Ecological response of tree saplings to simulated climate change along an elevational gradient (CLIMARBRE)

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Always treat people well; you never know what they are going through. What you see is just the tip of the iceberg.

Lausanne, 25th July of 2017

To my parents and to my baby Mushu

Summary

Switzerland will face higher temperature increases than the global average, which will have strong impacts on the mountain ecosystems. How tree species will respond to future climate change scenarios, and what mechanisms will they adopt, remains as a gap of knowledge in ecological research. Foresters will have to make short-term decisions and plan future managements under the great uncertainty of climate change and they demand answers to know if the current species will cope with the predicted climate change and to what extent the ecological goods and services will be affected (e.g. timber industry). The project CLIMARBRE was developed in order to ease and support their decision making by providing an advanced knowledge about the responses of beech and spruce regeneration to simulated climate change (specifically, warmer and drier conditions) in the wooded pastures of the Swiss Jura mountains. This project, which was built on the interface between fundamental research in forestry ecology and applied sciences, should attract the attention of foresters, managers of natural environments and of the general public.

By using transplantation along an elevational gradient, including four sites in the Jura mountains, "realistic" climate conditions were created to specifically simulated three potential future climatic scenarios from the IPCC (from A1B to A2). This space for time approach enabled the assessment of saplings' responses of beech and spruce to simulated climate change and their acclimation abilities. Saplings adapted to subalpine conditions at 1350 m were collected and transplanted towards lower altitudes exposing them to an average increase of 6.3^{o}C and a reduction in 30% of precipitation, at the lowest site throughout the study period.

The main findings include i) a longer growing season due to induced-elevation warming (downward shift) could not fully account for the species-specific positive growth responses; (ii) the contrasting species growth responses were linked to different sensitivities to elevated vapor-pressure deficits; (iii) models could better account for the growth response to warming after incorporating extreme climatic thresholds and their effects; iv) beech leaves showed an increase of xeromorphism through the increase of the cuticle thickness, vein network and smaller stomata, associated, to a higher leaf area v) which allowed it to grow in warmer conditions while coping with an increase of evaporative demand of the air; vi) and finally, the linkage between responses at tree, leaf, tissue and soil level, through a multiple level approach, improved the mechanistic understanding of these species capacities to respond to simulated climate change.

Keywords

Mountain ecosystems, response, Climate Change, elevational gradient, beech, spruce, saplings, acclimation, multiple level approach

Résumé

Dans le futur une augmentation des températures est attendue en Suisse et plus élevées que la moyenne au niveau mondial. Dans la recherche écologique, un manque de connaissances demeure quant à la réponse des arbres aux scénarios futurs de changement climatique et aux mécanismes qu'ils adopteront. Les forestiers devront prendre des décisions à court terme et planifier les futurs plans de gestion sous la grande incertitude du changement climatique. Ainsi, ils exigent des réponses sur l'habilité des espèces actuelles à résister aux changements climatiques prévus et dans quelle mesure les biens et services écologiques seront affectés (e.g. l'industrie du bois). Le projet CLIMARBRE a été développé afin de faciliter et soutenir leur prise de décision en fournissant une connaissance approfondie des réponses de la régénération du hêtre et de l'épicéa aux changements climatiques simulés (spécifiquement, conditions plus chaudes et plus sèches) dans les pâturages boisés des montagnes du Jura suisse. Ce projet, fondé à l'interface entre la recherche fondamentale dans l'écologie forestière et les sciences appliquées, devrait attirer l'attention des forestiers, des gestionnaires de milieux naturels et du grand public.

En utilisant la transplantation le long d'un gradient altitudinal dans quatre sites dans les montagnes du Jura, des conditions climatiques «réalistes» ont été créées pour simuler spécifiquement trois scénarios climatiques futurs potentiels du IPCC (de A1B à A2). Cette approche espace pour temps a permis d'évaluer les réponses des jeunes arbres du hêtre et de l'épicéa au changement climatique simulé et à leurs capacités d'acclimatation. Des plants adaptés à des conditions subalpines à 1350 m ont été recueillis et transplantés vers des altitudes plus basses, les exposant à une augmentation moyenne de 6,3 ° C et à une réduction de 30% des précipitations, au site le plus bas tout au long de la période d'étude.

Les principaux résultats comprennent i) une période de croissance plus longue en raison du réchauffement induit par l'élévation (décalage vers le bas) qui ne pouvait pas prendre pleinement en compte les réponses positives spécifiques à l'espèce; (ii) les réponses de croissance contrastées des espèces étaient liées à différentes sensibilités aux déficits élevés de pression de vapeur; (iii) les modèles pourraient mieux tenir compte de la réponse de la croissance au réchauffement après incorporation de seuils climatiques extrêmes et de leurs effets; iv) les feuilles de hêtre ont montré une augmentation du xéromorphisme par l'augmentation de l'épaisseur de la cuticule, du réseau de veines et des stomates plus petits, associés à une surface foliaire plus élevée v) qui lui a permis de pousser dans des conditions plus chaudes tout en faisant face à une augmentation de l'évaporation; vi) et enfin, le lien entre les réponses au niveau des arbres, des feuilles, des tissus et du sol, grâce à une approche à plusieurs niveaux, a amélioré la compréhension mécanique des capacités des espèces à répondre aux changements climatiques simulés.

Mots-clés

Écosystèmes de montagne, réponse, Changement climatique, gradient altitudinal, hêtre, épicéa, jeunes arbres, acclimatation, approche à plusieurs niveaux

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Chapter 1 General introduction

Spruce saplings growing among adult spruce trees in a wooded pasture (Col de Marchairuz)

P. Sanginés (2015)

> *"Global warming isn't a prediction. It is happening."*
>
> *(James Hansen)*

1.1 Forest ecosystems under climate change

Climate is a key factor shaping forest ecosystems; thus climate change is expected to have impacts on the phenology, growth, mortality and regeneration of tree species worldwide (Zimmermann *et al.*, 2014). In Europe, 38% of the total land area is covered by forests (European Parliament, 2017). This ecosystem not only provides with wood for the forest industry but also other ecological goods and services that range from erosion protection to recreation and tourism (Hengeveld *et al.*, 2013). The extent to which current trees and forests are able to cope with climate change will highly depend on the current site conditions and current stand properties (CH2014-Impacts, 2014). For instance, important changes will take place in sites where forest growth is currently limited by water availability or low temperatures (Way and Oren, 2010). Notable changes in biomass, forest composition and the provision of ecosystem services have already been evidenced at the low elevations in the Swiss inner-alpine valley (CH2014-Impacts, 2014), where the defoliation and mortality in scots pine has been associated to reduced precipitation and increased temperatures (Zweifel *et al.*, 2009).

Forecasting climate change impacts and to which extent forests are able to cope with it, remains today an important unsolved issue. It is extremely difficult to predict what the impacts of changing climate will be on the various tree species, and ecosystems at the various localities (Hengeveld *et al.*, 2013). In addition, forest management practices typically encompass many decades and are based on long-term climate change projections, which are highly uncertain and unprecise. Nevertheless, substantial effort has been put to provide foresters with the necessary decision-support tools for adapting forest managements to future climate change scenarios (IPCC, 2012).

1.1.1 Situation in Switzerland

Throughout the current century, temperate mountains in the northern hemisphere will experience the most intensive temperature rise, between two and three times higher temperatures (range +2.8ºC to +5.3ºC) than those recorded over the 20th century

(Nogués-Bravo *et al.*, 2007). Specifically, Switzerland will face higher temperature increases than the global average (MétéoSuisse, 2013), which will have strong impacts in the mountain ecosystems (IPCC, 2007). In the Swiss mountains, a rise in mean annual air temperature of 1.5ºC was observed during the 20th century (Beniston *et al.*, 1997) with associated increases in the frequency of extreme warm summers and prolonged droughts (Schar *et al.*, 2004; Beniston, 2009). More precisely, the CH2011 scenarios predict warmer summers (up to 6ºC until 2085) and precipitation reduction (up to 40%) to occur all over Switzerland (Modeling, 2011). At the regional scale, an increase of 1ºC in air temperature was observed in the past century in the Jura, within the climate change scenario A1B, and an increase of 1.1 to 3.5ºC is predicted towards 2060 (MétéoSuisse, 2013). Therefore, changes in summer soil water availability, plant phenology and growing season length, related to warming, will ultimately have repercussions in forest ecosystem distributions (Parry, 2000). Therefore, in the Swiss Jura region, the distribution of the two dominant tree species *Picea abies* (L.) H.Karst (Norway spruce) and *Fagus sylvatica* (L.) (European beech) will most likely be affected by future climate change projections. Currently, considerable concern has raised among forest managers and stakeholders to how climate change will affect the performance and distribution of these species, especially regarding Norway spruce, which is an evergreen conifer economically important in Switzerland (Cioldi *et al.*, 2010). According to the Swiss Federal Office for the Environment (OFEV) and the Swiss Federal Institute for Forest, Snow and Landscape research (WSL), the ecological goods and services provided by the Swiss forests will be compromised at the mid and long term. Therefore, it is imperative to develop sylvicultural practices that facilitate the transition of forests to future climate conditions (Brang *et al.*, 2016). For this reason, OFEV and WSL together launched a research program (2009-2017) entitled “Forests and climate changes” that aimed to gather and synthesize results from 42 research projects occurred all across Switzerland. The final report presents to what extent climate changes will affect forests and their ecological goods and services (Pluess *et al.*, 2016).

One of the research studies included in this program was the PorTree project (Zimmermann *et al.*, 2014), which generated maps of Switzerland (Figure 1-1) representing the climatic habitat suitability of these species, based on species distribution models (SDMs). They illustrate the habitat potential at certain time periods in the future, but do not give any indication on how fast these changes will occur.

Moreover, it is most likely that species will survive for a certain time in the areas that become unsuitable, so careful interpretation has to be done from these maps. Nevertheless, they provide managers with useful information of which species are likely to be suitable or not today and in the future.

Species distribution models project that spruce and beech will shift away from the Swiss plateau and towards higher elevations, as the growth conditions in the plateau will become unsuitable for these species (Zimmermann *et al.*, 2014). Therefore, the potential of conifer timber production might also shift from the Swiss Plateau to the Alps and the Jura (CH2014-Impacts, 2014). The disappearance of spruce on the Swiss Plateau (Figure 1-1) shows that the future climate in this region will become warmer (and partly also drier) than in any observed current distribution of Picea. However, according to Zimmerman *et al.* (2014), it is most likely that until 2050 the climate will not be extreme enough to drive spruce to extinction and that the out competition by immigrating competitor species, such as oaks or beech, will probably take 100s if not 1000s of years to occur.

Buttler *et al.* (2012) showed that for the Jura mountains, under simulated moderate climate change (IPCC-scenario B2, +4 K projected until 2100), spruce initially profits from higher temperatures, but around 2100, drought stress leads to tree cover fluctuations and partial forest breakdown. Pioneer tree species (rowan berry, *Sorbus aucuparia*) indicate the ongoing shift in the forest community towards a dominance of beech. For extreme climate warming (IPCC-scenario A1FI, +8 K projected until 2100), spruce is replaced by Scots pine.

1.2 Tree responses to climate change

Changes in environmental conditions (i.e. higher temperatures and more frequent drought periods) can expose plants to an increasing abiotic stress (i.e. water availability). Stress can be defined as "*an environmental factor that reduces the rate of some physiological process (e.g. growth or photosynthesis) below the maximum rate that the plant could otherwise sustain*" (Lambers *et al.*, 2008). In this context, plants present a wide range of responses to stress, which scope from alterations in gene expressions and cellular metabolism to changes in growth rate and plant productivity (Shao *et al.*, 2008).

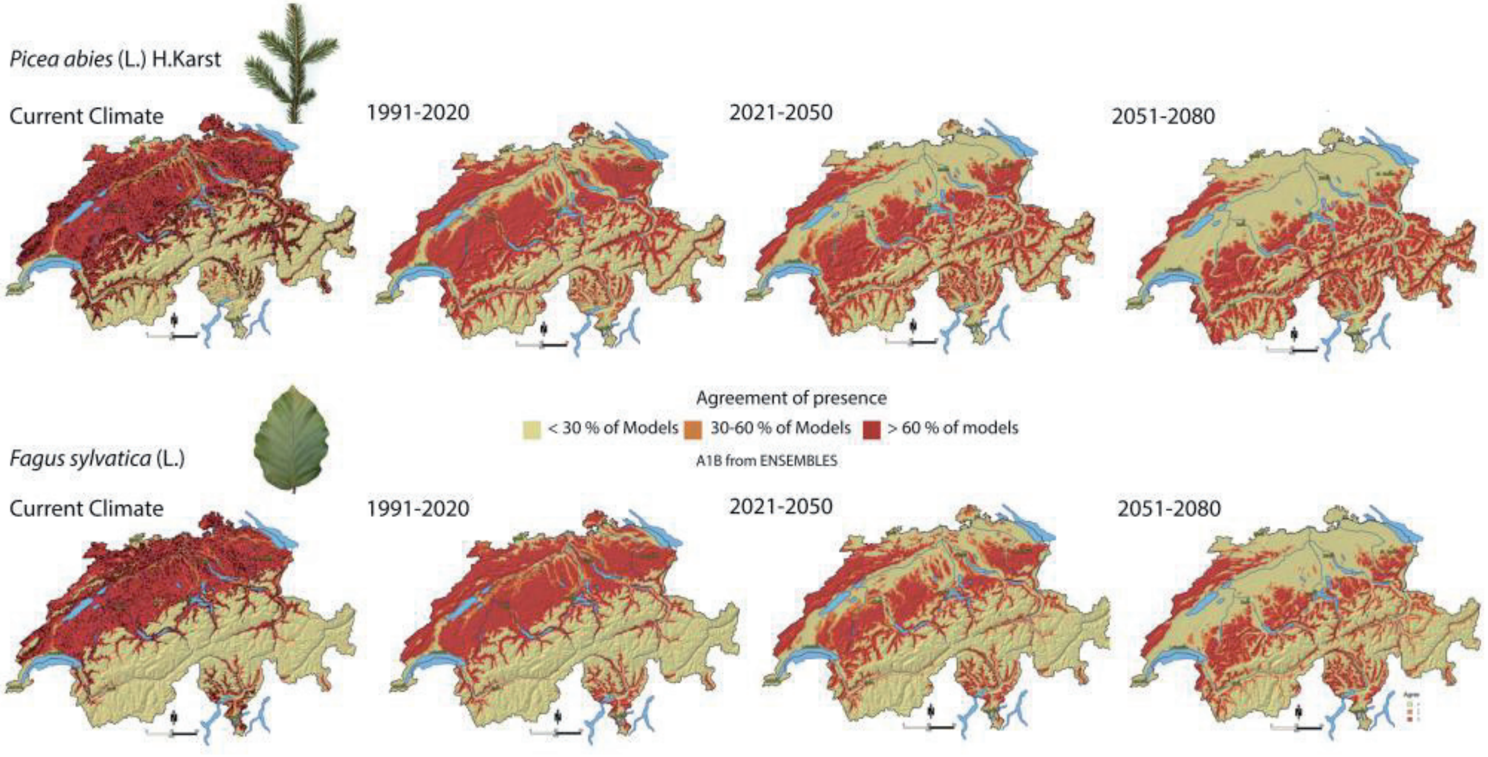
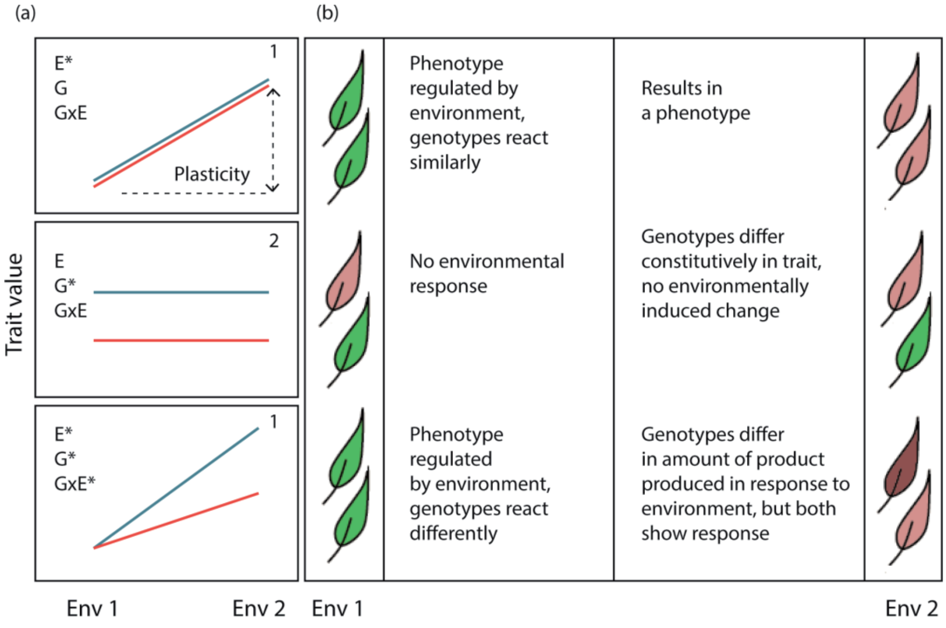


Figure 1-1 The first map from the left represents the current distribution (black dots) from the Swiss National Forestry inventory (LFI 1) and simulated habitat suitability under current climate as calibrated from forest inventory data across the Alps (MANFRED Project). The following three maps are the ensemble of projected future ranges of suitable habitat as modeled from six RCMs and six statistical models. Light yellow colors indicate that all climate and statistical model combinations project absence of the species, while dark red colors indicate presence. The orange colors indicate uncertainty regarding habitat suitability. *Source www.wsl.ch/lud/portree*

To evaluate the response of individual processes (e.g. the photosynthesis response) to stress, three different time scales have been established: (1) the **stress response**, (2) the **acclimation** and (3) the **adaptation** (Lambers *et al.*, 2008). In general terms, when an environmental factor has a negative effect on a plant process an immediate **stress response** appears during a time that can span from seconds to days. In order to compensate this decline in performance, plants adjust their morphological and physiological properties through **acclimation**, within the life time of an individual. As a result, the biochemical changes that occur induce changes on physiological processes, such as photosynthesis and growth rate, as well as changes in the morphology of organs (Sanginés de Cárcer *et al.*, 2017). Eventually, a population becomes **adapted** to a specific environmental stress when the compensation for the decline in performance is achieved through genetic changes (Lambers *et al.*, 2008).

Tree species in the forest ecosystems may respond to climate change in three different ways: (i) persistence in the modified climate, by adjusting to the novel conditions through phenotypic plasticity or adaptation through natural selection, (ii) migration following conditions to which they are adapted or (iii) extinction (Theurillat and Guisan, 2001; Nicotra *et al.*, 2010). Several studies showed how elevational upward shifts of species' ranges have occurred during the past century in response to current climate warming. As a way of example, Peñuelas and Boada (2003) have provided a detailed investigation about the elevational shift of beech (*Fagus sylvatica* L.) which has been progressively replaced by Mediterranean holm oak forest (*Quercus ilex* L.) in the Montseny Mountains (Spain). Moreover, the decline in growth and vitality of silver fir (*Abies alba* Mill.) has led this species to shift towards higher elevations and facilitating more drought tolerant species, such as beech and pubescent oak, to take over the lower elevations (Maxime and Hendrik, 2011). Thus, environmental changes cause the loss of some species and the gain of others, resulting in the change in species traits and interactions in plant communities; with cascading effects on the functioning of ecosystems and services they provide (Gimmi *et al.*, 2010; Wardle *et al.*, 2011). This will inevitably lead to a change in the composition of forests, timber production, and thus the forest economy and landscape in general (Peringer *et al.*, 2013). However, the ongoing climate warming might be too rapid for natural migration to successfully allow species to reach more suitable habitats (Rice and Emery, 2003). In this context, individual plants would need to remain in their current location and withstand environmental changes (Bonn, 2000; Petriccione, 2005; Allen *et al.*, 2010) through short-term phenotypic plasticity (Vitasse *et al.*, 2010).

