\text{C}$ (‰) values of different measures of the same sample. H182 and H785 are the name of the two selected beech trees and Q038 and Q074 are the name of the two selected oak trees.

Effect on $\delta^{13}\text{C}$ due to protocol itself

- Pencil marks effect

The ring boundaries were marked with a pencil under the binocular lens when preparing carrots before digitization. This carbonaceous material is a pollutant for isotopic measurements, so the marks have been removed with a scalpel during the identification of the pentads before their cutting. I voluntarily added two samples with the marks to see their influence on $\delta^{13}\text{C}$. These values are expected to be

more negative since they include fossil carbon. The following graph (**Figure III**) shows the evolution of $\delta^{13}\text{C}$ on two cores over time: the first point of each curve (in 1982) corresponds to the two samples with pencil marks. It is evident that this low pollution did not significantly reduce the value of $\delta^{13}\text{C}$ since the first points are above the others. Similarly, the carbon content is somewhat lower than that of the other samples. Pencil marks constitute a very small fraction of the totality of the wood. In conclusion, pollution with pencil marks is insignificant on this scale.

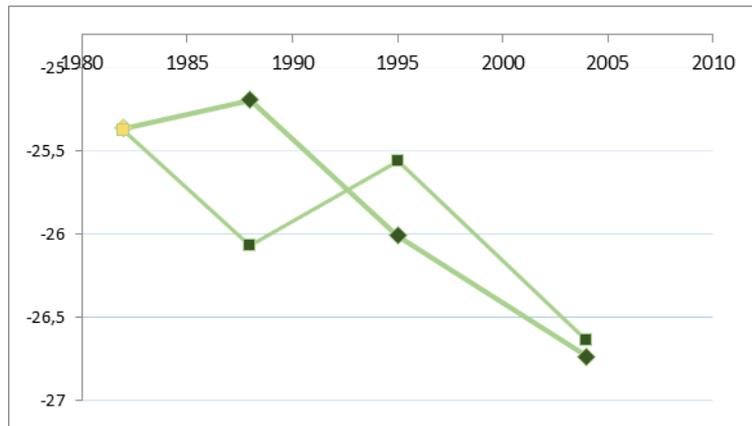


Figure III. Evolution of $\delta^{13}\text{C}$ on two beech cores over time. The first yellow point of each curve (in 1982) corresponds to the two samples with pencil marks.

- "Batch" effect during purification

Due to the large number of samples, I had to divide them into two lots: from 1 to 200 in the left soxhlet and above 200 in the right one. The samples were thus grouped during all phases of cellulose purification. The Pierroton maritime pine wood standards were also divided between these two batches, which makes it possible to test a possible "batch" effect. For this, I do not use the 5 standards ground with Genogrinder (see above). So there are 15 samples: 7 left and 8 right. The result of a t-test has shown that the difference between the averages is 0.078 ‰. It is less than the lowest confidence interval obtained in "Repeatability". Thus I can conclude that the purification did not bias the $\delta^{13}\text{C}$ measurements between the two batches of samples.