Phenotype is defined as "*the appearance of an organism resulting from both genetic and environmental influences*" (Nicotra *et al.*, 2010) (Figure 1-2). Therefore, "*phenotypic plasticity is the range of phenotypes that a single genotype can express as a function of its environment*". In fact, the main challenge of evolutionary ecology is to understand how small modifications in phenotypes can have an effect on the natural selection of individuals (Vitasse, 2009). Literature supports a remarkable range of plastic responses among plant species and populations to comparable environmental challenges (Valladares *et al.*, 2007). This wide range of responses will most likely have an effect on the inter-specific competition and distribution of species. Thus, phenotypic plasticity will play a key role to determine plant responses, both in short- and long-term, and requires the understanding of the phenotypic variation of individual plants for a given species or population (Nicotra *et al.*, 2010; Gratani, 2014).



Adapted from Nicotra *et al.* (2010)

Figure 1-2 (a) In the evolutionary and ecological literature, plastic responses are commonly presented as reaction norms. Here the blue and red lines indicate the reaction norms of two different genotypes responding to a change from a low light environment (Env 1) to a high light one (Env 2.) The extent of phenotypic change in response to a signal is its phenotypic plasticity. Asterisks in the panels denote whether there is a significant effect of environment (E) or genotype (G), and whether there is a significant genotype by environment interaction (G x E). (b) Visual example of plastic responses. Adapted from Nicotra *et al.* (2010).

In the following subsections tree responses have been discerned into three different topics: (1) growth and phenology (2) physiology (i.e. photosynthesis) and (3) morphology (Ahmad and Prasad, 2012).

1.2.1 Tree growth and leaf phenology

Growth responses to warming have been observed to vary between latitudes and between functional groups (i.e. between deciduous trees and conifers). While temperate forests seem to benefit from warmer conditions with an increase in growth (Way and Oren, 2010), Mediterranean and tropical ecosystems are likely to respond with a decline in biomass allocation (Ogaya and Peñuelas, 2007; Way and Oren, 2010). This is partly explained by the fact that the growth of trees in high latitudes or altitudes are usually temperature-limited while Mediterranean forests are mainly limited by water availability (Penuelas *et al.*, 2004; Way and Oren, 2010). A review with synthesized data from 63 studies, comprising trees from different functional groups and thermal niches, showed that deciduous species would be highly more responsive than evergreens to warming, revealing a more conservative response of the last (Way and Oren, 2010). However, the authors of this review also state the importance of taking into account other factors, such as water and nutrient availability, which also influence the growth response of trees to warming conditions.

Strong evidence is found in literature that an increase in biomass in temperate zones is related to a lengthening of the vegetative season. Most of the studies of terrestrial biological systems report consistent changes on plant phenology (the timing of seasonal events) and on the growing season lengthening (time span between leaf unfolding and leaf fall) due to global warming (IPCC, 2007). During the second half of the 20th century it has been reported that the length of the European annual growing season was in average of 10.8 days longer and it was mainly attributed to changes in air temperature (Menzel and Fabian, 1999). In particular, a lengthening of 2.7 days/decade was recorded in Switzerland during the same period (IPCC, 2007). Moreover, due to the important role the vegetative period has on the global carbon fixation, as well as in the related global water and nutrient cycles (Peñuelas and Filella, 2001), we can expect these processes to be affected by a longer duration of forest canopy.

In addition, recent studies performed in temperate forests show how the sensitivity of phenology to an increase in temperatures commonly leads to an advanced leaf unfolding (Vitasse *et al.*, 2011) and thus to longer growing seasons (Menzel, 2000). More precisely, in temperate-zone deciduous tree species, temperature is the main driver of phenology (Kramer *et al.,* 2000), although photoperiod is also known to play an important role in the budburst of some species, such as common beech (Basler and Körner, 2012). These shifts in phenology timing events have been reported for a widespread number of species and locations (Cornelius *et al.*, 2013; IPCC, 2007; Vitasse *et al.*, 2009).

Autumn phenology also plays a key role in the determination of the growing period. It has recently been hypothesized that a delay on leaf senescence could also lead to a longer growing season (Menzel and Fabian, 1999; Vitasse *et al.*, 2011). However, as senescence timing is poorly understood (Estrella and Menzel, 2006) further research is necessary in order to understand the mechanisms and the factors that trigger the process (i.e. photoperiod, content of nonstructural carbohydrates in leaves or decrease and temperatures during autumn). In addition, phenological responses to temperature can be species-dependent which could affect biotic interactions under climate warming (Vitasse *et al.*, 2009), leading to a change in species distribution. As a general conclusion, studies on growth rates and tree phenology are good indicators for assessing climate change impacts on temperate forests.

1.2.2 Ecophysiology

Atmospheric changes (i.e. partial pressure of CO_2 and temperature) associated with altitude (Körner, 2007) have an impact on the main physiological processes that control the carbon fixation (photosynthesis) and the transpiration in plants (stomata conductance) (Bresson *et al.*, 2009). Indeed, a reduction in the total and partial pressure of atmospheric gases has a significant impact on the respiration and gas exchange of plants (Bresson *et al.*, 2009; Körner, 2007).

Phenotypic plasticities in photosynthetic traits are found in certain plants (Berry and Bjorkman, 1980) under different environmental conditions. Discrepancies have been found between the results of studies that compared photosynthetic performances along an elevational gradient. For instance, some studies find no variation on photosynthetic rates, whereas others find that it decreases with elevation (Bresson *et al.*, 2009). The lack of consensus regarding the elevational effect on CO_2 assimilation rates is due to the absence of current comparative field studies (Körner *et al.*, 1986). For instance, some studies use CO_2 molar concentrations while others use CO_2 partial pressure. Moreover, gas exchange measurements based on CO_2 partial pressure are usually performed at ambient pressure, while very few studies have used CO_2 constant pressure (Bresson *et al.*, 2009). In fact, Bresson *et al.* (2009) found different results in photosynthetic capacities when performing gas exchange measurements along an elevational gradient at ambient and constant CO_2 partial pressure. These authors insist on the fact that this discrepancy may lead to controversial conclusions. They state the importance of keeping all microclimatic variables constant (CO_2 partial pressure, temperature, light and humidity) in order to compare the photosynthetic performance of populations at various elevations. The results of this study performed at uniform CO_2 partial pressure revealed an increase in the maximum assimilation rate with elevation for beech and oak. These results suggest that alpine species compensate harsh environmental conditions and short growing seasons by having a higher photosynthetic performance.

Photosynthetic assimilation rate is also strongly related with the leaf nitrogen content (Reich *et al.*, 1998; Hikosaka, 2004), as a high percentage of this component is located in the principal enzyme (ribulose-1,5- bisphosphate carboxylase/oxygenase) that catalyses the carboxylation process. In fact, some findings reveal an increase of photosynthetic capacity associated to an increase of nitrogen content per leaf area and increasing elevation (Körner *et al.*, 1986; Körner, 1989; Reich *et al.*, 1998). This suggests that plant's growth from higher elevations is not significantly restricted by nutritional status nor by the gas exchange capacity of leaves (Körner *et al.*, 1986; Körner, 1989).

The allocation of nitrogen within leaves is related to the photosynthetic nitrogen use efficiency (PNUE, photosynthetic capacity per unit leaf nitrogen), which is another important factor when studying the interspecific difference in photosynthetic capacity

(Hikosaka and Hirose, 2000; Takashima *et al.*, 2004). Lower PNUE values are usually found in evergreen more than in deciduous species, explained by the smaller allocation of nitrogen in photosynthetic apparatus. This puts into manifest the different strategies followed by trees, either focusing in persistence (toughness under constraint environments) in evergreen species, or productivity (photosynthesis) in deciduous species (Takashima *et al.*, 2004).

Stomatal conductance regulates the gas exchange and transpiration in vascular plants, thus influencing the carbon fixation and transpiration. The closure mechanism of stomata plays an important role in plants growing under drought conditions. However, non-stomatal limitations (i.e. mesophyll conductance, leaf diffusive resistance and metabolic impairments) may also affect the photosynthetic performance under drought stress. The response of the leaf's net assimilation rate (to sub-stomatal CO2 concentration, An-Ci curve) and chlorophyll *a* fluorescence (Fv/Fm) are two methods used for determining these non- stomatal limitations (Signarbieux and Feller, 2011).

1.2.3 Anatomy and morphology of tree foliage

Stress factors eventually manifest themselves as macroscopic leaf symptoms (Günthardt-Goerg and Vollenweider, 2007) when physiological and structural alterations are presented in the targeted tissues (Vollenweider and Günthardt-Goerg, 2005). For instance, related changes on the cell and tissue structure have been associated to drought (Olmos *et al.*, 2007) ozone concentrations (Kivimaenpaa *et al.*, 2001; Günthardt-Goerg *et al.*, 2013) and different light environments (Ashton and Berlyn, 1994). Moreover, several studies reveal correlations between certain morphological and anatomical adaptations (i.e. leaf size, stomata density, cuticle thickness, etc.), and physiological processes (i.e. net photosynthesis, water potential, etc.) (Ashton and Berlyn, 1994; Royo *et al.*, 2001; Gratani *et al.*, 2003). For *Quercus ilex* L. seedlings under high temperature conditions, an increase in water use efficiency and photosynthetic rate was correlated to higher leaf mass area and to the proportion of palisade in the mesophyll thickness (Gratani *et al.*, 2003). In addition, leaf structure (i.e. specific leaf area, SLA) has an influence in the maximum assimilation rate and leaf nitrogen (Amax-N) relationship (Reich *et al.*, 1998), which differs among different functional groups (i.e. deciduous and conifer). For instance, deciduous species have higher SLA than evergreen species. A relationship between leaf size and elevation has also been reported within different plant life forms in New Zealand. The main finding is a decreasing trend of leaf size and SLA towards higher elevations, suggesting that changes on these characteristics are related to temperature regimes (Körner *et al.*, 1986).

A decrease in the photosynthetic rate has also been associated to changes in stomata density and mesophyll proportions (Greenwood *et al.*, 2008). As way of example, stomata density, length and dry weight of pine needles increased with elevation up to 3000 m (Qiang *et al.*, 2003). Therefore, plants are able to manage and respond to environmental changes through phenotypic plasticity in morpho-anatomical traits (Bradshaw, 1965).

In the present research I intend to study the **acclimation abilities** to simulated climate change of spruce and beech through different **response levels** (individual, organ and tissue level), by comparing saplings from the same population growing in different environmental conditions. To achieve this, I will analyze the variation in morpho-anatomical traits and physiological processes of these species along an elevational gradient in the Swiss Jura Mountains.

1.3 Assessing future climate change impacts in the present

The evaluation of climate change impacts in the forest ecosystems is very challenging, as it takes several decades to observe an ecological response. However, we cannot afford waiting to observe how climate change is going to evolve and affect these ecosystems as the loss in ecological goods and services and associated consequences can be devastating.

Many modelling approaches exist to assess climate change impacts on trees and forests. Most of these are constrained to small comparable regions or to selected species. Hengeveld *et al.* (2013) list the following approaches frequently used: biogeochemistry models, population dynamics models with competition, demographic models of a single species, phenological models of single species and species distributions models. The main limitation of these approaches is their incapability of predicting the future fate of species at large spatial scale while predicting responses at fine spatial resolution that is useful for forest managements. Moreover, they generally do not take into account changes in climate variability and extremes as their predictions are based on the use of average changes of climate.

Other approaches, related to empirical studies, are the climate-controlled chamber experiments often conducted *ex situ*, which are valuable for understanding the impact of a single factor on tree physiology but does not represent real *in situ* conditions that involve many abiotic and biotic interactions that determine tree responses (Zimmermann *et al.*, 2009; De Boeck *et al.*, 2010; Körner *et al.*, 2016; Vicca *et al.*, 2016). However, elevational gradient experiments (Körner *et al.*, 1986; Vitasse *et al.*, 2010; Gavazov *et al.*, 2014; Kong *et al.*, 2014; Pescador *et al.*, 2015) are used in ecological research as a "natural" approach to the impacts of future climate change by means of "space-for-time/warming experiments" (Körner, 2007). Elevational gradients are characterized by steep changes in the physical environment such as temperature and atmospheric pressure. However, it is important to distinguish changes in climatic variables associated with altitude from those that are related to local peculiarities. Thus, a reduction in land area, decrease in atmospheric pressure (declines by c.a. 11 % for every km gain in altitude), reduction of atmospheric temperature (on average 5.5 K per km of altitude) and increasing radiation (under cloudless sky) are climatic factors relevant for organisms that are associated with altitude (Körner, 2007). Climatic trends that are generally not related to altitude are precipitation, moisture, wind velocity and seasonality. These two main group of drivers are generally not distinguished in transect experiments, leading to controversy conclusions.

The general use of monthly or annual climatic means to assess species responses to climate change has ignored the question of how climatic extremes could help to explain species distributions. However, climate change implies simultaneously changes in mean climatic variables and changes in extreme events (IPCC, 2007). Moreover, both adaptation and future responses of species to changing climates will certainly be affected by extremes in addition to means (Zimmermann *et al.*, 2009). Despite this fact, extreme climatic events can have a large effect on tree growth and have been rarely studied (Lendzion and Leuschner, 2008; Teskey *et al.*, 2015) and there is no accurate definition related to the existence of an "extreme" (Stephenson, 2008). An established definition would be "*an episode or occurrence in which a statistically rare or unusual*

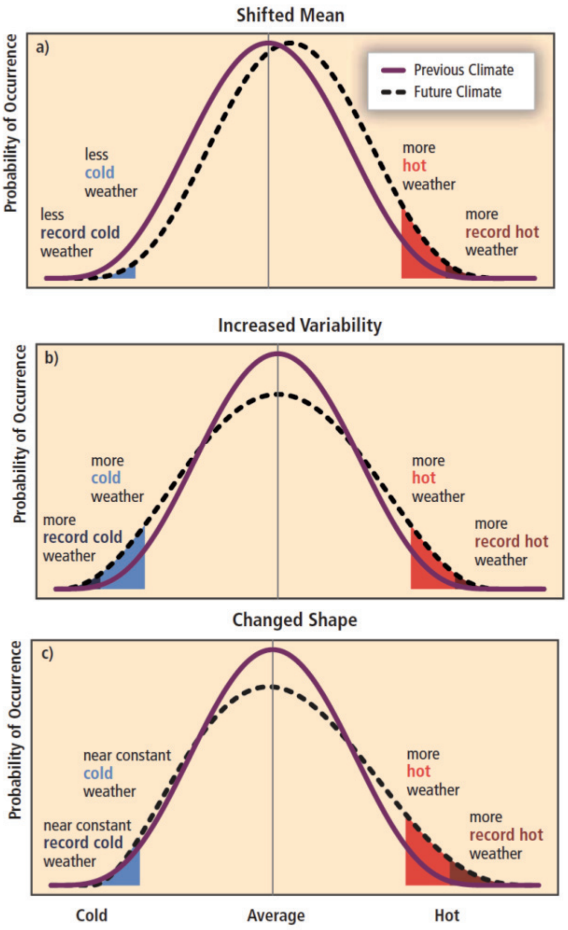


Figure1-3 The effect of changes in temperature distribution on extremes. Different changes in temperature distributions between present and future climate and their effects on extreme values of the distributions: a) effects of a simple shift of the entire distribution towards a warmer climate; b) effects of an increased temperature variability with no shift of the mean; and c) effects of an altered shape of the distribution, in this example an increased asymmetry towards the hotter part of the distribution (IPCC, 2012).

climatic period alters ecosystem structure and/or function well outside the bounds of what is considered typical or normal variability" (Smith, 2011). A special report of IPCC (2012) defined an "extreme climate or weather event" or "climate extreme" as "*the occurrence of a value of a weather of climate variable above (or below) a threshold value near the upper (or lower) ends of the range of observed values of the variable*". They clarify this definition by stating that it includes absolute thresholds as extreme events and give the example of specific critical temperatures for health impacts. Moreover, changes in extremes can be related to variations in the mean, variance or shape of probability distribution, or as combination of all (Figure 1-3).

To conclude, the intrinsic uncertainty of climate change implies that no single method is sufficient enough to predict and understand species responses. Therefore, it seems more appropriate to evaluate potential impacts of climate change from an array of methods (Zimmermann *et al.*, 2014) at multiple scales (Sass-Klaassen *et al.*, 2016).

1.4 Thesis overview

The silvopastoral ecosystems of the Swiss Jura Mountains are traditionally used as a source of forage and timber, but their sensitivity to land-use changes and climatic conditions puts them at risk (Buttler, 2014). The regeneration and growth of the two main species, spruce (Norway spruce) and beech (European beech), is crucial for structuring these ecosystems and the semi-wooded landscape, and in particular for maintaining dynamic coexistence in space and time, isolated trees, shreds of forests and pastures. It is therefore important to understand the processes of regeneration of trees for the sustainable use of these ecosystems and to maintain their multifunctional value, including their biodiversity. From the inevitable perspective of landscape transformation under climate change (Peringer *et al.*, 2013), adaptive management is crucial to ensure the expected ecological goods and services, including pasture forage, wood, biodiversity and beauty of the landscape. In this context, the response of young trees to climate change becomes more important.

The main objective of the present thesis was to better understand the mechanisms of response to simulated climate change of two of the main tree species in central European forests, European common beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) H. Karst). Because from a response at the individual level begins a cascade of ecological processes observed at the ecosystem level (Hansen *et al.*, 2001), our main source of information was the individual tree. Moreover, due to the complexity of tree responses to changing environmental conditions, I assessed the acclimation abilities at different levels of response (Figure 1-4). In other words, I intend to explain the growth patterns observed in beech and spruce saplings by looking at the phenology, morphology and physiology of tree foliage and the tree-soil-atmosphere relationship. This makes this project original as it integrates results from multidisciplinary approaches, which are commonly studied separately.

In addition to increases in air temperature, it has been predicted an increase in frequency and intensity of extreme events (IPCC, 2013), which will have stronger impacts on tree

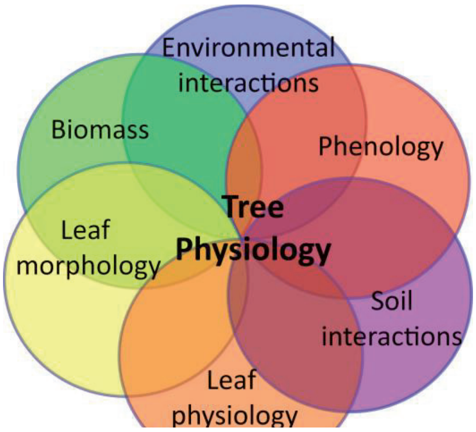


Figure 1-4 Levels of study in this thesis

physiology than gradual temperature shifts (Teskey *et al.*, 2015). Therefore, I assessed the impacts of extreme climatic variables on tree performance (i.e. tree growth) and evaluated their importance for the ecological understanding of tree physiology in increasing climatic variability.

Specifically, this thesis intended to give answers to the following research questions and to test the following hypothesis:

Q1: Will spruce and beech saplings acclimate to simulated climate change?

H1: Spruce and beech saplings will acclimate to simulated climate change.

Q2: Which is the main climatic driver (either temperature or precipitation) influencing the performance of these species within our latitudes?

H2: Within our latitudes, temperature is the main driver of saplings' responses, as precipitation is not a limiting factor in the Jura mountains.