11.4 Appendix S4

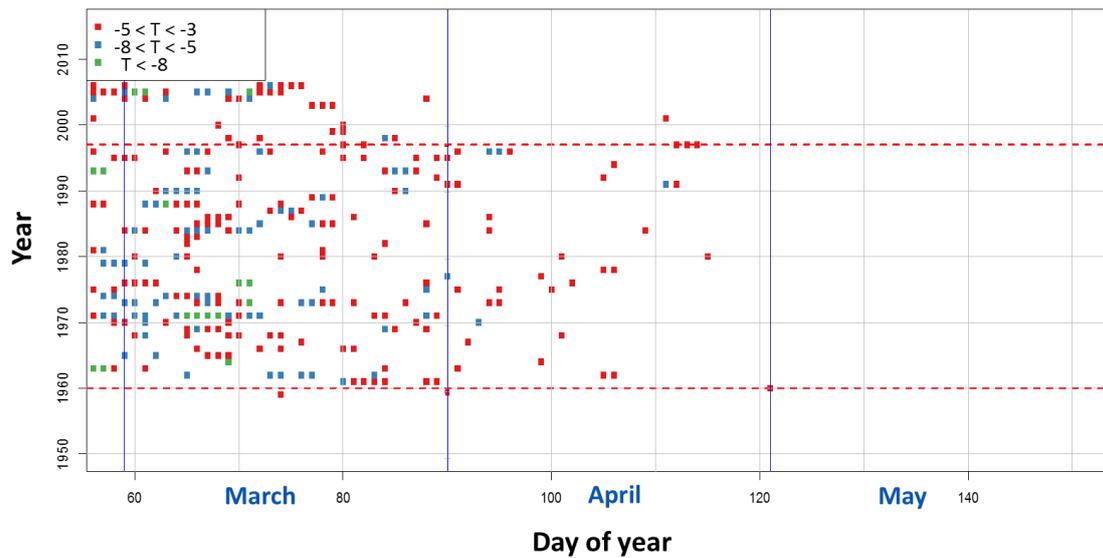


Figure IV. Plot of negative temperatures for each day from March to May (x-axis) between the year 1950 and 2015 (y-axis) in Budos ($44^{\circ}32'22''\text{N}$, $0^{\circ}22'45''\text{W}$), ca 18 km northwest of the study site. Red square correspond to negative temperature between -3 and -5 °C, blue square correspond to negative temperature between -5 and -8 °C and green square correspond to negative temperature less than -8 °C. Red dashed lines correspond to the years 1960 and 1997.

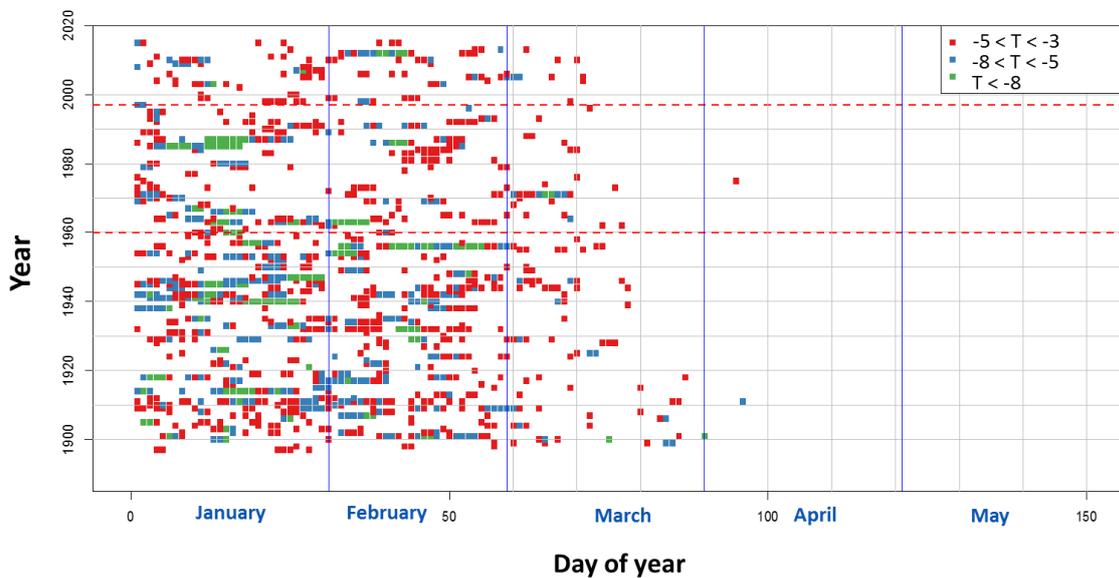


Figure V. Plot of negative temperatures for each day from January to May (x-axis) between the year 1900 and 2015 (y-axis) in Sauternes ($44^{\circ}32'22''\text{N}$, $0^{\circ}22'45''\text{W}$), ca 17 km north of the study site. Red square correspond to negative temperature between -3 and -5 °C, blue square correspond to negative temperature between -5 and -8 °C and green square correspond to negative temperature less than -8 °C. Red dashed lines correspond to the years 1960 and 1997.