Q3: Will phenotypic plasticity (at different structural levels) lead to species-specific responses and, therefore, to different degrees of acclimation to the simulated climate change?

H3: Beech will present a higher phenotypic plasticity than spruce.

Q4: Do extreme climatic variables improve the interpretation of species responses to climate change?

H4: Climatic extremes have stronger impacts on tree growth than gradual changes in climate and will explain better tree growth responses.

To address these research questions, I assessed the acclimation abilities of beech and spruce saplings native to the montane belt of the Jura Mountains by performing a transplantation experiment along an elevational transect (Figure 1-5). This "space-for-time" approach (Körner, 2003) is based on the variation of environmental conditions (e.g. temperature) with elevation within a very short distance, enabling to simulate the impacts of future climate change scenarios on vegetation in the present.

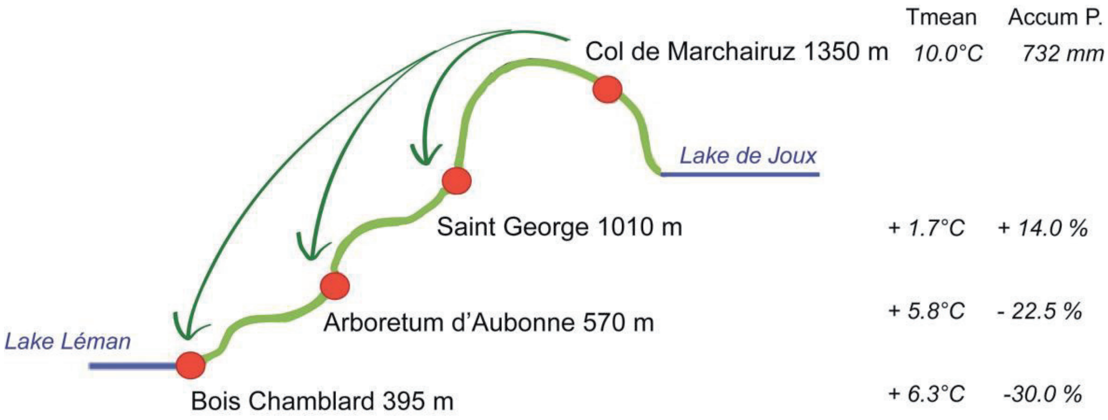


Figure 1-5 Experimental design of the translocation of saplings native of Col de Marchairuz to three lower recipient sites. The mean temperature and the accumulative precipitation during the growing season is presented for the control site at 1350 m, while for the recipient sites the difference in temperature and precipitation is presented. Values correspond to the average of the data collected between the years 2013, 2014 and 2015.

Saplings of beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) H.Karst.) were collected at the donor site (1350 m) immediately before the budburst of the 2012 growing season. The surface of collection was assumed to be reduced enough to ensure the same provenance of saplings. The saplings had similar sizes (average height of 33 cm for beech and 31 cm for spruce) and were excavated with intact root systems and soil. They were then transplanted to 20-L pots and randomly reallocated in four plots, one at each altitude, in open spaces and far enough from the forest to avoid shade from surroundings adult trees. Half of the saplings were transplanted a second time to 40-45-L pots in autumn 2014, two years after the first transplantation, due to the potential limitation of growth by the initial pots. The other half was harvested for analysis. The soil of the harvested saplings was used for the transplantation of the remaining individuals to maintain the same soil characteristics for each species, treatment and site.

The experimental design was a generalized block with repeated measures (2013, 2014 and 2015). The four blocks were considered as fixed and corresponded to the four sites at altitudes of 1350, 1010, 570 and 395 m. Ten replicate saplings per species (beech and spruce) and treatment (non-irrigated and irrigated) were randomly allocated within each block. The experimental unit was a pot with one sapling, which was randomly placed on a grid with a spacing of 0.3 × 0.9 m to avoid light competition. The pots were recessed belowground, and a geotextile cap was placed at the top and bottom of the pots to reduce both the evaporation of soil water and the penetration of roots into the soil of the site. All sites were equipped with wireless meteorological stations (Sensorscope, Climaps. available at: https://www.climaps.com/) that continuously recorded climatic parameters (precipitation and air temperature), enabling us to add water weekly during the growing season to ensure equal amounts of precipitation at the donor and recipients sites for the irrigated treatment. The saplings in the non-irrigated treatment were subjected to the local environmental conditions of each site. The purpose of the irrigation treatment was to identify the effect of rainfall for studying the responses of the saplings to temperature alone (Figure 1-6).

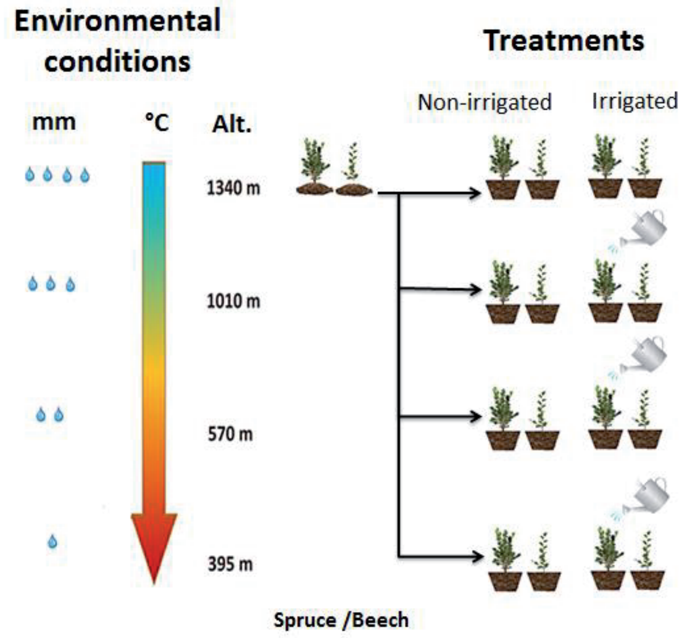


Figure 1-6 Experimental design: treatments applied along the elevational gradient

To summarize, I assessed the vitality of saplings by measuring the annual tree growth **(Chapter 2**) through a specific allometric equation which allowed estimating the biomass increase of each individual tree in a non-destructive way. I also assessed to what extent a longer growing season did increase tree growth **(Chapter 2**). In order to explain the species-specific growth responses observed, I sought to further understand the mechanisms of response at the leaf level **(Chapters 3 and 4)**. I characterized the short-term responses of foliar traits in beech and spruce saplings through phenotypic plasticity **(Chapter 3)**. This allowed me to assess the potential plasticity of the foliage of these species to respond to simulated climate change and give indications of their acclimation abilities at leaf level. Finally, any changes at micro-morphological traits of the leaf (e.g. stomata density and stomata size) may have a cascading effect on physiological process such as the regulation of leaf gas exchange that allows carbon fixation. For this reason, through a multiple-level approach I related ecophysiological measurements (e.g. photosynthesis capacity) in foliage and soil conditions with the growth performance of saplings **(Chapter 4).** Additionally, I tested for beech the legacy effects of advanced or delayed budburst on the budset timing **(Chapter 5).** This high resolution growth and physiological monitoring provides with needed data to assess the dynamic responses of trees to stress factors (Sass-Klaassen *et al.*, 2016). Therefore, this study can improve the parametrization of mechanistic models predicting future species distributions. Specifically, the results from the project CLIMARBRE will serve to calibrate the spatial and dynamic WoodPam model, which has already been used to predict the evolution of landscapes and tree species in the Jura Mountains (Peringer *et al.*, 2013).

This thesis is divided in chapters corresponding to the different manuscripts prepared for submission to peer-reviewed journals (Table 1-1). In addition, to introduce each chapter a brief introduction is presented at the beginning and references are presented at the end of the dissertation. Supplementary material is presented at the end of the dissertation, as appendices.

Table 1-1 Overview of manuscripts included in this thesis

Chapter	Research topic	Journal	Submission status
2	Tree growth	*Global Change Biology*	Under revisión
3	Leaf morphology	*Environmental and Experimental Botany*	In press[1]
4	Multi-level approach	*Undefined*	In preparation
5	Phenology	*Global Change Biology*	In press[2]

[1] P. Sanginés de Cárcer, C. Signarbieux, R. Schlaepfer, A. Buttler, P. Vollenweider, *Responses of antinomic foliar traits to experimental climate forcing in beech and spruce saplings*, Environmental and Experimental Botany, Volume 140, August 2017, Pages 128-140, ISSN 0098-8472, https://doi.org/10.1016/j.envexpbot.2017.05.013.

[2] Signarbieux C, Toledano E, Sanginés de Carcer P, *et al. Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring*, Glob Change Biol. 2017;00:1–12. https://doi.org/10.1111/gcb.13740

Chapter 2 Tree growth responses

Growth contrasts between beech saplings at control site (left) vs the lowest site (right)

P. Sanginés (2015)

> *“Of all the circumstances that affect the habitation of plants, temperature is undoubtedly the most essential”*
>
> *(Lamark, de Candolle, Flore française, 1805)*

Growth of individual trees is directly related with the productivity of a forest, which will most likely be challenged by increasing temperatures and more frequent extreme events. Therefore, it is important to understand and assess climate-growth relationships to adapt future managements. Forest practices that take into account the suitability of species to future climate conditions will most likely reduce the risk of timber loss related to climate change impacts.

Tree growth is determined by various site and environmental factors such as temperature, water, nutrients and light availability, as well as, inter and intra-specific competitions. In this chapter we focused in assessing species-specific growth responses to variation in temperature, precipitation and evaporative demand of the air.

The estimation of aboveground biomass in adult trees is generally obtained by allometric equations that relate diameter (at breast level) and tree height with biomass. Experimental studies performed in saplings tend to use also these variables as an estimation of tree growth. However, at early stages of a tree, strong differences in growth patterns can be observed between species. For instance, spruce presents a more conservative growth strategy than beech, which tends to growth faster in height. Moreover, because of the different functional groups they belong to (i.e. evergreen conifer vs. broadleaf deciduous), they also allocated biomass differently. For this reason in this study additional saplings, not included in the experiment, were harvested to build a biomass model that would accurately estimate the aboveground biomass of both species. We found that the measurement of total volume, combining the diameter and the length of stem and four longest branches, was the best approach to estimate the biomass increase of the saplings of both species.

This chapter is divided into two sections. In the first (2.1), the procedure for preliminary model selection is presented. In the second (2.2), growth responses of beech and spruce saplings subjected to changing environmental conditions is presented as the following submitted scientific article:

Article 1: **Vapor-pressure deficit and extreme climatic variables limit tree growth**

Sanginés de Cárcer, P, Vitasse, Y, Peñuelas, J, Jassey, V.E.J., Buttler, and Signarbieux, C.

Under revision in Global Change Biology (17.7.2017)

2.1 Models estimating the biomass of beech and spruce saplings

Precise biomass estimations are essential for assessing climate change. Tree growth is a central part in this study so preliminary models, relating biomass with non-destructive measurements, were carefully evaluated to select the most parsimonious one. A detailed description of the methodology is presented in *section 2.2*. Here I focus in the

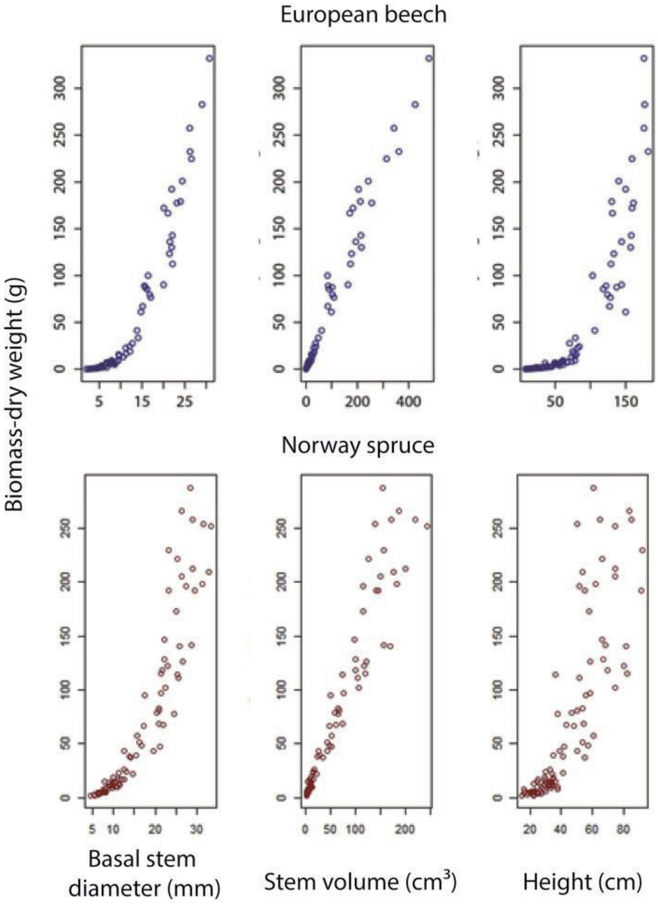


Figure 2-1 Correlation between selected independent variables and biomass

comparison of four different preliminary models.

Previous to the model comparison, I graphically analyzed the relationship between explanatory variables and aboveground biomass (Figure 2-1). Visually, there is an increase in the variability (heteroscedasticity) of biomass with increase tree size (funnel shape), which is a common pattern observed in biology. A log transformation of the data solved this problem and satisfied the assumption of constant variance of residuals.

To be able to compare the different models I followed several criteria to select the optimal allometric equation for the biomass estimation of each species:

1. the highest value of the adjusted coefficient of determination (R^2)
2. the lowest root-mean-square error (RMSE)
3. significances of F-test < 0.05
4. the regression model that best estimates the biomass of both species
5. the practical use of the model (cost of the independent variable measurements) and biological meaning

I present in Table 2-1 the preliminary allometric equations developed for each species and a summary of the statistical analysis. Close relationships ($R^2 > 0.9$) were observed between the explanatory variables (D, H, Vs, Vt) and the estimated aboveground woody biomass (AGB), and all models were highly significant. However, models 4 and 8 presented the lowest root-mean-square errors. Moreover, in Figure 2-2, I further assessed the accuracy of all models by comparing them to the linear regression $y=x$, that would represent the 100% accuracy of the biomass estimation.

Because I aimed for a model that best explained the morphology and different growth strategies of both species (e.g. beech has a tendency to grow more in height than spruce), I chose the total volume model (Vt-B) as the final one. Unlike the model considering only the basal diameter, this final model takes into account both the fast growth in height of beech and the lateral growth of spruce, which is very notable at the sapling stage. In fact, from Figure 2-2 the model including exclusively stem diameter underestimated considerably the actual biomass for beech while overestimating that for spruce. Meanwhile, the total volume model (Vt) slightly overestimated, at same extent, the actual biomass for both species.Table 2-1 Statistical summary of models for estimating aboveground wood biomass (B) as a function of basal diameter D (mm), stem volume Vs (cm3), stem length H (cm) and total volume (stem and branches) Vt.

Beech

#	Model	Coefficients			R^2	RMSE	Signif.
		c	a	b			
1	Ln (B) = c + a Ln (D)	-4.48	3.08		0.959	0.273	***
2	Ln (B) = c + a Ln (Vs)	-0.47	0.99		0.987	0.153	***
3	Ln (B) = c + a Ln (D) + b Ln (H)	-5.69	1.91	0.91	0.985	0.166	***
4	Ln (B) = c + a Ln (Vt)	-0.54	0.96		0.974	0.134	***

Spruce

#	Model	Coefficients			R^2	RMSE	Signif.
		c	a	b			
5	Ln (B) = c + a Ln (D)	-3.23	2.52		0.942	0.287	***
6	Ln (B) = c + a Ln (Vs)	0.46	0.93		0.963	0.228	***
7	Ln (B) = c + a Ln (D) + b Ln (H)	-4.49	1.85	0.82	0.965	0.223	***
8	Ln (B) = c + a Ln (Vt)	0.09	0.98		0.975	0.118	***

Signif. Codes: 0 `***` 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

All coefficients are significant

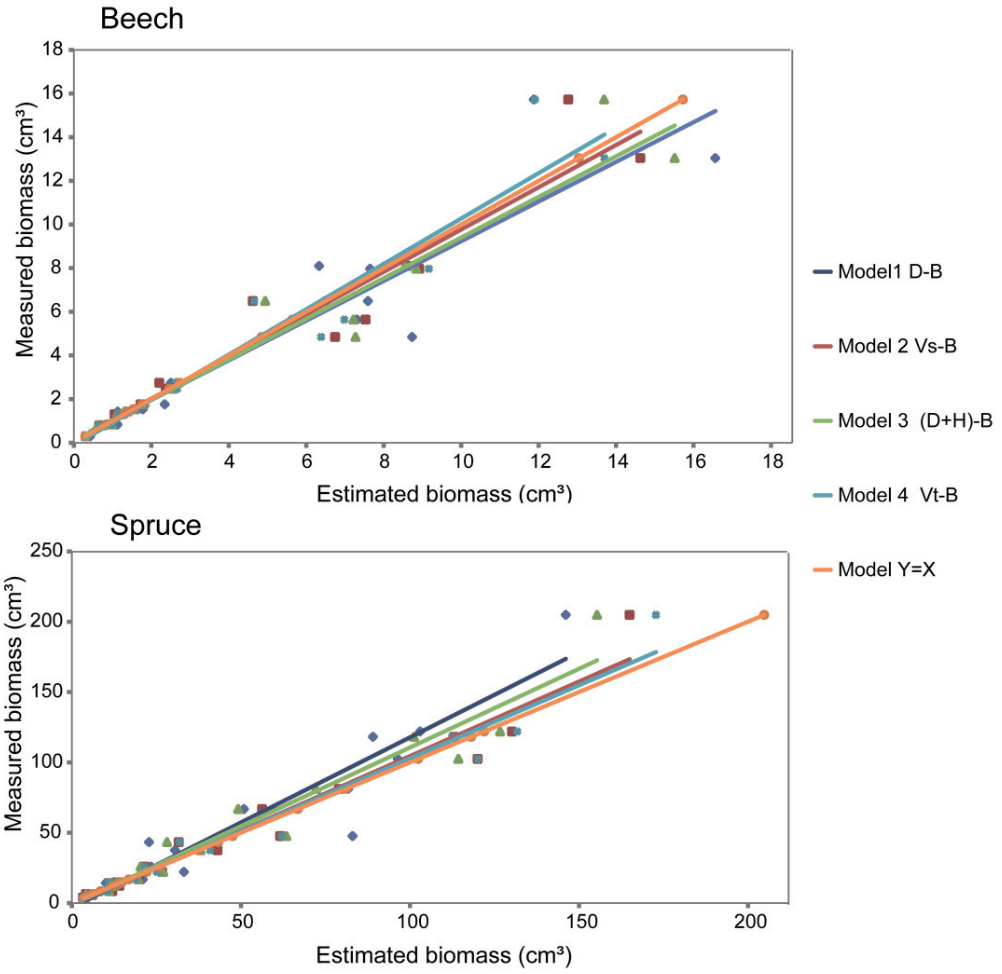


Figure 2-2 Comparison of the accuracy of the different biomass models

Finally, I improved the selected model for each species by the harvest of additional saplings (to sum 95 individuals) to cover the diameter class range presented in the field experiment. Moreover, I validated the models with a sum 20 saplings of each species harvested from the field experiment during fall 2014. The final models I used for assessing the response of tree growth to changing environmental conditions (section 2.2) were for beech, B = exp ^ (1.012535 * (Ln(V))-0.585528), with an R^2adj of 0.995 and $P < 0.0001$ and for spruce, B = exp^1.00926 * (LnV), with an R^2adj of 0.997 and $P < 0.0001$.

2.2 Vapor-pressure deficit and extreme climatic variables limit tree growth

2.2.1 Abstract

Assessing the effect of global warming on forest growth requires a better understanding of species-specific responses to climate change conditions. Norway spruce and European beech are among the dominant tree species in Europe and are largely used by the timber industry. Their sensitivity to changes in climate and extreme climatic events, however, endangers their future sustainability. Identifying the key climatic factors limiting their growth and survival is therefore crucial for assessing the responses of these two species to ongoing climate change. We studied the vulnerability of beech and spruce to warmer and drier conditions by transplanting saplings from the top to the bottom of an elevational gradient in the Jura Mountains in Switzerland. We (1) demonstrated that a longer growing season due to warming could not fully account for the positive growth responses, and the positive effect on sapling productivity was species-dependent, (2) demonstrated that the contrasting growth responses of beech and spruce were mainly due to different sensitivities to elevated vapor-pressure deficits, (3) determined the species specific limits to vapor-pressure deficit above which growth rate began to decline and (4) demonstrated that models incorporating extreme climatic events could account for the response of growth to warming better than models using only average values. This results support that the sustainability of forest trees in the coming decades will depend on how extreme climatic events will change, irrespective of the overall warming trend.

Keywords

Tree growth, spruce, beech, climate change, vapor-pressure deficit, extreme events, wooded pastures

2.2.2 Introduction

Climate change is currently escalating so rapidly that many trees may not be able to adapt (Rogers, Jantz, and Goetz, 2017). In addition to the gradual global warming, the frequency and severity of extreme events such as heat waves, heavy precipitation, summer droughts and cold spells are expected to increase in the coming decades (IPCC, 2013; Schar *et al.*, 2004), which may ultimately determine future tree distributions (Zimmermann *et al.*, 2009). Extreme events can have strong impacts on tree growth and survival, due to typically stronger responses and shorter response times than for normal climatic events (Hanson, Palutikof, Dlugolecki, and Giannakopoulos, 2006; Kreyling, Jentsch, and Beierkuhnlein, 2011). Forest researchers must estimate the resilience of forests to expected climate change and extreme climatic events to guide sustainable forest management (Lindner *et al.*, 2014). An increasing number of studies are therefore testing the impact of extreme events on forest growth (Ciais *et al.*, 2005; Teskey *et al.*, 2015), some under controlled conditions (Lendzion and Leuschner, 2008). Experiments are often conducted *ex situ* (e.g. in climate-controlled chambers), which is valuable for understanding the impact of a single factor on tree physiology but does not represent real in situ conditions that involve many abiotic and biotic interactions that determine tree growth (De Boeck, Dreesen, Janssens, and Nijs, 2010; Körner *et al.*, 2016; Vicca *et al.*, 2016; Zimmermann *et al.*, 2009).

Increased tree growth has been correlated with warmer temperatures (Way and Oren, 2010) and longer growing seasons (Keenan, 2015; Menzel and Fabian, 1999; Piao, Friedlingstein, Ciais, Viovy, and Demarty, 2007; Signarbieux *et al.*, 2017). However, divergent responses to warming among co-existing tree species have been also widely reported (C. Allen *et al.*, 2010; Carnicer, Barbeta, Sperlich, Coll, and Penuelas, 2013), reflecting different physiological needs and growth strategies. A change in environmental conditions due to altitude is one of the factors leading to this divergence. For instance, tree growth during the extremely hot and dry summer in 2003 in the Swiss

Alps increased at high altitudes but decreased at low altitudes (Jolly, Dobbertin, Zimmermann, and Reichstein, 2005). This contrasting growth response was explained by the differences in resource, temperature and water limitations between lower and higher elevations.

Shifts in the onset of spring phenology, due to increasing temperatures, generally are related to an increase in growing season length (Keenan, 2015). It has been reported that leaf unfolding of European woody species has advanced by about 13 days during the period 1982-2011 in Europe, which together with delayed autumn phenology has contributed to extend the growing vegetative period (Fu *et al.*, 2014) by 24 days during the same period (Kolářová, Nekovář, and Adamík, 2014). Moreover, it has been hypothesized that the length of the growing season affects productivity to a larger extent in angiosperms than in conifers (Carnicer *et al.*, 2013). However, Körner (2017) argued that longer growing seasons may contribute to higher annual tree growth, but only to a certain limit, which is not yet clearly identified (Delpierre, Guillemot, Dufrêne, Cecchini, and Nicolas, 2017).

The stomatal response of trees to changing environmental conditions is complex and it is a process which is still not well understood (Damour *et al.*, 2010). The closure of stomata at midday is regulated by the water available in the soil, leaf and atmosphere, and it is highly species-specific (Bond and Kavanagh, 1999). Stomatal responses to increasing evaporative demand of the air seem to be another explanation for the contrasting growth responses between functional groups. Carnicer *et al.* (2013) reviewed various hypotheses that could account for the contrasting responses of growth to temperature in Mediterranean angiosperm and coniferous trees. They included a hypothesis involving the effect of eco-physiological and hydraulic traits on tree growth. More specifically, they suggested that different sensitivities of stomatal conductance to vapor-pressure deficit (VPD) lead to different growth responses. Several studies have been performed in order to understand the mechanisms triggering stomatal closure in response to vapor pressure deficit (Sellin, 2001; Brodribb and McAdam, 2011; Mott and Peak, 2013) and agree that stomata typically close at high VPD and open at low VPD (McAdam and Brodribb, 2015). The sensitivity of VPD to changes in air temperature differs among plant functional groups (Ogaya and Peñuelas, 2007; Way and Oren, 2010) and underlies the strategies optimizing carbon uptake with reduced water loss

(Franks and Farquhar, 1999). For instance, the positive response of growth to increased temperature in angiosperms could be due to a narrower hydraulic safety margin and a higher capacity to reverse embolisms (Carnicer *et al.*, 2013). The higher hydraulic safety margin in conifers implies an earlier response of stomatal closure before cavitation (Carnicer *et al.*, 2013), at a cost of reducing carbon uptake. Therefore, vapor-pressure deficit (VPD) can limit tree growth (C. D. Allen, Breshears, and McDowell, 2015), but its importance has not been fully recognized (Lendzion and Leuschner, 2008). Leaf-to-air VPD is expected to increase with the predicted increase in air temperature (Novick *et al.*, 2016), with subsequent impacts on plant transpiration and photosynthesis. Reciprocal common garden experiments along altitudinal transects have been suggested to be a powerful tool for testing ecological responses to changes in environmental conditions (Carnicer *et al.*, 2013; Körner, 2007), such as increasing temperature and evaporative demand of the air. This type of experiment is based on the variation of environmental conditions (temperature, atmospheric pressure, etc.) with elevation, simulating climate change conditions without needing to wait decades to observe an impact and therefore predict responses and adapt forest managements. Most studies of the impacts of climate change on vegetation are based on changes in the averages of climatic variables (Miyamoto, Griesbauer, and Scott Green, 2010), such as the mean annual or summer temperature. Extreme climatic events can have a large effect on tree growth but have been rarely studied (Lendzion and Leuschner, 2008; Teskey *et al.*, 2015) and there is no accurate definition related to the existence of an “extreme” (Stephenson, 2008). In this study, we defined “extreme” according to IPCC (Murray and Ebi, 2012), i.e. we quantified climate extremes by determining specific thresholds above which tree growth could be largely affected. We thus analyzed the effects of changes in climatic factors on the growth of beech and spruce saplings and compared the variances of the data for averages vs. extremes. Specifically, our main questions were: 1) how does species-specific growth respond to warmer and drier conditions, 2) to what extent does a longer growing season increase tree growth, 3) how does an elevated VPD affect tree growth and 4) what benefit does the study of “extreme conditions” have on a mechanistic understanding of the responses of tree growth under various environmental conditions? The novelty in this study is that we used a ‘natural warming experiment’ to assess how trees adapted to cold and wet environments respond to warmer and drier conditions by a translocation experiment along a transect across an elevational gradient. Generally, elevational gradient experiments compare populations of a same species but growing at different elevations, or use climate chambers to control climatic variables, without taking into account the effects of extreme climatic variables. In our approach, we transplanted beech and spruce saplings from a donor site at a high elevation to three recipient sites at lower elevations to assess the effects of warmer and drier conditions on growth of individuals adapted to cold and wet environments. We focused on the saplings of the two dominant sylvopastoral species of the Jura Mountains, Norway spruce and European common beech. A good understanding of regeneration and its consequences under conditions of climate change are crucial for both species conservation and the sustainable and adaptive management of landscapes (Buttler 2014). These two species are also among the dominant trees in central Europe and are key to the timber industry, so forest managers need to know whether they will be sustainable in the coming decades.

2.2.3. Materials and methods

2.2.3.1. Study site and elevational gradient

The conditions of climate change were simulated using an elevational gradient along a south-facing slope of the Jura Mountains in Switzerland. This space-for-time substitution (Körner, 2003) simulated a climatic gradient, i.e. an increase in temperature and a decrease in precipitation towards lower altitudes. A detailed description of the site selection is given by Gavazov *et al.* (2014). Briefly, the donor site was at Combe des Amburnex (N46°54′, E6°23′; 1350 m a.s.l.), with an oceanic climate, a mean annual temperature and precipitation of 4.5°C and 1750 mm, respectively, and a permanent snow cover from November to may (K. S. Gavazov, Peringer, Buttler, Gillet, and Spiegelberger, 2013). The three recipient sites were at St.-George at 1010 m a.s.l. (N46°52′, E6°26′), Arboretum d'Aubonne at 570 m a.s.l. (N46°51′, E6°37′) and Les Bois Chamblard at 395 m a.s.l. (N46°47′, E6°41′). Combe des Amburnex was the control site with native climatic conditions, so this climatic gradient covered three possible warming scenarios of the Intergovernmental Panel on Climate Change (K. S. Gavazov *et al.*, 2013; K. Gavazov *et al.*, 2014): moderate at 1010 m a.s.l. (on average + 2°C and 20 % rainfall reduction), intermediate at 570 m a.s.l. (+ 4°C and 40 % rainfall reduction) and extreme at 395 m a.s.l. (+ 5°C and 50 % rainfall reduction). In this study we mainly focused on the impact of changes in air temperature and precipitation in the tree growth of saplings. However, we acknowledge that there is a decrease in total atmospheric pressure and partial pressure of gases with altitude, as well as an increase in radiation under cloudless sky due to a decrease in atmospheric turbidity (Körner, 2007). Sanginés *et al.* (2017) showed that temperature gradients had a major effect on the morphological changes of leaves as compared to changes in partial pressure. Regarding solar radiation, the actual dose received by a plant will also depend on scattering elements such as clouds, which generally increase with altitude in mountain regions (Körner, 2007). Therefore, we assume that the increase in solar radiation at high elevations during the growing season is, to some extent, compensated with the associated increase in cloudiness. To support this assumption, we visually inspected the data of solar radiation recorded by meteo-stations placed at our study sites and observed similar July solar radiation averages and same trends along the spatial-temporal gradient (data not shown).

2.2.3.2. Experimental design and species

Saplings of beech (Fagus sylvatica L.) and spruce (Picea abies (L.) H.Karst.) were collected at the donor site (1350 m) immediately before the budburst of the 2012 growing season. The surface of collection was assumed to be reduced enough to ensure the same provenance of saplings. The saplings had similar sizes (average height of 33 cm for beech and 31 cm for spruce) and were excavated with intact root systems and soil. They were then transplanted to 20-L pots and randomly reallocated in four plots, one at each altitude, in open spaces and far enough from the forest to avoid shade from surroundings adult trees. Half of the saplings were transplanted a second time to 40-45-L pots in autumn 2014, two years after the first transplantation, due to the potential limitation of growth by the initial pots. The other half was harvested for analysis. The soil of the harvested plants was used for the transplantation of the remaining individuals to maintain the same soil characteristics for each species and site.

The experimental design was a generalized block with repeated measures (2013, 2014 and 2015). The four blocks were considered as fixed and corresponded to the four sites at altitudes of 1350, 1010, 570 and 395 m. Ten replicate saplings per species (beech and spruce) and treatment (non-irrigated and irrigated) were randomly allocated within each block. The experimental unit was a pot with one sapling, which was randomly placed on a grid with a spacing of 0.3 × 0.9 m to avoid light competition. The pots were recessed belowground, and a geotextile cap was placed at the top and bottom of the pots to reduce both the evaporation of soil water and the penetration of roots into the soil of the site. All sites were equipped with wireless meteorological stations (Sensorscope, Climaps. available at: https://www.climaps.com/) that continuously recorded climatic parameters (precipitation and air temperature), enabling us to add water weekly during the growing season to ensure equal amounts of precipitation at the recipient and donor sites for the irrigated treatment. The saplings in the non-irrigated treatment were subjected to the local environmental conditions of each site. The purpose of the irrigation treatment was to identify the effect of rainfall for studying the responses of the saplings to temperature alone.

2.2.3.3. Biomass estimation: in situ measurements of growth rate

Growth was monitored twice (before budburst and after senescence) for all saplings and for three consecutive growth periods (2013, 2014 and 2015) to evaluate the effects of the changes in environmental conditions on aboveground biomass. Overall growth was estimated by dasometric measurements of the stem and four main branches. The four longest branches for each sapling were identified and tagged to allow continuous monitoring. Stem and branch diameters (basal and apical) were measured using an electronic caliper with an accuracy of 0.01 mm. The basal diameter *(Sb)* of the stem was an average of two perpendicular measurements approximately 1-2 cm from the base of the root collar. The basal diameter of a branch (*Bb)* was recorded at the base. The apical diameter of the branches *(Ba)* and the stem *(Sa)* were measured below the dormant bud. Stem length *(H)* and branch length *(l)* were measured with a ruler from the base to the below winter bud (accuracy of 0.1 cm).

2.2.3.4. Biomass models: sampling and independent variables

We expressed tree growth as total aboveground woody biomass using an allometric equation. This equation estimated the aboveground woody biomass from non-destructive measurements of easily measured variables (e.g. basal diameter). This model was constructed using a total of 95 additional saplings per species collected from the donor site and harvested. The allometric equation based on these additional saplings served to estimate the biomass of the saplings included in the study in a non-destructive way. Tree components (stem, four longest branches, remaining branches and roots) were separated in the laboratory and placed in paper bags. *Sa, Sb, H, Ba, Bb* and *l* were measured on the stems and main branches, which were then oven-dried at 65°C to constant weights. These independent variables and their combinations (e.g. stem diameter and height) were then correlated with the total dry weight (g) using linear regressions to obtain the most parsimonious model.

2.2.3.5. Biomass models: model construction and validation

Several criteria were followed for selecting the optimal allometric equation for each species: (1) the highest adjusted coefficient of determination (R^2adj), (2) the lowest root-mean-square error RMSE, (3) $F < 0.05$, (4) the regression model with the best biomass estimates for both species and (5) and the practicality of the model (cost of measuring the independent variables).

The selected model indirectly estimated the aboveground biomass using the total volume of the stem and the four main branches (Eq. 1). This method was the best for quantifying and comparing the aboveground biomass of the two species with different growth patterns (i.e. beech growing in height and spruce producing more branches). The model developed was:

$$\mathrm{Ln(B) = c + aLn(V)}$$

Equation 1 Estimation of aboveground woody biomass

Where B is the aboveground woody biomass (g), V is the total volume of the four main branches and stem (cm^3) and c is the intercept and a is the slope coefficient of the regression line. The allometric equation for beech was B = exp ^ (1.012535 * (Ln(V))-0.585528), with an R^2adj of 0.995 and $P < 0.0001$. The model for spruce was B = exp^1.00926 * (LnV), with an R^2adj of 0.997 and $P < 0.0001$. The model selected for each species was then validated with half of the saplings used in the study harvested in autumn 2014 during the transplantation to larger pots.

We used these models to calculate the initial and final biomasses of each sapling for each growing season. The difference between the final and initial biomasses was divided by the initial biomass to normalize for sapling size, allowing the removal of any possible effect of sapling size.

2.2.3.6. Definition of length of the growing season

Onset of the vegetative period

Phenological variables were observed along the elevational gradient during the entire study period. Leaf emergence was monitored in spring every 2-3 days by the same observer. The developmental stages that were chosen were based on Vitasse (2009). The stages for beech were: (0) dormant buds, (1) swollen and/or elongated buds, (2) budburst and (3) at least one fully unfolded leaf. The stages for spruce were: (0) dormant buds, (1) expanded buds with new green visible behind the transparent cupule and (2) unfolded needles. The date of leaf unfolding was defined as the date when 50% of the buds had reached this stage.

End of the vegetative period

Leaf colouring and/or leaf fall in autumn were the criteria used to assess the senescence of beech leaves and therefore the end of the vegetative period. Senescence was defined as the time when 50% of the leaves of a sapling were no longer functional, i.e. either coloured or fallen, using the equation (Vitasse, 2009):

$$x_t = \frac{a_t \times (100 - \beta_t)}{100} + \beta_t$$

Equation 2 Calculation of the amount of senescent leaves

Where x_t is the percentage of coloured or fallen leaves for a sapling at time t, α_t is the percentage of coloured leaves at time t and β_t is the percentage of missing leaves at time t.

Norway spruce is an evergreen coniferous tree, so determining the exact end of the vegetative period is challenging. We therefore also monitored budset for both species three times per week from August to October in 2014 and 2015. The stages recorded were: (0) ongoing leaf development, (1) newly formed green and soft buds, (2) small and brown buds and (3) elongated (> 1 cm) and brown buds. The time of budset was recorded when 50% of the buds of a sapling had reached stage 3. The two proxies used for the definition of the cessation of primary growth were compared for beech to further understand bud formation and hardening before winter.

Length of the growing season

We defined the length of the beech growing season as the number of days between the date of leaf unfolding and the date of leaf senescence. We assumed that spruce ended its primary growth at beech senescence at the latest (see Results). The mean dates of beech senescence were thus calculated per site and year and assigned to the corresponding site and year of the spruce saplings. The length of the spruce growing season was consequently defined as the number of days between needle unfolding and the corresponding mean date of beech senescence.

2.2.3.7. Defining mean climatic variables

We took into consideration three mean climatic variables to explain the tree growth observed (Table 1): mean air temperature during the growing season (Tmean), the precipitation rate per day (Rain mm/day) and the soil moisture by measurements of volumetric water content in the soil (VWC, m3/m3) during the growing season between the months of May and July of each year (primary growth was mainly suppressed at the end of the summer (August) when the winter bud formation takes place; Figure 2-3. For the VWC measurements, we used sensors 5TM (Decagon S.A) placed at 20cm soil depth measuring at hour resolution. Soil moisture was also monitored weekly by means of a TDR probe (Time Domain Reflectometry) from May to September but these data were not considered for the mean climatic variables as they covered only the vegetation season 2013 and 2014 (See Figure S1).

2.2.3.8 Defining climatic extremes

There is no accurate definition related to the existence of an “extreme” (Stephenson, 2008). An established definition would be “an episode or occurrence in which a statistically rare or unusual climatic period alters ecosystem structure and/or function well outside the bounds of what is considered typical or normal variability” (Smith, 2011). A special report of IPCC (Murray and Ebi, 2012) defined an “extreme climate or weather event” or “climate extreme” as “the occurrence of a value of a weather of climate variable above (or below) a threshold value near the upper (or lower) ends of the range of observed values of the variable”. They clarify definition by stating that it includes absolute thresholds as extreme events and give the example of specific critical temperatures for health impacts.