11.5 Appendix S5

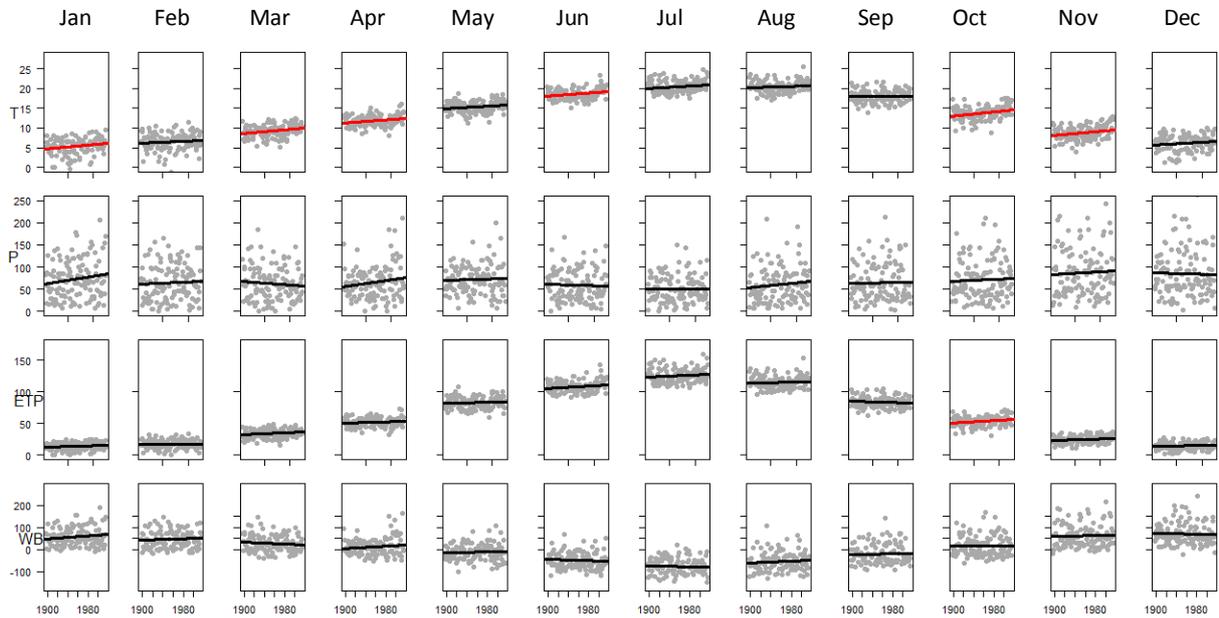


Figure VI. Mean temperature (T in °C), precipitation (P in mm), evapotranspiration (ETP in mm) and water balance (WB in mm) for each month from 1897 to 2015. Black and red lines indicate respectively, a non-significant and a significant correlation between the corresponding climate factor and the date.

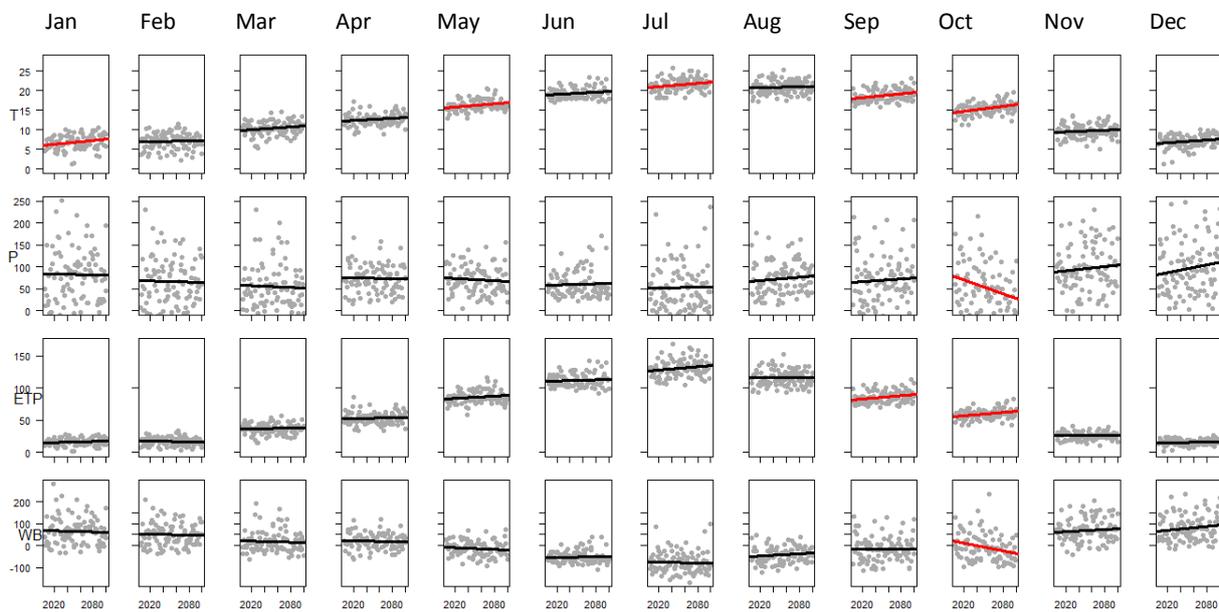


Figure VII. Mean temperature (T in °C), precipitation (P in mm), evapotranspiration (ETP in mm) and water balance (WB in mm) of the future climate scenario RCP4.5 for each month from 2006 to 2100. Black and red lines indicate respectively, a non-significant and a significant correlation between the corresponding climate factor and the date.