In this study, we defined “extreme” as done by IPCC (Murray and Ebi, 2012) in a special report about managing the risks of extreme events. Therefore, we established impact-related thresholds in beech and spruce performance for saplings growing in the Swiss Jura. The thresholds were based in thermal-hydric requirements of each species. Vapor pressure deficit (VPD) is a measure of the difference between the pressure exerted by the moisture currently in the air and the pressure at saturation. We calculated the VPD, as a measure of the drying power of the air, as follows:

$$VPD = (1 - (RH/100)*SVP$$

Equation 3 Vapor pressure deficit

where RH is the relative humidity and SVP is the saturated vapor pressure for a given temperature.

A VPD threshold of 1.5 kPa was chosen to represent the approximate value above which stomata close in both species (Kurjak et al., 2012; Lendzion and Leuschner, 2008; Zweifel, Bohm, and Hasler, 2002). We used this VPD to represent the extremely dry air during an extended summer drought. Heat waves and cold spells were represented by two predefined temperature thresholds: above 25°C and below 5°C. The superior threshold (25°C during the vegetative period), was based on the thermal requirements of the two species (Gelete, 2010). Additionally, photosynthesis temperature-response curve performed in saplings growing at the extremes sites revealed that from 25°C the net photosynthesis starts to decline for both species. The lower limit was established at 5°C as it appears to be the limit for higher plant tissue growth (Körner, 2008). We thus calculated i) the accumulation of hours over threshold 25°C (AOT25) during the growing season and ii) the accumulation of hours below threshold 5°C (ABT5). We also calculated the number of events with thirty consecutive days without rain during the growing season at each site (P30).

2.2.3.9. Statistical analysis

General additive mixed effects models (GAMMs) were used to explore the response of aboveground tree biomass to changing climate over time. Briefly, GAMMs allow the change in mean biomass to follow any smooth curve, not just a linear form or a sequence of unrelated estimates. The form of the predictor function is the principal difference between the classical generalized mixed-effects models and GAMMs. All models were fitted according to a Gaussian distribution. We assessed five fixed effects (mean climate and extreme events) and one random effect (site nested into date) to take into account the inflation of the residual degrees of freedom that would occur if we were using repeated measurements within sites as true replicates. Several combinations of models were tested to find the most parsimonious model that would best explain the biomass response. We used Akaike's Information Criterion (AIC) (Akaike, 1973), R^2adj and the normality of residuals to compare the different models. As explanatory variables, we included soil moisture (m^3/m^3) along with the other "average" variables Tmean (^oC), Rain (mm/day) and the defined "extreme" temperature variables AOVPD1.5 (hours) and ABT5 (hours). According to Dormann's *et al.* review (2013), correlation coefficients between variables of $|r|>0.7$ is an appropriate indicator for when collinearity begins to severely distort model estimation. Therefore, for all models, we ensured that none of the explanatory variables were correlated between each other with a Pearson's correlation coefficient higher than 0.6, which gives us relatively good confidence that collinearity among predictors is not affecting our inference. Using effect size (Eq.4) allowed to quantify the impact of changing environmental conditions on the growth of the transplanted saplings. All statistical analyses were performed in R 3.1.2 using the mgcv package.

$$Effsize[i] = \frac{x[i]-\overline{x_c}}{\sigma x_c}$$

Equation 4 Standardized effect size

where x is the variable considered for each observation [i], $\overline{x_c}$ and σx_c are the mean and standard deviation of the control population.

We measured the interannual climatic variability along the elevational gradient to identify the factors influencing tree growth (Table 2-2). The wireless meteorological stations continuously recorded the climatic parameters needed for calculating the duration of extreme events. Data from nearby stations were used when necessary to complete the data set (Agroscope, 2016). The climatic variables were calculated individually for each sapling as a function of the growing season.

2.2.4. Results

2.2.4.1. Interannual climatic variability

The elevational gradient provided a distinct climatic gradient, with warmer and drier conditions towards the lower sites (Table 2-2). The mean annual temperature increased by an average of 5.5°C between the highest and lowest sites during the study period. Precipitation was 20 to 47% (data not shown) lower at the two lower sites compared to the donor site. The number of cold days (ATB5) was generally considerably higher at 1350 and 1010 m than at 570 and 395 m, and the number of warm days (ATO25) had the opposite trend. The lower altitudes had warmer conditions, but precipitation was not linear along the elevational gradient. Precipitation varied widely between years, and the saplings received considerable rainwater during spring and summer, despite the lower rainfall at the lower altitudes (especially in 2015).

Higher mean temperatures during the 2015 growing season led to a higher evaporative demand compared to the two previous years. For example, the accumulation of hours of VPD > 1.5 kPa (AOVPD1.5) in the growing season 2015, were 107, 220, 444 and 366, respectively at 1350, 1010, 570 and 395 m, but were 41, 33, 65 and 92 h, respectively, at the same sites in the previous year (Table 2-2). Mean temperature, however, was not always a good indicator of the dryness of the air. The mean temperatures at the lowest site during the 2013 and 2014 beech growing seasons were 16.5 and 16.1°C, respectively, but AOVPD1.5 was 2.5-fold higher in 2013 than 2014. Similarly, the mean temperatures at 570 m during the 2013 and 2015 beech growing seasons were 17.6 and 17.2°C, and the corresponding AOVPD1.5 were 160 and 444 h, respectively.

Comparison of soil moisture along the gradient

Globally, we observed a soil moisture gradient between the highest (1350 m and 1010 m) and the lowest (395 m and 570 m) sites (Table 2-2, see also supplementary Figure. S2-1). This gradient was notable during the growing season 2015.

2.2.4.2. Phenological responses to the simulated conditions of climate changeSpring phenology shifted along the elevational gradient for both species (Figure 2-3). Budburst was delayed towards the higher altitudes by averages of ~4.8 d 100 m-1 (± 0.16) and 4.0 d 100 m-1 (± 0.42) for beech and spruce, respectively. Autumn phenology, i.e. budset and leaf senescence, tended to occur later at the lower altitudes, but the pattern was more variable and less pronounced than for budburst. Overall, a decrease in elevation extended the growing season. The growing season length (GSL) at 1350 and 395 m over the three years averaged 115 ± 10 d (mean ± 1 SE) and 179 ± 3 d for beech and 113 ± 10 and 165 ± 3 d for spruce, respectively. Interestingly, the interannual variation of GSL was higher at the donor than the lowest site for both species, at ± 10 and ± 3 at 1350 and 395 m, respectively. The time lag between budset and senescence varied between years. For example, budset for both species in 2014 was very advanced compared to beech senescence, but budset and senescence in 2015 occurred at nearly the same time.

2.2.4.3. Growth responses to the simulated conditions of climate change

Exposure to the warmer and drier conditions at the lower altitudes for three years had contrasting effects on beech and spruce saplings from 1350 m (Figure 2-4). The growth of the spruce saplings did not differ significantly along the elevational gradient, but beech growth increased significantly between the donor and lower altitudes (Figure 2-4a). Growth increased more for spruce than beech at 1350 m but more for beech than spruce at 395 m. Standardizing the growth data by the growing season length produced similar patterns, which varied with altitude and species (Figure 2-4b).

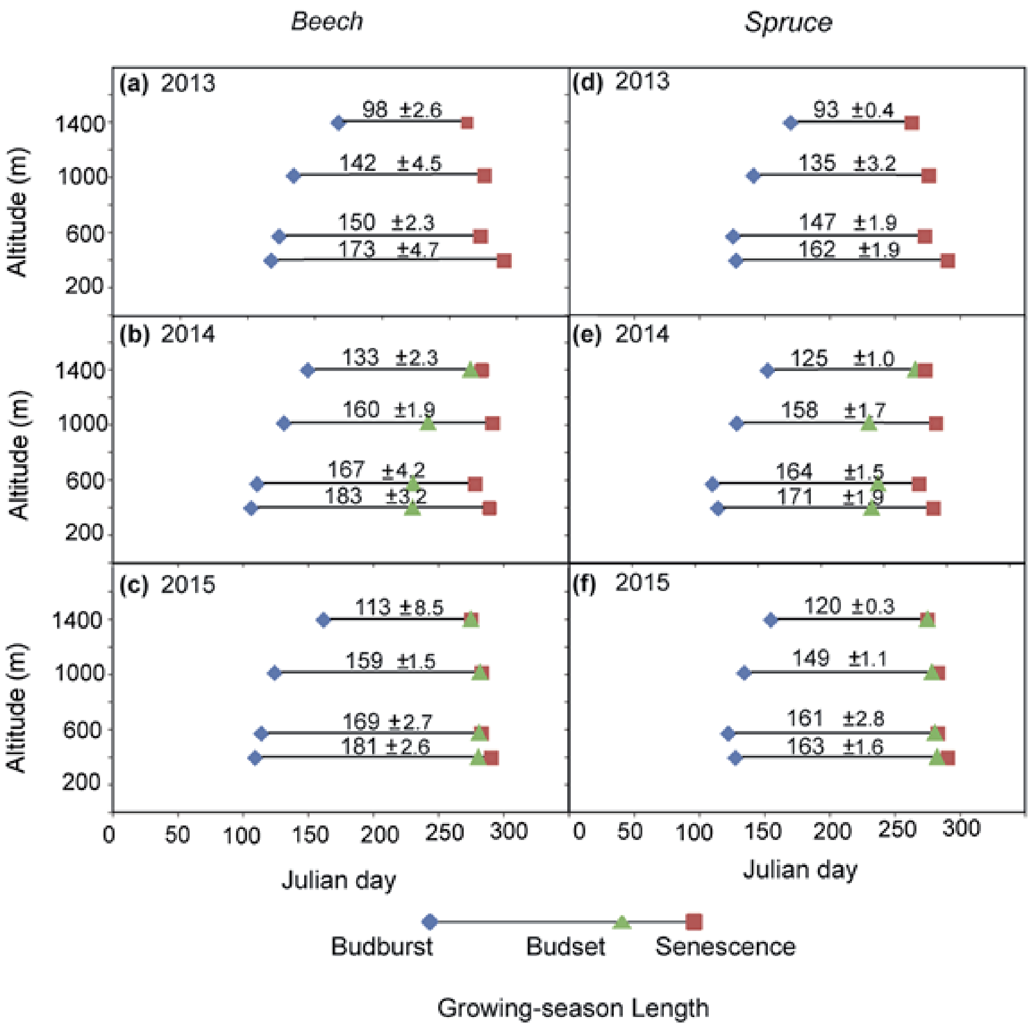


Figure. 2-3. Time of budburst, budset and leaf senescence for the beech and spruce saplings along the elevational gradient in 2013, 2014 and 2015. The length of the growing season was defined as the time between the dates of budburst and senescence, represented by the numbers above the black lines (mean ± 1 SE). We pooled the treatments (irrigated and non-irrigated), because irrigation did not have a significant effect on the phenological dates. The number of replicates for each species and altitude were thus 20 in 2013 and 2014 and 10 in 2015.

Table 2-2: Interannual climatic variability along the elevational gradient during the study period. Mean annual air temperature and mean growing season length (GSL) was calculated for each species (F.s., beech; P.a., spruce), site, altitude and year. Average volumetric water content in the soil (VWC, m^3/m^3), measured at hour resolution by sensors 5TM (Decagon S.A) placed at 20cm soil depth, during the period from May to July. For each length of growing season (GSL, days), we calculated the corresponding precipitation rate, number of hours with vapor-pressure deficit above 1.5 kPa (VPD > 1.5), number of hours of temperature below 5°C (T < 5°C), number of hours of temperature above 25°C (T > 25°C), number of hours of temperature below or equal to 0°C (T ≤ 0°C), and the number of events with more than 30 consecutive days without rainfall (P ≥ 30).

Altitude (m)	Year	Mean annual temp. (°C)	GSL (d)		May-July		Growing season													
					Soil VWC (m^3/m^3)		Mean temp. (°C)		Precipitation rate (mm/d)		VPD >1.5 kPa (h)		T < 5°C (h)		T > 25°C (h)		T ≤ 0°C (h)		P ≥ 30 (# events)	
			F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.	F.s.	P.a.
1350	2013	5.0	98	93	0.38	0.31	12.7	12.5	4.4	4.6	28	16	204	204	23	17	3	3	0	0
	2014	5.9	133	125	0.40	0.32	11.3	11.5	4.2	4.3	41	41	189	148	6	6	0	0	0	0
	2015	6.1	113	120	0.36	0.36	12.8	12.9	3.0	2.9	107	41	244	148	83	6	15	0	0	0
1010	2013	5.2	142	141	0.44	0.39	10.9	11.0	4.7	4.5	48	48	209	196	86	86	2	2	0	0
	2014	7.7	158	159	0.54	0.22	12.8	12.8	4.8	4.8	33	33	95	95	34	34	0	0	0	0
	2015	7.9	159	147	0.15	0.18	14.1	14.3	4.0	3.9	220	208	124	95	195	195	0	0	0	0
570	2013	9.8	152	148	0.20	0.20	17.6	17.7	1.9	2.0	160	160	35	35	186	186	0	0	1	1
	2014	10.9	166	164	0.17	0.20	15.7	15.9	3.3	3.2	65	65	15	15	115	115	0	0	0	0
	2015	10.6	174	166	0.10	0.17	17.2	17.5	3.8	3.2	444	441	10	6	447	447	0	0	0	0
395	2013	10.1	171	157	0.16	0.20	16.5	16.8	3.9	3.9	228	228	20	13	284	284	0	0	0	0
	2014	11.4	182	169	0.14	0.16	16.1	16.5	3.1	3.2	92	92	23	1	77	77	0	0	0	0
	2015	11.5	182	163	0.07	0.20	17.4	17.9	1.8	1.1	366	365	12	11	466	466	0	0	1	1

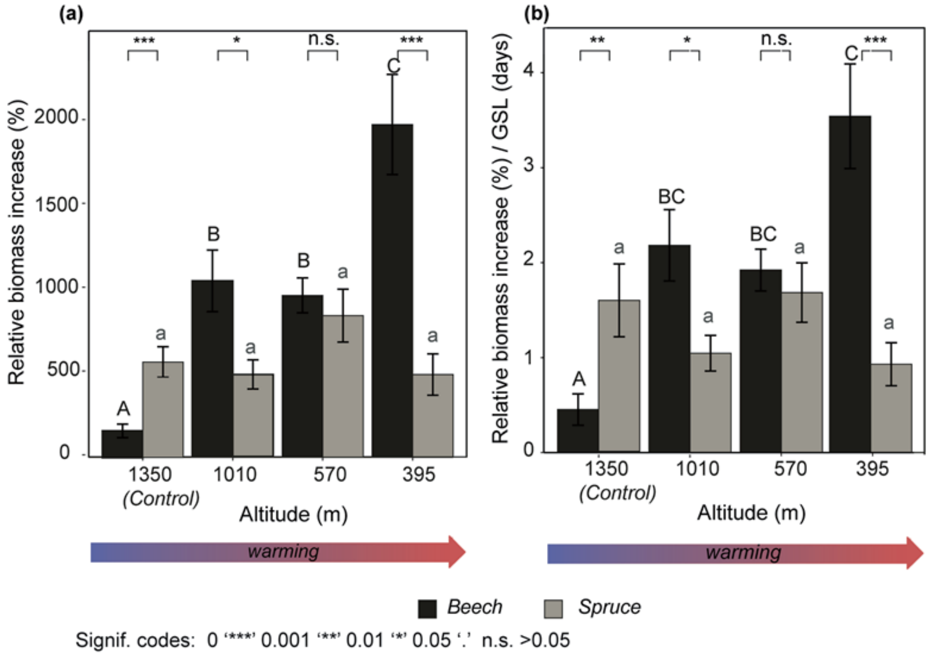


Figure 2-4 Relative increase in biomass after three growing seasons (2013-2015) at the control site (1350 m) and the three recipient sites. (a) Relative biomass increase and (b) relative biomass increase standardized by the length of the growing season, which varied along the gradient and for each species. The data for the irrigated and non-irrigated treatments were pooled because irrigation did not have a significant effect ($P < 0.05$) on the increase in biomass during this period. Different letters represent significant differences within a species, uppercase for beech and lowercase for spruce, along the gradient identified by an ANOVA. The asterisks represent significant differences between the means (± 1 SE) for each species at an altitude ($n = 5$) identified by a Tukey's post hoc test.

2.2.4.4. Effect size for tree growth under warmer and drier conditions

The transplantation to warmer and drier conditions generally had a positive impact on beech growth and a negative impact on spruce growth. The growth of the beech saplings from 1350 m differed significantly between 2013 and 2015 (Figure 2-5a), but the tree effect size was similar along the gradient in 2014, with an overall very positive effect relative to the donor site. We also observed a species-specific effect size at the same altitude. The effect size was mostly positive for beech but was negative for spruce (except in 2014). The irrigation treatment significantly mitigated the lower soil moisture, thus negative impact of warming on spruce growth in 2015 ($p = 0.001$).

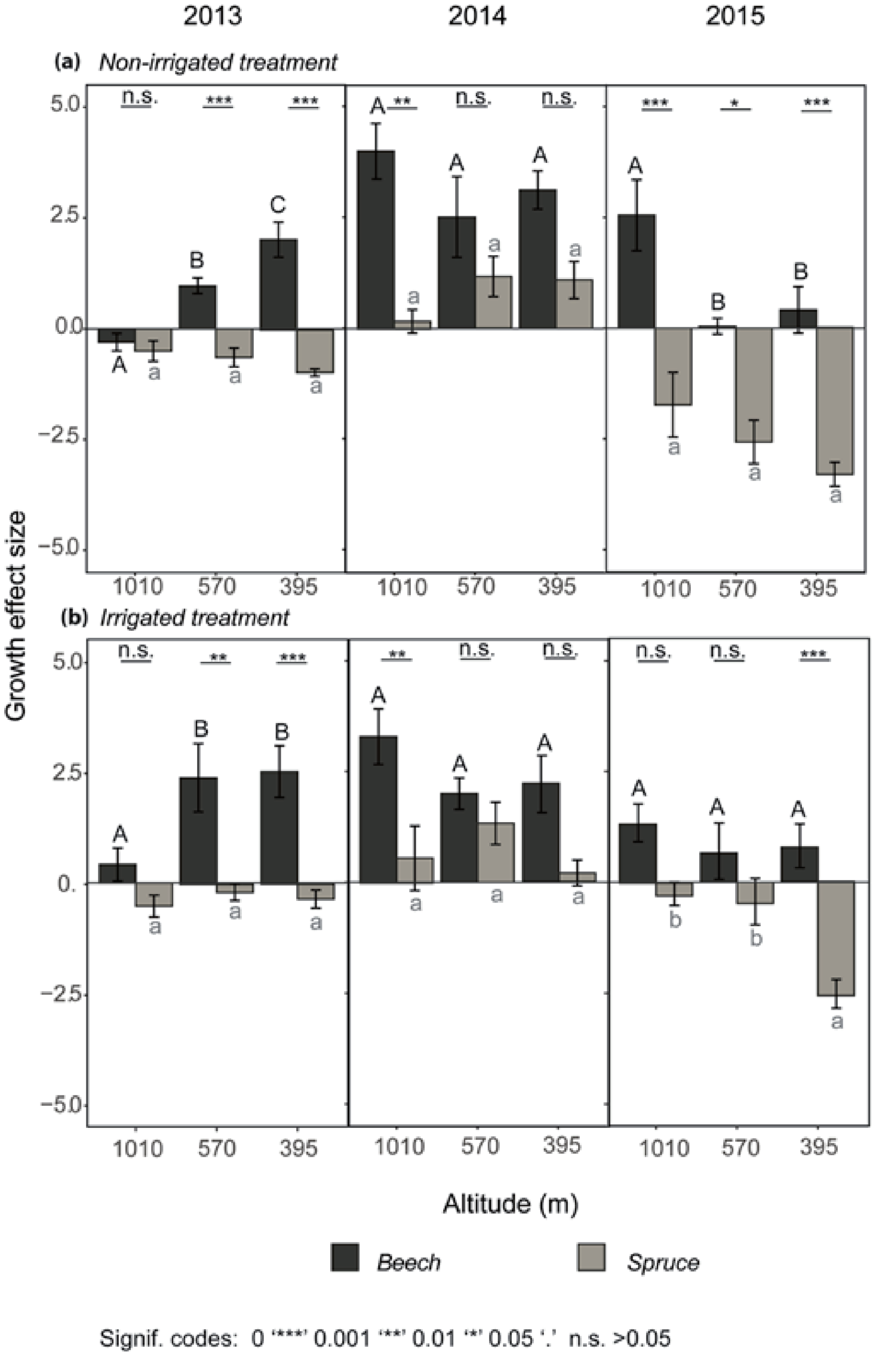


Figure 2-5 The effect size (1350 m as the control site) of the biomass increase along the gradient and throughout the three years for both species and for the non-irrigated (a) and irrigated (b) treatments. The larger the absolute value, the higher the impact of the local conditions on the relative biomass increase (standardized by GSL). Different upper- and lowercase letters represent significant differences within a species and year, respectively, identified by Tukey's post hoc tests. Significant differences between the species at each altitude are indicated by asterisks above each graph. The biomass annual increase (Δ % d-1) was calculated for each sapling.

2.2.4.5. Impacts of the extreme climatic conditions on sapling growth

The relationship between effect size for growth and the measured extreme conditions identified a distinct interannual climatic variability (Figure 2-6). The AOVPD1.5 was lowest in 2014, whereas 2015 had the longest period of dry air during the growing season, with more than 400 h of AOVPD1.5. The effect size was negative beyond 300 h of AOVPD1.5 for beech and beyond 100 h for spruce. The effect size for cold days was negative for beech in 2013, with growth decreasing as the number of cold days increased. In contrast, the effect size for spruce had no clear pattern, suggesting that other variables limited its growth. The irrigation treatment mitigated the negative effect of increasing VPD, especially for spruce during the dry 2015 growing season.

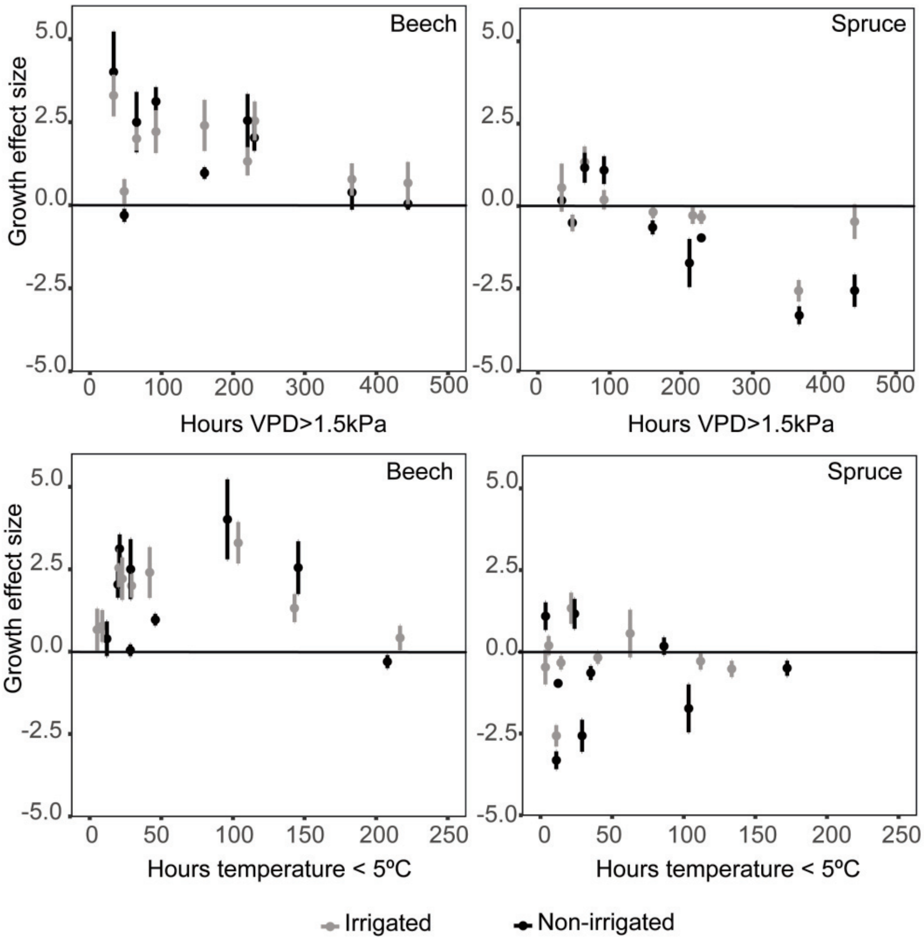


Figure 2-6 Effect size (1350 m as the control) for biomass increase compared to the number of hours of VPD > 1.5 kPa and the number of hours with T < 5°C for both species and treatments. The larger the absolute value, the larger the impact of the extreme on the increase in biomass (standardized by GSL and initial biomass). Each dot is the mean at a site ± 1 SE, with n = 10 for 2013 and 2014 and n = 5 for 2015.

The results from GAMM models of beech showed that the model including only mean variables (i.e. Rain, T_{mean} and Soil VWC; model 1) was the less accurate for explaining beech saplings' growth. However, when considering the extreme variables (i.e. ABT5 and AOVPD1.5), we observed an important increase in the explanation of beech biomass with a $R^2 > 0.75$ (model 2). Moreover, replacing the AOVPD1.5 by Soil VWC, in order to answer the question whether it is VPD and not soil moisture that best explains the growth patterns observed, showed a significant drop in the model performance for both species (models 3 and 6). Models explained in lesser extent the growth patterns of spruce than those of beech, with still a higher model performance when considering only the extreme climatic variables ($R^2 = 0.40$; model 5) and none of the models presented a significant effect of soil moisture (Table 2-3).

Table 2-3: Results from the GAMM model comparing means and extremes for beech and spruce. Biomass was used as the response variable explained by the climatic variables Rain (amount of precipitation per day during the growing season), AOVPD1.5 (number of hours during the growing season with VPD > 1.5 kPa), ABT5 (number of hours during the growing season with temperatures < 5°C), T_{mean} (mean temperature during the growing season) and Soil VWC (May-July). All explanatory variables were measured during the growing season. The model selection was based on the Bayesian information criterion (BIC), the Akaike information criterion (AIC) and log likelihood (logLik).

Beech

Model	Response variable	Explanatory variable	Signif var.	R-sq(adj)	df	AIC	BIC	logLik
1	sqrt (Biomass)	Rain	n.s.	0.15	10	140.9	152.7	-60.5
		T_{mean}	***					
		Soil VWC	n.s.					
2	sqrt (Biomass)	ABT5	***	0.77	8	110.9	120.3	-47.5
		AOVPD1.5	***					
3	sqrt (Biomass)	ABT5	***	0.50	8	124.8	134.2	-54.4
		Soil VWC	**					

Spruce

Model	Response variable	Explanatory variable	Signif var.	R-sq(adj)	df	AIC	BIC	logLik
4	Biomass	Rain	n.s.	0.34	10	267.6	278.1	-124.8
		T_{mean}	**					
		Soil VWC	n.s.					
5	Biomass	ABT5	***	0.40	8	256.8	266.2	-120.4
		AOVPD1.5	***					
6	Biomass	ABT5	n.s.	0.04	8	271.1	277.4	-126
		Soil VWC	n.s.					

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

2.2.5. Discussion

Transplanting saplings to lower elevation provides crucial insights on how trees in their juvenile life stage will face climate change. The analysis of growth response over contrasted climatic conditions from year to year allowed us to differentiate responses due to mean over extreme climatic conditions. Here, focusing on growth performances, we showed that beech saplings may benefit to warmer conditions and even drier conditions, whereas spruce seems already constrained by water limitation and air dryness (VPD) at low elevations of the Jura mountains. The higher sensitivity of spruce to increasing VPD, compared to beech, likely explain its limited growth at the lower elevations. This study highlights the importance to account for the effects of extreme climatic events when assessing the impact of climate warming on tree performance because these events are likely to deviate from the overall expected growth response to change in the mean climatic conditions.

Contrasting growth responses of beech and spruce saplings exposed to simulated climate change

Beech and spruce saplings' growth was differently affected when transplanted towards lower elevations during the three monitored years. In these drier and warmer conditions, beech growth was significantly enhanced, whereas spruce growth was similar to the growth at the native higher elevation site. The extension of the growing season may explain such tendencies. Our phenological observations showed a lengthening of the growing season towards the lower altitudes for both species, regardless of the proxy used for the end of the growing season (budset or beech senescence). The time lag between budset and senescence varied between years. For example, budset for both species was substantially advanced in 2014 compared to beech senescence, but budset and senescence occurred at nearly the same time in 2015, likely influencing the effective period of nutrient mobilization. The growing season was nonetheless consistently longer at the lower altitudes, which may partly account for the increase in beech biomass at the lower altitudes, as also suggested by Lenz *et al.* (2014), but does not account for the patterns of spruce growth. However, we found similar responses to warming when standardizing the growth data by the length of the growing season. Increasing the length of the growing season thus had a positive effect on tree growth only to a certain limit, which was species-dependent. This suggests that additional factors (e.g. air temperature, VPD, nutrient turnover) than phenological variations promoted beech growth while limiting spruce growth at lower elevations.

The continuum of soil-plant-atmosphere is critical for tree growth and their respective impacts in tree growth are very difficult to disentangle. However, depending on the site conditions there is always one factor being more limiting than the others. In this study, we aimed to find which factors explained best tree growth variation during years with contrasted climate and at different elevations. Because the two species are known to be sensitive to drought, one may expect that the transplantation of saplings to lower elevations with warmer and drier conditions would expose them to water deficits and limit their growth. However, we found that saplings were not water limited at the lower sites during the growing seasons 2013 and 2014, which was also supported by the ecophysiological measurements of leaf water potential (see supplementary Table S2-1).

To answer the question as to whether it is VPD and not soil moisture that best explains beech growth responses, we can compare the performance of models 2 and 3 and see that the replacement of the VPD explanatory variable (AOVPD1.5) by the soil moisture explanatory variable (Soil VWC) even decreases the overall performance of the model. Therefore, by including the soil moisture variable (model 3), we did not add any valuable information to the model. To sum up, model 2, which includes only "extreme variables" (ABT5, AOVPD1.5) best explained the growth response of beech with a R^2 of 0.77. All explanatory variables were significant; the residuals of the model followed a normal distribution, and AIC and BIC were the lowest compared to the other models. Generally, models explained in lesser extent the growth patterns of spruce than the growth patterns of beech, suggesting that other factors, not taken into account in this study, may explain part of spruce's biomass variance, and none of the models presented a significant effect of the soil moisture. Following the same logical procedure than beech, we found that model 5, including only the "extreme" variables, had a higher R^2 adj coefficient (0.4). In conclusion, GAMM models showed that soil moisture was not a significant factor explaining tree growth in this humid area of study. Interestingly, the "extreme" climatic variables, ABT5 and AOVPD1.5, significantly explained tree growth and even more than the models integrating exclusively "average" variables, such as mean temperature.

Growth rates between years revealed an interannual variability within species (Figure S2-2). The growth patterns of beech showed that in 2013 and 2014 tree growth was higher at the lowest elevation compared to the control site. In the growing season 2015, beech saplings grew at the same rate along the gradient except at 1010 m. In contrast, spruce showed a decrease in growth rate at lower altitudes compared to the control site, for years 2013 and 2015. Yet, interestingly higher growth rates were observed at lower altitudes (Figure S2-2 blue rectangle) during the growing season 2014, in spite of the lower soil moisture and lower amount of precipitation recorded during this year. Regarding atmospheric conditions, average temperatures during the growing season hardly differed in 2013 and 2014, but significantly differed in the amount of hours during which saplings were exposed to elevated VPD. It has been hypothesized that VPD may trigger stomatal closure to avoid an excess of water loss due to high evaporative demand of the air (Carnicer *et al.*, 2013). The degree of sensitivity of stomatal closure to elevated VPD is highly species-specific. Two main hydraulic functional groups have been distinguished in the literature depending on their strategies to cope with higher evaporative demand (Bond and Kavanagh, 1999; Carnicer *et al.*, 2013). Isohydric trees (e.g. spruce) avoid drought-induced hydraulic failure via stomatal closure, reducing the carbon assimilation (McDowell *et al.*, 2008; Carnicer *et al.*, 2013). This greater stomatal control maintains a relatively constant day-time leaf water potential (see supplementary Table S2-1). This allows them to prevent leaf water potential from falling below a threshold associated with cavitation (McDowell *et al.*, 2008; Pangle *et al.*, 2015) Typically, anisohydric tree species are associated with a higher ability to reverse embolisms leading to this narrower hydraulic safety margins compared to isohydric species (Carnicer *et al.*, 2013). Therefore, the difference in amount of hours with elevated VPD to which saplings were exposed in this study may cause important physiological responses and cannot be disregarded.

Our findings show that beech and spruce respond differently to high VPD. Leaf water potential of these species also revealed different patterns of regulating water transpiration (see supplementary Table S2-1). It is known from literature that species may exhibit intraspecific variation in degree of anisohydricity or isohydricity (Cocozza *et al.*, 2016) meaning that no species is strictly anisohydric or isohydric. However, in our study, beech did present a more anisohydric behavior than spruce (see supplementary Table S2-1). Therefore, we suggest that the different responses to elevated VPD could be linked to a difference in isohydric behavior.

This hypothesis is in agreement with a previous study carried out in a mixed forest in Pennsylvania, where the authors assessed the stomatal sensitivity to VPD of seven co-occurring temperate tree species (Meinzer *et al.*, 2013). They found that ring-porous species had a lower stomatal responsiveness to VPD than the diffuse-porous and coniferous species. In this paper, they suggest that these findings are linked to the isohydric behavior of the diffuse-porous and coniferous species, and the rather extreme anisohydric behavior in oaks.

Our results show that increasing VPD limits tree growth even before soil moisture starts to be limiting. Moreover, tree growth reduction is greater when both VPD and soil moisture reach limiting thresholds. The key finding of this paper is the demonstration of the different degree of sensitivity of these species to increasing VPD. The degree of isohydricity of these two species is likely to be linked to this different sensitivity.

Many other authors have hypothesized that contrasting growth responses between angiosperms and gymnosperms are due to the different sensitivities of their stomatal conductance to vapor pressure deficit, leading to contrasting growth responses (Martínez-Ferri *et al.*, 2000; Brodersen *et al.*, 2010; Carnicer *et al.*, 2013; Coll *et al.*, 2013; Meinzer *et al.*, 2013). In contrast, Martínez-Vilalta and Garcia-Forner (2016) have recently argued that water potential regulation and stomatal behavior are decoupled across species, so it remains today as an open debate whether isohydric and anisohydric trees lead to different responses to VPD. Further research in this topic is warranted.

The use of means and extremes for analyzing the impact of interannual climatic variability on the growth responses of the saplings

Growth responses are commonly correlated with mean temperatures (Bowman, Williamson, Keenan, and Prior, 2014; Jump, Hunt, and Peñuelas, 2006; Lévesque, Rigling, Bugmann, Weber, and Brang, 2014; Miyamoto *et al.*, 2010; Way and Oren, 2010). Mean annual temperature in our study differed by ca. 5.5°C between the native and lowest sites. Growth of saplings inhabiting high elevation (1350 m) are likely to be limited by temperature and we expect warmer temperatures to elicit positive effects on growth, in the absence of water stress (Way and Oren, 2010). Yet, growth was not enhanced for spruce when transplanted towards lower elevations in contrast to beech. This difference or response may root in different sensitivity of the two species to extreme climatic conditions, in particular to high VPD, which can be observed by analyzing growth response of the two species to interannual climatic variations.

For instance, cold spells at the beginning of the season were about twice as long in 2013 as in the other two years. Important precipitation deficits were further observed in the two lowest sites for more than 30 consecutive days (at 570 m in 2013 and at 395 m in 2015) compared to the native site. Thus, the mean interannual climatic variability of 2°C did not fully explain tree growth pattern because it hides substantial variations in the extreme climatic values and their frequency. The mean temperature during the growing season at the lowest site (395 m) was similar in 2013 and 2014, with 16.5 and 16.1°C, respectively, which was 3.8 - 4.8°C higher than at the donor site. The quantification of the effect size, however, identified contrasting growth responses for spruce between these two years: the effect size was negative in spruce's growth response to warming in 2013 whilst a positive effect size was found in 2014. Spruce saplings were subjected to 228 hours of AOVPD1.5 at the lowest site in 2013, which is more than twice as much as in 2014 (92 hours). An exceedance in VPD above the threshold of 1.5kPa can stimulate the closure of stomata in spruce (Kurjak *et al.*, 2012; Zweifel *et al.*, 2002). Assuming that there is a reduction in carbon up take, or even suppression depending on the degree of stomatal closure, these results suggest that spruce growth was limited by a large amount of VPD hours above 1.5 kPa in 2013 but not in 2014. Spruce is more sensitive to VPD increases than beech as it closes rapidly its stomata to reduce hydraulic conductivity before substantial cavitation occurs. By contrast, stomatal conductance in beech, an angiosperm, can remain high even at very high evaporative demands due to its higher capacity to reverse embolisms (Carnicer *et al.*, 2013).

Saplings' growth transplanted at 1010 m were likely constrained by temperature (Koch, 1958; Modrzyński and Eriksson, 2002). There was a strong contrasting growth response for beech between 2013 and 2014 at this site associated to the 2°C difference in mean temperature during the two growing seasons, i.e. warmer temperature during summer 2014 may have contributed to enhance beech growth. Conversely, the growth of the saplings at the lowest sites was likely strongly limited by water in 2015. Rainfall was

47% lower at 395 m, accompanied by an increase in AOVPD1.5 of 259 h compared to the donor site. A water deficit also occurred at the treatment level; irrigation had a lower negative impact on the growth response, i.e. the impact of a water deficit in 2015 for spruce was mitigated by the irrigation treatment ($P = 0.001$).

Difference in species sensitivities to extreme conditions due to different tolerance thresholds

The quantitative assessment of the impacts of an increase in the AOVPD1.5 and ABT5 on tree growth provided new insights for assessing climate-growth relationships. The higher sensitivity of spruce than beech to increasing VPD could account for the limited growth of spruce at the lower altitudes. We used a scatterplot of VPD vs. growth effect size to determine the approximate upper limit threshold for each species. The lower tolerance limit of spruce (~100 h) compared to beech (~300 h) partly could account for the contrasting growth responses between these species. The negative impacts of extreme conditions also depended on the conditions of the site, i.e. the impact on growth was not the same at 1010 and 395 m for the same duration of increased VPD, mainly due to the differences in the limitation of tree growth at higher and lower altitudes (temperature vs. water deficit). The absence of a clear response of spruce to the length of cold spells during the growing season suggested that spruce growth was limited by other variables. In contrast, beech growth was negatively affected by an ABT5 above 200 h during the growing season. The large range of responses of both species under this threshold of 200 h indicated that lower temperatures were not the main limitation to growth at the recipient sites. Our results suggest that both spruce and beech are limited by increasing air dryness but present different degrees of tolerance. The final GAMMs identified VPD as the main explanatory variables of the increases in biomass for both species. As second main explanatory variable the precipitation rate during the growing season (mm/day GS) was determinant factor for beech while the number consecutive days without precipitation during the growing season was for spruce. This leads to a differentiation between the main factor which is common for both species (VPD) and more species-dependent factors related to precipitation and consecutive days without rain.