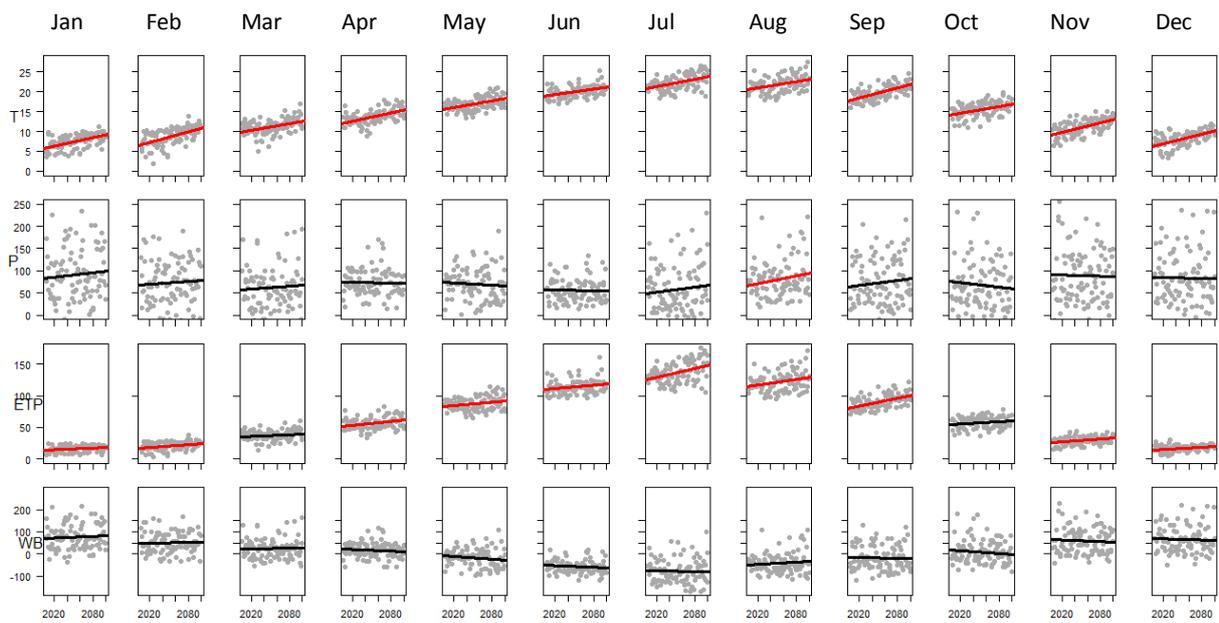


Figure VIII. Mean temperature (T in °C), precipitation (P in mm), evapotranspiration (ETP in mm) and water balance (WB in mm) of the future climate scenario RCP8.5 for each month from 2006 to 2100. Black and red lines indicate respectively, a non-significant and a significant correlation between the corresponding climate factor and the date.

General discussion

Forests worldwide are at risk from increasing temperatures and drought caused by modern anthropogenic climate change (Carnicer et al., 2011; van Mantgem et al., 2009; van Mantgem and Stephenson, 2007). The present-day persistence of tree populations at the low-latitude range margin of species ranges relies largely on their situation in climate refugia, that is, areas that locally mitigate the effect of unsuitable environmental conditions that dominate the greater surroundings (Keppel et al., 2012). A better understanding of the ecological and evolutionary mechanisms that refugial populations underlie is crucial for understanding their functioning and adopting suitable conservation measures (Hampe and Jump, 2011; Fady et al., 2016). This thesis attempted to unravel long-term population dynamics as well as short-term demographic processes of an emblematic refugial beech population located at the xeric range margin of the species. Conducting such a study is particularly tricky for long-lived organisms such as forest trees (Petit and Hampe, 2006). For this reason, I combined analyses of the adult population genetic structure (to explore the existence of genetic groups within the stand), paternity analyses of seeds (to study the contemporary movement of pollen), and dendrochronological analyses (to assess the influence of the local environment on tree growth and vigour) with the goal to achieve novel and interesting insights upon the history of this peculiar population and its functioning under current environmental conditions.

1 GENE FLOW AND THE POSTGLACIAL HISTORY OF THE CIRON BEECH POPULATION

In **chapter 1**, I could identify the existence of two genetic clusters within the adult beech population of the Ciron. The first cluster is mainly located in the central part of the population, where de Lafontaine et al. (2014a) had found beech charcoal remains older than the Last Glacial Maximum (LGM, ca. 19-26.5 kyr BP; Clark et al., 2009) and de Lafontaine et al. (2013) had already detected singular genotypes. The cluster hence is likely to represent the extant remainder of an ancient beech population that would probably have persisted in the Ciron area since the last interglacial period. The second cluster was more frequent towards the both ends of this riparian population, where de Lafontaine et al. (2013) had observed genotypes that resemble those of other beech populations in the region. Those genotypes probably

originate from pollen immigration after the LGM (Petit et al., 2005; Kremer et al., 2012). The first genetic cluster exhibited lower genetic diversity than the second one, a difference that would be in line with their different putative origins and histories. The result might also be interpreted as a signal that the ancient cluster still continues to decrease in abundance (Cornuet and Luikart, 1996). This opens questions upon the future fate of this cluster and the conservation of its genetic heritage. Furthermore, I detected a well perceivable spatial gradient between the two clusters, whose formation and maintenance could most easily be explained with an unusually limited within-population gene flow.