We conclude that elevated vapor deficit limits tree growth. Our results showed that (i) a longer growing season due to induced-elevation warming (downward shift) could not fully account for the species-specific positive growth responses; (ii) the contrasting species growth responses were linked to different sensitivities to elevated vapor-pressure deficits; (iii) models could better account for the growth response to warming after incorporating extreme climatic events and their effects. On the top of that, for the first time we determined the threshold above which tree growth starts to decline for each species when soil moisture was not limiting. It is however likely that if soil moisture would have been lower during these high VPD conditions saplings growth would have been further reduced. Finally, the inclusion of climate extremes will likely improve models predicting species distribution under future climatic conditions (Zimmermann *et al.*, 2009). The potential extrapolation of this approach and results, through further research on adult trees, will be crucial for a better understanding of forest response to climate change and for adapting forest management to the predicted increase in duration and in frequency of extreme climate conditions.

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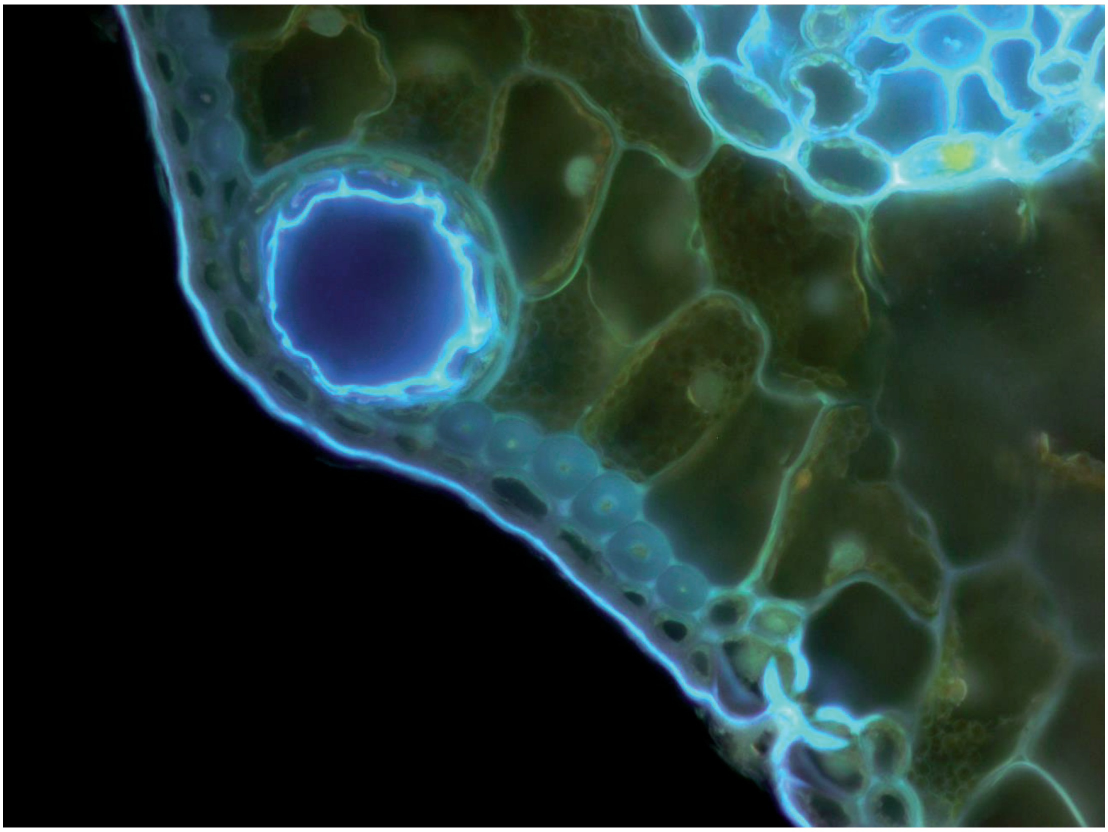
Chapter 3 Foliage plasticity



Picture: Microscope cross section (x60) of spruce needle. Close up of epidermis tissue and stomata

P. Sanginés, January 2014

“We sometimes underestimate the influence of little things”

(Charles W Chestnutt)

Stress factors eventually manifest themselves as macroscopic leaf symptoms (Günthardt-Goerg and Vollenweider, 2007) when physiological and structural alterations are presented in the targeted tissues (Vollenweider and Günthardt-Goerg, 2005). For instance, related changes on the cell and tissue structure have been associated to drought (Olmos *et al.*, 2007), ozone concentrations (Kivimäenpää *et al.*, 2001; Vollenweider *et al.*, 2013) and different light environments (Ashton and Berlyn, 1994). That is why leaf traits are a good proxy for tracking environmental changes and disturbances (Nicotra et al., 2010).

In the following chapter I present published results regarding the phenotypic plasticity of leaves and needles to simulated climate change. These findings give a further understanding of the responses of foliage at the structural level that will be further related, in chapter 4, to physiological functioning of leaves (e.g. photosynthetic capacity).

Article 2: **Responses of antinomic foliar traits to experimental climate forcing in beech and spruce saplings**

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3.1 Abstract

Global warming is predicted to have a strong impact on mountain ecosystems. Subalpine sylvopastoral systems are very sensitive to climate change, which puts their future sustainability at stake. These ecosystems are mostly dominated by spruce and beech, and therefore their regeneration abilities are critical in this context. The main objective was to characterize the short-term responses, of foliar traits in beech and spruce saplings through phenotypic plasticity with regard of actual scenarios of climate change. Therefore, we transplanted saplings from a cold environment at 1350 m a.s.l. in the Swiss Jura Mountains to three recipient sites at lower altitudes along an altitudinal gradient, in the experimental framework of a space-for-time substitution approach and measured morpho-anatomical foliar traits. The results revealed for beech an increase of xeromorphism through the increase of the cuticle thickness, vein network and smaller stomata, associated, surprisingly, to a higher leaf area. This antinomic response allowed beech to grow in warmer conditions while coping with an increase of evaporative demand during summer. Spruce did not present as much plasticity as compared to beech due to its inherent xeromorphic traits. Our findings further suggest a strong correlation between the timing of the leaf development, extreme conditions and tree growth. These contrasting strategies may lead to the competitive advantage of beech over spruce under climate change.

Keywords

Tree regeneration, altitudinal gradient, temperature, precipitation, foliar traits, xeromorphy, plasticity.

3.2 Introduction

The world's climate is expected to change significantly over the next century with an overall increase of temperature, changes in precipitation and greater frequency of extreme climatic events, leading to more climate variability (IPCC, 2002). Global warming is predicted to have a particularly strong impact on mountain ecosystems (IPCC, 2007). The observed trend in Swiss mountains during the 20th century for a 1.5°C rise in mean annual air temperature (Beniston *et al.*, 1997) is paralleled by increased event frequency of extreme summer temperature maxima and prolonged droughts (Schar *et al.*, 2004; Beniston, 2009). Throughout the current century, these tendencies are predicted to become more pronounced, and northern hemisphere temperate mountains will experience the most intensive temperature rise with a rate of warming typically of about two to three times higher (range +2.8 °C to +5.3 °C) than that recorded over the 20th century (Nogués-Bravo *et al.*, 2007). For example, at the tree line in the Swiss Alps, an increase of 2.5 °C of the mean air temperature has been observed during the 20th century, but a marked increase of 1°C was observed since 1980 (Gehrig-Fasel *et al.*, 2007). Alongside, predicted hot spells and a 30% diminishing precipitation during the growing season will intensify droughts in Central Europe

(Beniston *et al.*, 2007; C2SM, 2011). These will lead to changes in summer soil water availability, plant phenology, and growing season length, which would ultimately have repercussions for ecosystem distributions and functioning (Parry, 2000; FOEN/FSO, 2011). Temperate forests seem to benefit from warmer conditions with an increase in growth (Way and Oren, 2010), while Mediterranean and tropical forests are likely to respond with a decline in biomass allocation (Ogaya and Peñuelas, 2007; Way and Oren, 2010). This is partly because the growth of trees at high latitudes or altitudes is usually temperature-limited, while Mediterranean forests are mainly limited by water availability (Peñuelas *et al.*, 2004; Way and Oren, 2010).

In mountain ecosystems, plants are more sensitive to climate change and may respond to it in three different ways: (i) persistence in the modified climate, (ii) migration to more suitable climates or (iii) extinction (Theurillat and Guisan, 2001). The change in species range, and in particular upward shifts of species, has already been demonstrated (Walther *et al.*, 2005; Peñuelas *et al.*, 2007; Lenoir *et al.*, 2008; Wardle *et al.*, 2011). Nevertheless, ongoing climate warming may be too rapid for natural migration to successfully allow species to reach more suitable habitats (Rice and Emery, 2003). In this context, individual plants would need to remain in their current location and withstand environmental changes (Bonn, 2000; Petriccione, 2005; Allen et al., 2010) through short-term phenotypic plasticity (Vitasse *et al.*, 2010)**,** defined as the ability of a genotype to develop different phenotypes in response to environmental changes (Bradshaw, 1965). These short-term responses of plant traits will be important in assessing plants' persistence under climatic change (Nicotra et al., 2010) as acclimation is the first step prior adaptation (i.e. genetic changes in morphological and physiological traits). Through acclimation, individual plants compensate the decline in performance due to a stress factor by adjusting their morphology and physiology (without genetic changes), and usually occurs within days to weeks within the lifetime of an individual (Lambers *et al.*, 1998). Foliage organs, particularly during foliage development, are thus most responsive to environmental constraints, especially regarding the water availability (Dickson and Tomlinson, 1996; Fink, 1999). Hence, some desert plants have a dry and wet season foliage with distinct morphology (Fahn and Cutler 1992) and a spring drought treatment caused the formation of smaller and more xeromorphic needles in *Pinus canariensis* (Grill *et al.* 2004). Once the foliage organs and their tissues have completed elongation and differentiation however, only the cell physiology and structure remain responsive to primarily acute environmental stress (Fink, 1999; Wyka *et al.*, 2007). Moreover, Wyka *et al.* (2007) tested the ability of spruce and beech foliage to shade acclimation during two years of study (2003 and 2004).

Leaf functional traits have been proven to provide good indicators for tracking the impact of environmental changes on plants (Nicotra et al., 2010). Indeed, leaf phenotypic plasticity has been recognized as a key process by which plants cope with rapid climate changes (Nicotra *et al.*, 2010; Vitasse *et al.*, 2010; Stojnić *et al.*, 2015). However, the extent to which phenotypic plasticity manifests in response to new climate conditions remains unclear. It was shown that some species will be affected more than others in terms of growth due to differing sensitivities to stress conditions (Davis and Shaw, 2001). Ultimately, foliar trait changes will affect tree performance and physiology (Niinemets *et al.*, 1998) and this could lead to changes in wood production and even forest species composition, potentially impacting important sectors of forest economy, as being demonstrated in the Swiss Jura mountains (Peringer *et al.*, 2013). In this region, sylvopastoral systems serve as a traditional source of forage and timber in the subalpine vegetation belt, but their sensitivity to historic land-use and climate change puts their future sustainability at stake (Buttler, 2014). The two dominant species in this ecosystem are *Picea abies* (L.) Karst (Norway spruce) and *Fagus sylvatica L.* (European common beech). Beech and spruce are mesomorphic species (Ellenberg, 1988) with foliage showing mesomorphic and xeromorphic traits, respectively. Beech is a anisohydric deciduous species (Zang *et al.*, 2014) that presents a large geographical distribution in Europe due to its tolerance to a wide range of environmental conditions, but its water requirement lies between 550-2000 mm, thus defining this species as sensitive to drought (Gelete, 2010). Spruce, an isohydric evergreen conifer (Zang *et al.*, 2014), naturally occurs in low temperature regions (Modrzyński and Eriksson, 2002) and is also susceptible to summer drought making it more predispose to bark beetle attack (Netherer *et al.*, 2015).

Stress can be defined as “an environmental factor that reduces the rate of some physiological process (e.g., growth or photosynthesis) below the maximum rate that the plant could otherwise sustain” (Lambers *et al.*, 2008). In this context, plants present a wide range of responses to stress, which scope from alterations to gene expression and cellular metabolism to changes in growth rate and plant productivity through macro and micro-morphological symptoms (Fink, 1999; Vollenweider and Günthardt-Goerg, 2005; Shao *et al.*, 2008). Körner *et al.* (1986) showed that leaf size and SLA decreased with higher altitudes, as a consequence of lower temperatures, which has been confirmed by several other studies (Scheepens *et al.,* 2010; Woodward 1983). Typical and in some instance specific changes to the cell and tissue structure are observed in response to stress factors such as drought (Olmos 2007; Vollenweider *et al.* 2016), elevated ozone levels (Kivimaenpaa *et al.*, 2001) or shading of foliage (Ashton and Berlyn, 1994)**.** Moreover, plants growing in water-limited environments, either as a result of rain deficit (e.g. chaparral), winter drought (e.g. cold habitats), physiological dryness (e.g. saline soils, acid bogs) or high rates of evaporation, usually exhibit xeromorphic adaptations such as e.g. thick cuticula, thickened epidermis and hypodermis, sunken stomata or compact mesophyll (Khan, 2002)**.**

In this study, the main objective was to characterize the phenotypic plasticity of foliar traits in spruce and beech saplings, with regard to actual scenarios of climate change. Therefore, we transplanted saplings from one population per species growing in a cold environment at 1350 m a.s.l. in the southern Swiss Jura mountains to three recipient sites at lower altitudes along an altitudinal gradient. We thus exposed sapling to warmer and drier climate conditions for 2 years (2013-2014), with contrasted weather during the vegetation season, using a space-for-time substitution approach (Körner, 2003b). We assessed the species-specific foliar plasticity measuring macro- and micro-morphological foliar traits. We hypothesized that i) leaf traits of spruce and beech would develop more xeromorphic characteristics in response to transplantation and warmer and drier conditions as a consequence, indicating an overall higher evaporative demand, and ii) the phenotypic plasticity of the mesomorphic beech leaves would be higher, as compared to that of already xeromorphic spruce needles.

3.3 Materials and methods

3.3.1 Study sites and altitudinal gradient

An altitudinal gradient was used along the south-facing slope of the Jura Mountain in Switzerland to simulate climate change impacts. Specifically, the increase of temperature and the decrease of precipitation were achieved through a down-hill transplantation experiment, i.e. using a space-for-time substitution approach (Körner, 2003b). It is known that there is also a decreasing in total atmospheric pressure and partial pressure of gases with elevation. However, the observed decrease of partial pressure of CO_2 does not occur in isolation but together with that of other gases, and its direct influence on plants may be diminished or enhanced by three factors as cited by Körner (2003b): “(1) the oxygen partial pressure decreases as well, hence photorespiration, (2) “thinner” air allows CO2 molecules to diffuse faster through stomata and the intercellular spaces in the leaf and (3) the air temperature drops and so does leaf temperature, which counteracts (2) and enhances (1)”. So, whenever molecular gas diffusion comes into play, reduced temperature (slowing diffusion) is counteracting the effect of reduced pressure alone (increasing the rate of diffusion) (Körner, 2007). Therefore, we estimate that other factors, such as temperature regimes, have a superior effect on the leaf morphology than changes in partial pressure of gases. As morpho-anatomical changes are integrative in nature, an eventual effect of changing air pressure is likely to be too weak to have any significant consequences. Hence, changes in the partial pressure of gases are primarily a concern with regard to leaf gas exchanges.

A detail description for site selection is given in Gavazov *et al.* (2014). Briefly, the donor site was located at Combe des Amburnex (N 46°54’, E 6°23’, 1350 m a.s.l) within the boundaries of the Parc Jurassien Vaudois natural area. In this subalpine area, Norway spruce is generally the dominant tree species of the pasture-woodland landscape, especially on shallow humic soils at stony sites, while on deeper soils beech is also present, together with spruce. At lower altitudes spruce expends its distribution beyond the limits of natural range, favored over beech by forest managers due to economic reasons.

The region is characterized by an oceanic climate, with a mean annual temperature and rainfall of 4.5ºC and 1750 mm, respectively, and presents a permanent snow cover from November to May (Gavazov *et al.*, 2013, 2014). The three recipient sites were situated at: St.-George at 1010 m a.s.l. (N 46°52’, E 6°26’), Arboretum d’Aubonne at 570 m a.s.l. (N 46°51’, E 6°37’) and Les Bois Chamblard at 395 m a.s.l. (N 46°47’, E 6°41’). Thus, by establishing Amburnex as the control site with native climate conditions, we covered three possible IPCC warming scenarios, according to Gavazov *et al.* (2013, 2014): moderate at 1010 m a.s.l (on average +2°C and 20% rainfall reduction), intermediate at

570 m a.s.l (+4°C and 40% rainfall reduction) and extreme at 395 m a.s.l (+5°C and 50 % rainfall reduction). These data for each site were spatially interpolated from nearby weather stations (source: MeteoSwiss, Agroscope 2016). The temperature increments were chosen in accordance with the moderate A1B and the intensive A2 climate change scenarios outlined in the IPCC report (Meehl *et al.*, 2007) and the predicted concurrent decrease in precipitation for temperate regions (Frei *et al.*, 2006; C2SM, 2011).

3.3.2 Experimental Design

Saplings of beech and spruce were collected in spring 2012 in the Amburnex site in two areas that were limited enough in surface to assume that the saplings belonged to a same population (provenance). Saplings of beech were taken in a clearing within a beech dominated forest, while spruce saplings were taken in a spruce dominated wooded pasture. Saplings of similar size (average height of 33 cm for beech and 31 cm for spruce) were dug out with their intact soil, transplanted in 20 L pots and reallocated in the four common gardens, respectively at each altitude within the same day.

The experimental design is a generalized block design with repeated measures. The four blocks, considered as fixed, are the four study sites at altitudes 1350, 1010, 570 and 395 m a.s.l. Within each block, the two levels of the factor treatment (irrigated and non-irrigated) and the factor species, also with two levels (beech and spruce), each combination with 10 repetitions, were allocated at random to the 20 pots of the block. The aim of the irrigation treatment was to disentangle the rainfall effect and study the responses of leaf to temperature *per se*. In order to achieve this, at each recipient site, water was added weekly during the growing season in accordance to the rainfall at the donor site. The factor year is repeated within pot and has 2 levels (2013 and 2014). The experimental unit is a pot with one plant. Within each site, pots were placed randomly on a grid with 0.3 x 0.9 m spacing to avoid light competition. Pots were pushed into the soil and, in order to reduce soil water evaporation and to avoid roots' breaking through the pot, a geotextile cap was placed on top and at the bottom of the pot. All sites were equipped with wireless meteorological stations (Sensorscope, Climaps. available at: https://www.climaps.com/.) that recorded continuously climate parameters (i.e. precipitation and air temperature) and allowed to calculate vapour pressure deficit

(VPD). VPD is highly sensitive to air temperature increases (Novick *et al.*, 2016) and reflects the air dryness and therefore the potential water loss through stomatal conductance. When necessary, data from nearby stations were used to complete the data set (Agroscope, 2016).