The actual existence of such a limited gene flow is a major finding of **chapter 2**. Wind-pollinated forest tree species are broadly assumed to experience regular effective pollen dispersal spanning several to many km (reviewed in Kremer et al., 2012). Yet in the Ciron population mating occurs predominantly between neighbours. A marked dominance of short-distance pollen flow has recently also been described from small refugial populations of Pedunculate oak (*Quercus robur*) (Moracho et al., 2016). The pattern observed in the Ciron beech population is however even more remarkable, because neighbouring trees tend to be closely related (half-sibs on average). This population thus contradicts the widespread notion that selfing or mating between relatives are rather the exception in forest trees (e.g. Hamrick and Godt, 1996; Petit and Hampe, 2006; see also Gaüzère et al., 2013). Curiously, my paternity analysis detected abundant mating between relatives but not a single selfing event. The outstanding spatial genetic structure (SGS) of the adult Ciron beech population indicates that the gene flow patterns of the analysed cohort of beechnuts (i.e., the 2013 fruiting event) were not an exception. The development of such an extensive SGS, even if favoured by the linear structure of the population, certainly requires a number of generations with population dynamics that are not mixed up by disturbance events – that is, conditions typical of long-term climate refugia (Hampe and Petit, 2005). On the other hand, the observed SGS nicely links the observed patterns of pollen flow with the postglacial formation of the two genetic clusters reported in chapter 1. The only apparent contradiction arises from the fact that the paternity analysis did not detect any immigration event whereas the study in chapter 1 revealed a strong signal of immigration. However, this divergence could simply be explained with the relative

rarity of pollen immigration at ecological (chapter 2) but not at evolutionary or biogeographical (chapter 1) timescales.

Refugial tree populations are typically small and their effective size is likely to be even smaller (Hampe and Jump, 2011). Theory predicts that such populations should be prone to genetic erosion, inbreeding and ultimately extinction (Nieminen et al., 2001; Willi et al., 2006; Kawecki, 2008). However, the global levels of genetic diversity in the Ciron beech population are not particularly low. Secondary contact and the gene immigration through long-distance pollen immigration that putatively underlies the second genetic cluster have apparently been able to maintain its standing genetic variation and corresponding potential for microevolutionary adaptation (Ouborg et al., 2006; Willi et al., 2006; Aitken et al., 2008; Kremer et al. 2012).

Pollen movement and mating represent the very first step in the life cycle of trees and the initial template for regeneration, whose success is decisive for the persistence of refugial tree populations. It was beyond the possibilities of this thesis to study further steps in the regeneration process, although I could confirm during my numerous field campaigns that the Ciron beech population counts with numerous tree seedlings and saplings, indicator of ongoing tree recruitment. The Ciron refugium hence appears to offer conditions within the regeneration niche of beech (Jackson et al., 2009). However, the adult niche may also undergo phases of sensitivity, for example during drought periods. The dendroecological approach applied in this thesis thus completes the genetic study in that it seeks a better understanding of the dynamics of the adult Ciron beech population under current (and future) climatic conditions.

2 GROWTH AND PHYSIOLOGICAL RESPONSE OF ADULT TREES TO MODERN CLIMATE

My investigations of tree radial growth and intrinsic water use efficiency (iWUE) reported in **chapter 3** covered the past ca. 200 years, that is, the period since the industrial revolution after 1850. The results of the dendroecological analyses and modelling clearly show that the long-term growth trends of beech were not stable over time but increased before the

1980s and slightly decreased since then. Such a pattern depicting a positive trend followed by a decline is coherent with studies on beech from more central parts of its distribution range (Badeau et al., 1996; Bontemps et al., 2009; Cavin and Jump, 2016). On the other hand, the relative weakness of the observed decline is also fully in line with findings of a recent extensive study of beech radial growth along a latitudinal gradient (Cavin and Jump, 2016), who reported that the highest drought sensitivity and lowest resistance are found in populations from the range core and not in those from the southern margin. These authors argued that this could be due to the fact that marginal populations are growing in refugial areas which provide a relatively stable local climate. Evidently, the refugial Ciron population fully sustains this hypothesis.

On the other hand, I observed a continuous increase of the iWUE since the 1860s. This increase was more pronounced over the second half of the 20th century. Such trend is also known from other beech stands (Duquesnay et al., 1998; Waterhouse et al., 2004; Peñuelas et al., 2008; Brienen et al., 2017). However, the observed positive trend in iWUE was not accompanied by a similar amplitude of increase in tree growth, as reported in other studies (Silva et al., 2010; Peñuelas et al., 2011). This divergence suggests that the increment in iWUE is more likely to be related to climatic drivers such as warming and drought (Jump et al., 2006; Jump et al., 2007; Peñuelas et al., 2008; Piovesan et al., 2008) than to the fertilization effect of increased atmospheric CO₂.

Chapter 3 also produced direct empirical evidence that climate – more precisely: the water balance during the growing season – plays a major role for patterns of among-year variation in tree growth (see also Lebourgeois and Mérian, 2011). Broadly similar trends in Pedunculate oak, which I investigated for comparison, confirm that the growth dynamics of beech within the Ciron valley are particular to the site (and not the species) and hence likely to be driven by its local environment. More specifically, they are more likely related to the effect of climate than to local endogenous or exogenous perturbations such as silviculture, pathogens or insect outbreaks. This conclusion underlines the importance of maintaining the special microclimate of the Ciron valley in a future warmer world (Wigley, 2005).

On the other hand, my model-based projections of future beech growth trends could also invite to some optimism. Based on my empirical data of tree growth-climate relationships and on the most recent IPCC scenarios (IPCC 2014), I found that even a certain amount of climate warming will have relatively little negative effects on beech growth over the next few decades, because the key climatic driver – water balance during the growing season – is predicted to change relatively little in the region. Given that the Ciron beech population must already have experienced and survived very different climatic conditions between the LGM and today (de Lafontaine et al., 2014a), one might hence conclude that its future is not in imminent danger from modern climate change. Such a conclusion must however been taken with much caution as it is laden with many uncertainties, including for instance the very simple model used for my projections, the neglected effects of climatic extreme events, or the interplay between climate and other threads such as fires, pathogens or perturbations from silviculture.

While the major trends that I observed and their drivers are well in line with what has been reported in the literature, two further phenomena deserve however a particular mention. First, the fact that late frosts probably caused strong growth declines in two years of study (1960 and 1997) nicely illustrates the complexity of tree growth-climate relationships. Both local long-term meteorological data and global climate projections (IPCC, 2014) indicate that late frosts will decrease in the area, so similar events should become exceptional. Second, the fact that I detected some (weak) differences in the radial growth of trees belonging to each of the two genetic clusters represents rare empirical evidence for a possible genetic basis, and hence microevolutionary potential, of tree growth-climate relationships. This aspect clearly deserves further investigation (see also Bosela et al., 2016).

3 IMPLICATIONS FOR THE CONSERVATION AND MANAGEMENT OF THE CIRON BEECH POPULATION

One strength of this thesis is that it investigated the characteristics and intrinsic dynamics of a refugial population in great detail and partly adopting novel integrative perspectives. The obtained knowledge allows to make some predictions about the

population's performance and viability over the next several decades under a future warmer and drier climate, and how it can be supported by a well-adapted conservation management. Evidently, the present thesis can only offer a very partial picture that needs to be completed by future investigations.

To start with a positive and somewhat unexpected aspect, my dendroecological studies revealed that tree growth and performance in the Ciron beech population clearly respond to climatic triggers, but I also inferred that the population should be able to withstand a certain amount of future global warming without major difficulties. This prediction clearly represents a relief, because populations of long-lived organisms such as forest trees can persist over long periods despite irregular regeneration as long as they manage to maintain a roughly stable long-term population growth rate without excessive adult mortality (Jackson et al., 2009; Hampe and Jump, 2011). The finding also implies that other threats than global climate change are more imminent and hence relevant for the viability of the Ciron beech population. And that it is worth to invest in tackling these threats because the population is not predestined to doom over the coming few decades (as one might actually expect for long-term refugial populations).

My studies revealed the existence of two genetic clusters in the population, of which one shows a particular genetic composition and probably also represents a singular history (see debate in Huntley 2014, de Lafontaine et al., 2014b). The putatively 'ancient' genetic cluster exhibits a somewhat reduced genetic diversity. Certain evidence suggests that trees from this cluster could perform differently (in terms of radial tree growth) under certain microenvironmental conditions than those from the putative 'immigrant' cluster. At present, the 'ancient' cluster appears to be experiencing a dilution by pollen originating from other populations in the region. This dilution is however slowed down by very restricted within-population gene flow that has not only contributed to the development of a strong spatial genetic structure but also implies frequent mating between close relatives. As a consequence of mating patterns, seeds from the same mother tree are genetically relatively little diverse but relatively unique compared to those from other trees.

This complex of new knowledge can now be used for defining conservation targets and measures. Concerning the targets, the major question could be whether to focus on conserving genotypes from the putative ancient genetic cluster. One can argue that its singular history and persistence under very different environments converts it into a rare forest genetic resource that might contain genotypes of relevance when the Ciron population needs to adapt to future conditions (Hampe and Petit, 2005; Fady et al., 2016). However, it is important to underline that we do not really know to which extent the identified genetic cluster actually represents the ancient population and whether much of the original heritage has already been lost. In addition, the ongoing admixture of the two clusters will finally result in their disappearance as distinguishable units. Therefore it could be more rewarding if conservation measures target rather the ecological processes that make the Ciron population outstand than specific genotypes. For instance, the continued existence of the quite unique mating system that has generated the peculiar genetic structure of the population relies on a high density of adult trees and an intact forest vegetation structure along the Ciron gorge (Ouayjan and Hampe, 2018). Both factors are closely linked with the silvicultural management of the area. The increasing awareness of the stand's conservation value can certainly help with securing the protection of the stand and its immediate surroundings. Conserving the individuals within their natural environment will allow the evolutionary forces that occur within the system to maintain genetic diversity and modify allele and gene frequencies (Lefèvre et al., 2013). Thus, securing the tree density within the Ciron valley may help the population to evolve new adaptive trait variants without putting the long-term persistence of the population at risk (Fady et al., 2016). Furthermore, facilitating a successful natural regeneration will be the easiest and least cost-intensive way to support the viability of the Ciron beech population. The observed strong SGS indicates that not only pollen dispersal but also seed dispersal appear to be very ineffective. This would imply a quite low ability of beech to colonize new places, or to re-establish on orphaned ones. Planting activities could therefore help to close current local distribution gaps. If possible, such plantations should use plant material in a way that respects the current genetic structure. On the contrary, campaigns for restoring beech stands where they were formerly present but have now disappeared could collect the material for propagation in a way that maximizes the genetic diversity of the

planted material (Ouayjan and Hampe, 2018), since there is no need of recreating a specific genetic structure in the newly created stands.

On the other hand, at regional scale, it appears important to elaborate a conservation plan not only for the large Ciron stand but also for the different islets of smaller beech populations found in the region (Timbal and Ducouso, 2010), in order to secure their connectivity. The fact that I did not detect any immigration event in the study described in chapter 2 indicates that even today pollen exchange is relatively rare. Yet even rare events are important for maintaining (and eventually increasing) populations' genetic diversity and for reducing the risk of inbreeding depression (Oddou-Muratorio et al., 2005). Finally, at a continental scale, the described genetic composition of the Ciron beech population further recommends it as a highly suited candidate for campaigns of assisted migration. The program GIONO organised by the ONF already takes advantage of this opportunity that could help with transporting valuable forest genetic resources towards other parts of the species range for helping them to adapt to a future climate (McLachlan et al., 2007; Aitken and Whitlock, 2013). Further activities in this sense would be desirable.

Finally, many further investigations are clearly needed to better understand the functioning of tree refugial populations in their peculiar environments and to elaborate precise management policy for these stands. The slower growth, poorer wood quality and lower economic value of peripheral forest population, compared to those from the temperate area, does by no means not make them less valuable (Lindner et al., 2010). Their importance lies instead on the unique genetic resources that they harbour as a result of their history (Petit et al., 2003; Hampe and Petit, 2005) and the similarly unique biological communities and ecosystems that they maintain (Ellison et al., 2005; Woolbright et al., 2014).

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Les changements climatiques en cours sont amplement considérés comme étant principalement dus aux émissions anthropiques de gaz à effet de serre. En particulier, les températures mondiales devraient continuer à augmenter au cours des prochaines décennies et cela crée des interrogations sur le sort des arbres et des forêts. En effet, les forêts couvrent près de 30% de la surface terrestre et fournissent de multiples services aux systèmes naturels et à l'Homme.

Lors d'une évolution rapide de l'environnement, les populations d'arbres forestiers peuvent connaître trois destins : (1) l'extinction, (2) la persistance grâce à l'adaptation aux nouvelles conditions, et (3) la migration en suivant spatialement leurs niches écologiques. Il existe de nombreuses preuves sur la dynamique passée des taxons d'arbres en réponse aux changements climatiques. Cependant, le réchauffement climatique moderne se produit si rapidement que les changements environnementaux en cours pourraient dépasser la capacité de réponse des populations d'arbres. De nombreuses espèces ligneuses subissent déjà une augmentation de la mortalité et une diminution de la croissance et de la reproduction dans les populations proches de la marge chaude de leur aire de répartition. Cependant, certaines populations marginales persistent dans des zones qui offrent un microenvironnement particulièrement stable et favorable : les refuges climatiques. Ces zones ont permis la persistance à long terme de la population dans des régions autrement inhospitalières. Leurs populations présentent souvent une composition génétique unique en raison de leur persistance prolongée en isolement relatif. Ainsi, l'extinction de ces populations implique une réduction drastique de la diversité génétique.

Les refuges climatiques sont des zones où « les paramètres physiographiques peuvent soutenir les climats régionaux autrefois dominants qui ont été perdus (ou sont en train de se perdre) ». Ils peuvent aider à atténuer les effets négatifs d'un climat régional défavorable sur les populations d'arbres. Cependant, leur petite taille et leur répartition clairsemée dans le paysage, poseront des contraintes sur les performances des populations. Les dynamiques intrinsèques qui ont permis aux populations de refuges de persister localement sont encore mal connues. Nous ignorons par exemple comment les modèles de reproduction peuvent influencer les composantes clés pour la persistance à long terme de la population, comme le maintien de la diversité génétique, le potentiel adaptatif et, en fin de compte, l'évolution. De même, nous savons peu de choses sur la façon dont la variation microclimatique au sein des

environnements de refuges influence la physiologie des arbres et les variations spatio-temporelles de la croissance et de la mortalité des arbres. Par ailleurs, la survie passée des populations d'arbres de refuges n'implique pas qu'elles soient résistantes aux menaces du changement climatique moderne. Par conséquent, il est nécessaire de mieux comprendre le fonctionnement de ces populations et anticiper leurs réponses aux changements globaux.

Cette thèse de doctorat étudie la structure démographique et génétique d'une population naturelle de Hêtre (*Fagus sylvatica* L.) située dans un refuge climatique, en marge chaude de la distribution de l'espèce dans le sud-ouest de la France. Cette population persiste depuis la dernière période glaciaire sur les pentes des gorges karstiques de la rivière Ciron à 50 km de Bordeaux, en Gironde. L'étude du fonctionnement et du devenir de cette population a utilisé deux approches complémentaires : l'écologie moléculaire et la dendroécologie. Cette combinaison d'approches a permis d'aborder à la fois les processus démographiques à court terme et la dynamique de la population à long terme. Les questions majeures de la thèse ont porté sur (1) la façon dont le contexte de refuge affecte les patrons d'accouplement et la diversité génétique au sein de la population, et (2) comment le changement climatique moderne influence la croissance des arbres.

Le premier des trois chapitres évalue la structure génétique et la diversité de l'ensemble de la population d'arbres adultes ($n = 932$) afin de déduire son histoire postglaciaire. L'étude a révélé que cette population semble constituer un gradient génétique entre deux pôles génétiques, que l'on peut probablement interpréter comme deux « groupes ». Le premier est principalement situé dans la partie centrale de la population. Il est susceptible de représenter la descendance d'une ancienne population de hêtres qui aurait persisté depuis la dernière période interglaciaire. Le second groupe est plus fréquent vers les deux extrémités de cette population linéaire et serait la conséquence de l'immigration de pollen depuis le dernier maximum glaciaire. Cette étude a également révélé que le premier groupe génétique présentait une diversité génétique inférieure au second groupe, ce qui serait cohérent avec leurs différentes origines et histoires putatives.

Le deuxième chapitre de la thèse étudie le système d'accouplement et le mouvement du pollen au sein de la population. Pour cela, l'analyse a porté sur l'appariement de graines avec des arbres mères sélectionnés ($n = 30$) tout le long de la population. L'étude a montré que l'accouplement prédominant entre voisins génétiquement apparentés a

entraîné une structure génétique spatiale très forte. Ce phénomène aide à expliquer le brassage lent des deux groupes génétiques identifiés dans la population.

Le troisième chapitre de la thèse consiste en une analyse dendroécologique basée sur un tiers de la population adulte de hêtres ($n = 317$) et de 79 chênes pédonculés (*Quercus robur* L.) échantillonnés pour comparaison. Les études sur les cernes annuels et la modélisation basée sur les projections climatiques révèlent que la croissance du hêtre, ainsi que celle du chêne, a été relativement peu affectée par des conditions climatiques de plus en plus sèches. La croissance radiale a connu une forte augmentation entre 1860 et 1920, puis elle a atteint un plateau. Depuis les années 1980, la croissance a légèrement diminué (davantage pour le chêne), et cela ne sera probablement pas accentué à l'avenir d'après les scénarios climatiques futurs pour la région. En outre, les analyses utilisant les isotopes stables du carbone montrent une grande hétérogénéité de performance entre les arbres en termes d'efficacité d'utilisation d'eau, et de croissance radiale. Cela est en partie expliqué par la position topographique des arbres dans la vallée, et pourrait également être influencé, dans une faible mesure, par leur génotype.

Ces travaux multidisciplinaires ont permis d'élaborer des prédictions probables d'évolution de la performance et la viabilité de la population pour les prochaines décennies, et d'envisager de les soutenir par une gestion de conservation bien adaptée. Elle permettrait de sauvegarder les ressources génétiques uniques que cette forêt représente du fait de son histoire, ainsi que les riches communautés biologiques qui composent cet écosystème.