3.3.3 Leaves and needles sampling and morpho-anatomical observations

In order to assess the phenotypic plasticity of foliar traits in spruce and beech saplings with regard to actual scenarios of climate change, foliar macro-and micromorphological traits were comprehensively assessed. Therefore, triplicate samples were collected on August 6th 2013 and 4th 2014 at the donor as well as recipient sites. Only undamaged and fully expanded foliage was collected, avoiding second-flush leaves. Macro-morphological measurements (i.e. lamina fresh and dry weight) were immediately performed in the laboratory on the day following sampling. Foliage fresh weights and surface (LICOR-3100 area meter) were measured prior to oven-drying at 65ºC until constant mass. Two discs of beech leaves and three mid-segments of spruce needles were fixed by infiltration using 2.5% glutaraldehyde buffered at pH 7.0 with 0.067 M Soerensen phosphate buffer and stored at 4 ºC until further processing. In beech leaves, the intercellular volume (Vair) was determined using Va/Vf (%), where Va (ml) is the leaf air volume and Vf (ml) the total volume of fresh leaf (Koike, 1988). Within beech leaves, the veinlet network was assessed, mounting two fresh unstained disks (7mm diameter) per beech sample in water and observing in diascopic light using 5x objective and a Leica DMRB microscope. Micrographs were taken using the Lumenera INFINITY 2 ANALYZE camera and Lumenera software (Lumenera Corporation, Canada). Light intensity and color settings were standardized with a view to high quality and comparable images (i.e. exposure= 13.42, gain=3.16, gamma=2.07, saturation=0.91 and hue=8). The transparent network of 2nd to 4th order veinlets *versus* green assimilative patches in one selected zone per micrograph was quantified using color image analysis and adapting procedures from the WinCELL software (Regent Instruments Inc., Canada). The size and density of stomata was determined using nail varnish imprints, the 5x and 40x objectives and aforementioned microscope, imaging system and software. In spruce needles, only the amount of stomata lines per needle segment was estimated, using a binocular. For both species, the size of tissues and tissue composition of foliar organs were assessed, using hand-microtomed 30 μm (spruce) and

70 µm (beech) cross-sections of hydrated and fixed foliar material. Sections were mounted in water, either directly or after staining for contrasting cuticula against cell wall material, using either Sudan Black B (modified according to Gerlach, 1984) for spruce needles or Alcian Blue (modified according to Arend *et al.,* 2008) for beech leaves. Sections were observed and photographed using the 10x to 100x objectives and aforementioned microscope and imaging system. The width of cuticula and leaf tissues was directly measured on the microscope using the Lumenera software. Due to the anisotropy of needles, the tissue area in the case of spruce samples was determined, using image analysis and the measurement tools in the Adobe Photoshop software (Cs5, version 12.0.0.0, Adobe Systems Inc.). Altogether, twenty morpho-anatomical variables were measured on beech leaves, and thirty-eight on spruce needles. Detailed description of variables is provided in Table 3-1.

3.3.4 Growth estimation

In order to estimate the overall growth of each sapling, dasometric measurements of the stem and four main branches were recorded. For each individual, the four longest branches were initially selected and identified to allow a continuous monitoring. Stem and branch diameters (basal and apical) were measured in mm using an electronic caliper (accuracy of 0.01 mm) while stem and branch length was determined with a measuring ruler, from the base to below the winter bud (accuracy of 0.1 cm). The total volume was calculated geometrically by considering these components as circular truncated cones. In order to remove tree size effect, volume increments (final volume – initial volume) were standardized by the initial biomass at the beginning of the growing season, obtaining a relative volume increment for each sapling during two growing seasons (2013 and 2014).

Table 3-1 Description of macro and micro-morphological variables measured on foliage of spruce and beech

Study	Species	Variable	Acronym-Units
Macro-morphology	Beech	Leaf mass per area	LMA [mg cm^{-2}]
		Leaf area	La [cm^{2}]
		Intercellular space	Vair [%]
	Spruce	Leaf mass area	LMA [mg mm^{-2}]
		Needle length	L [mm]
		Frequency of stomata lines	Lst [lines mm^{-1}]
Micro-morphology	Beech	Vein network	Wall [%]
		Stomata density	Std [mm^{-2}]
		Stomata area	Sta [µm^{2}]
		Upper cuticle	S.C. [µm]
		Upper epidermis	S.E. [µm]
		Palisade parenchyma I	P.P.I [µm]
		Palisade parenchyma II	P.P.II [µm]
		Spongy parenchyma	S.P. [µm]
		Lower epidermis	I.E. [µm]
		Lower cuticle	I.C. [µm]
	Spruce	Cross-section	Cross.sect. [mm^{2}]
		Cuticle thickness	C [µm]
		Epidermis	Ep [%]
		Resin ducts	RC [%]
		Mesophyll	M [%]
		Endodermis	En [%]
		Transfer	Tr [%]
		Phloem	Ph [%]
		Xylem	Xy [%]

3.3.5 Statistical analysis

We used repeated-measures three-way ANOVA’s to detect the effects of altitude (1350, 1010, 570 and 395 m a.s.l.), treatment (TP and T) and year (2013 and 2014) on the macro- and micromorphological adjustments of leaves and needles at organ, tissue, cell (stomata) and subcellular (cuticula) level to changing environmental conditions. Focus was put on traits potentially responsive to varying water availability (Fahn and Cutler, 1992). The full data set was visually inspected for normality and homoscedasticity of variance prior to analysis and log-transformed if necessary. All final models were checked for homogeneity of the residuals. The same data were also explored with a correlation matrix in order to detect correlations between morpho-anatomical response variables (Supporting Information Figure S3-3 and Figure S3-4). Based on these two univariate methods, we selected the best response variables to be considered in a further step, following two criteria: 1) avoiding as much as possible collinearity between response variables in further statistical analyses and 2) considering their importance for a good understanding of morpho-anatomical plasticity and for answering the hypotheses. Results from ANOVA's for the twelve traits variables which have been retained for both beech and spruce are given in supplementary Supplementary Information Tables S2-1 and S2-2.

To further understand the potential effects of changing climatic conditions with varying altitude on the foliar morphology and anatomy, we compared the multivariate data set of foliar traits with the more extreme temperature conditions during warm and cold spells, as well as with rainfall and vapor pressure deficit (VPD) during the vegetation period. Hence, proxies in the form of the amount of days above and below a given temperature threshold, during the period elapsed between budburst and sampling (mid-August), were calculated for each sapling. This sampling period largely covered that of foliage elongation and differentiation at all sites. Beech always presented an earlier budburst than spruce, especially at lower altitudes. We selected two specific temperature thresholds to represent extreme conditions considering the thermal requirements of each species (Gelete, 2010): (i) number of days during which the daily maximum temperature was above 25 ºC (extreme warm conditions) and (ii) number of days during which the daily minimum temperature was below 5 ºC (extreme cold conditions). We assessed the relationships between selected morphological and anatomical traits of leaves from tree saplings and the environmental variables by means of RDA ordination, using as explanatory variables extreme warm conditions (1), extreme cold conditions (2), rainfall (3) and number of days with VPD higher than 1.5 kPa (4) during the leaf development. Canonical axes and explanatory variables were tested by means of ANOVA's. Separate analyses were performed for each species and year after observing a clear differentiation between years 2013 and 2014. All analyses were carried out using R v3.2.3 (R Core Development Team, 2016) and vegan package.

Finally, we quantified the degree of plasticity of traits through a Phenotypic Plasticity Index (Valladares *et al.*, 2000) based on the maximum and minimum means of the traits [(maximum mean-minimum mean)/maximum mean]. This index enables the standardization of the phenotypic plasticity of different traits in a certain species, and thereby facilitates their comparison (Valladares *et al.* 2006). Since it assumes normality, all variables were transformed using the “Hellinger method”. This index ranges from 0, implying no plasticity, to 1, which represents the maximum plasticity. We calculated the index for each leaf and needle variable and then grouped these variables in three indices for each species; the first index included only macro-morphological traits, the second index included the micro-morphological traits and the third index included all the studied traits (see Table 3-1). These indexes were calculated as the average of the included variables of each group and enabled us to assess the degree of plasticity between these two morphological levels. Non-parametric, Wilcoxon 2- sample test was then applied in order to compare the mean plasticity values of the different groups of traits.

3.4 Results

3.4.1 Overall climatic conditions at each site during the leaf development

At the donor site at 1350 m a.s.l., giving the long-term average of mean annual temperature and precipitation (4.5°C and 1750 mm, respectively), the years 2013 and 2014 were warmer than the long-term average with 5.0 and 5.9°C, respectively, while the mean annual precipitation was similar in 2013 with 1706 mm, but not in 2014, which was much drier with 1124 mm (Table 3-2).

The transplantation downhill in the three recipient sites exposed the saplings to an expected increase in ambient air temperature and reduced precipitation (Table 3-2). In 2013, the increase in mean temperature during the vegetation period, compared to the donor site, was +0.5, +6.7 and +6.3°C at 1010, 570 and 350 m a.s.l., respectively, with an equivalent reduction in precipitation of -0.1, -44.4 and -19.7 %. In 2014, the changes in mean temperature for the same period and sites were +2.3, +5.6 and +6.3°C, while the associated changes in precipitation were -18.2, -12.3 and -26.2% compared to the donor site. When considering the difference between the donor site (1350 m a.s.l.) and the lowest recipient site (395 m a.s.l.) during the two growing seasons 2013 and 2014, the mean temperature increase was 6.3ºC at the lowest site, and at the same time precipitations were reduced by 20 to 26% compared to the donor site. An inter-annual variability in climatic conditions within each site during the leaf development was detected, with an inverse climatic trend between the season of spring and summer in 2013 and 2014. While the onset of spring 2014 was characterized by warmer and drier environmental conditions compared to spring 2013, in summer 2014 the weather was generally milder and wetter as compared to 2013, despite mean temperature in 2014 being higher than in 2013.

At the highest site, a sum of 86 to 107 days with daily minimum temperatures below 5 ºC were recorded during the two vegetation periods 2013 and 2014 (Table 3-2). These values decreased consistently along the gradient with up to six to thirteen days at the lowest site. While at the highest site there were fewer than 10 days with daily maximum temperatures above 25ºC, this reached a maximum of 46 days at the lowest site in 2013. We defined VPD as the mean of the ten highest daily values during the growing season. Some studies report that the closure of stomata of Norway spruce starts between 1 and 1.5 kPa (Zweifel *et al.*, 2002; Kurjak *et al.*, 2012) but, up to date, the determination of this VPD threshold remains limited to some plant species. During this study, the threshold of 2 was reached at 570 m in 2013, and in both years at the lowest site.

3.4.2 Variations of leaf traits under changing environmental conditions

Foliar response of beech

A significant site effect (repeated anova, p-value < 0.05), i.e. altitude, was found for half of the variables considered, while a strong year effect (p-value < 0.01) was observed for most variables except for stomata density and area (Supplementary Information Table S3-1). However, no significant effect was found for the irrigation treatment (T and TP), therefore for the following analyses these data were pooled together to increase the robustness of the analysis.

Table 3-2 Temperature, precipitation and vapor pressure deficit at the four altitudes along the altitudinal gradient in 2013 and 2014

Altitude [m]	Year		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean annual T (°C)	Mean T during the GS (°C) (Δ °C)	Number of days during GS with daily minimum T < 5 °C	Number of days during the GS with daily maximum T > 25 °C	Total annual P (mm)	Total P during the GS (mm) (Δ mm, %)	VPD during the GS, mean of the 10 daily max. values (KPa)
	2013	T	-2.8	-6	-0.3	**3.8**	**4.6**	**10.6**	**15.4**	**12.8**	**9.8**	9.4	2.5	-0.3	5	9.5	107	7			1.08
1350	2014	[°C]	-0.3	-0.6	2.1	**4.9**	**6.6**	**12.3**	**12.2**	**10.7**	**10.3**	8.6	4.9	-0.9	5.9	9.5	86	1			1.74
	2013	P	112	105	100	**172**	**200**	**129**	**134**	**52**	**195**	208	214	85					1706	882	
	2014	[mm]	81	65	32	**65**	**103**	**95**	**197**	**127**	**63**	134	113	49					1124	650	
	2013	T	-1.1	-3.7	0.4	**5.9**	**7.4**	**12.3**	**13.5**	**12**	**8.8**	7.2	0.1	-1	5.2	10 (+0.5)	46	21			1.9
1010	2014	[°C]	0.5	0.8	4.3	**7.5**	**9**	**14.5**	**14.2**	**13.1**	**12.3**	9.9	5	0.7	7.7	11.8 (+2.3)	42	7			1.93
	2013	P	88	113	112	**179**	**201**	**71**	**200**	**74**	**158**	183	229	149					1757	883 (-0.1)	
	2014	[mm]	168	263	72	**84**	**144**	**34**	**248**	**155**	**103**	163	146	84					1664	768 (-18.2)	
	2013	T	0.7	-0.5	3.4	**9.4**	**10.8**	**16.2**	**21.4**	**20.6**	**19**	11.3	4.4	0.8	9.8	16.2 (+6.7)	22	29			2.06
570	2014	[°C]	3.4	3.6	7.4	**10.7**	**12.2**	**17.8**	**16.9**	**16.7**	**16.4**	13.5	7.8	4.5	10.9	15.1 (+5.6)	10	22			1.94
	2013	P	50	98	125	**157**	**160**	**70**	**39**	**0**	**64**	172	150	165					1250	490 (-44.4)	
	2014	[mm]	134	231	62	**70**	**106**	**99**	**166**	**105**	**24**	111	113	47					1268	570 (-12.3)	
	2013	T	1.9	0.5	3.9	**9.7**	**11.3**	**16.6**	**21.5**	**19.6**	**15.8**	12.3	5.5	2.1	10.1	15.8 (+6.3)	13	46			2.4
395	2014	[°C]	3.8	4.3	7.6	**11.4**	**13**	**18.8**	**18**	**17.4**	**16.4**	13.4	7.8	4.4	11.4	15.8 (+6.3)	6	19			2.06
	2013	P	57	97	116	**132**	**135**	**92**	**169**	**54**	**126**	194	148	131					1451	708 (-19.7)	
	2014	[mm]	102	152	46	**53**	**94**	**61**	**148**	**96**	**28**	113	107	39					1039	480 (-26.2)	

Months considered for vegetation period are in bold. T stands for temperature, P for precipitation, GS for growing season and VPD for vapor pressure deficit.

Morphological changes that have been observed on the leaves along the gradient during the growing seasons 2013 and 2014 are given in Figure 3-1. Overall, more variables presented significant responses between sites in 2013, while in 2014 the variables were less responsive. Particularly in 2013, leaf area and lower cuticle increased towards lower altitudes, while stomata area and upper epidermis decreased along the gradient. Similar but less consistent trends of increase towards lower altitudes were observed for vein network and palisade parenchyma. Leaf mass per area, stomata density and spongy parenchyma increased their values from the highest site up to the intermediate lower site at 570 m but then significantly decreased at the lowest site. In 2014, the trend was similar to 2013 for leaf mass area, while other trends appeared for intercellular space which decreased towards lower altitude and for upper cuticle, which increased along this gradient.

Response of needles

Repeated measures ANOVA revealed more variables responding significantly to site, i.e. altitude effects as compared to beech, while less variables responded to the year effect (Supplementary Information Table S3-2). No significant irrigation treatment (T and TP) was observed, except for phloem tissue, and therefore, as done previously for beech, data from both treatments were pooled together, excluding the phloem.

Morphological changes that have been observed on the needles, which developed in warmer and drier conditions during the growing seasons 2013 and 2014 are given in Figure 3-2. Contrarily to what was observed for beech, more variables measured on spruce responded in 2014, but trends were often similar between years (Figure 3-2). Albeit the altitudinal gradient did not fully mirror the variable responses, variables such as resin ducts, endodermis and transfer tissues tended to decrease towards lower altitudes, especially in 2014, but the frequency of stomata line and the xylem did also in 2013. Interestingly, at the second highest site (1010 m), the cross section and mesophyll proportion was lowest, especially in 2013, while epidermis, endodermis and transfer tissue had the highest values. As for beech, leaf mass area was highest in the intermediate low site (570 m), in both years.

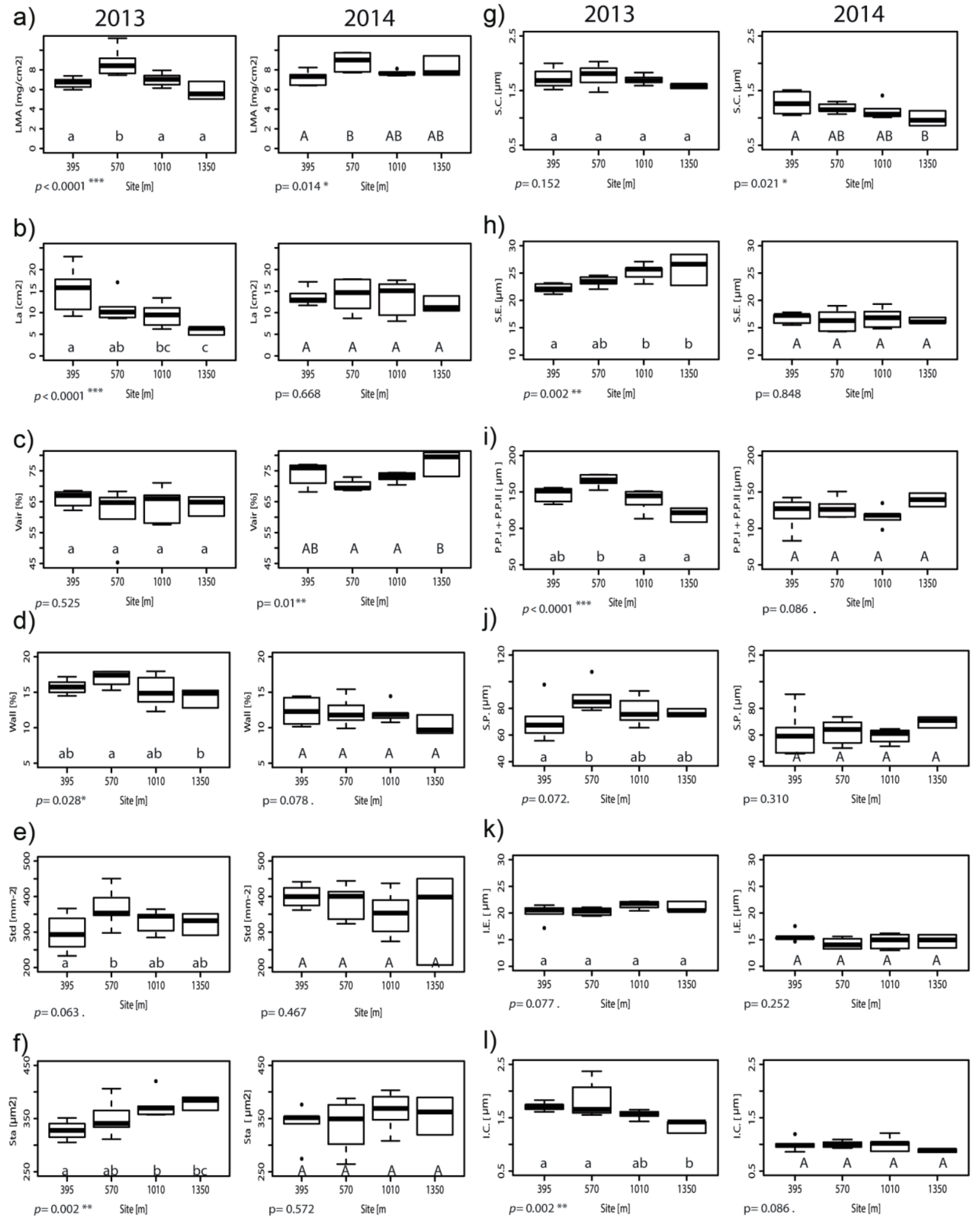


Figure 3-1 Variation along the gradient of the twelve selected variables for beech (see Table 3-1) during two consecutive years. Mean (N=3) ± 1xSE are indicated. Letters indicate the results of the post-hoc Tukey test after one-way Anova (p-value < 0.05), with site as factor and treatments T and TP pooled together to increase robustness (as no difference was found, see Supplementary Information Tables S1). When normality was not achieved, non-parametric tests were performed, namely for LMA and P.P

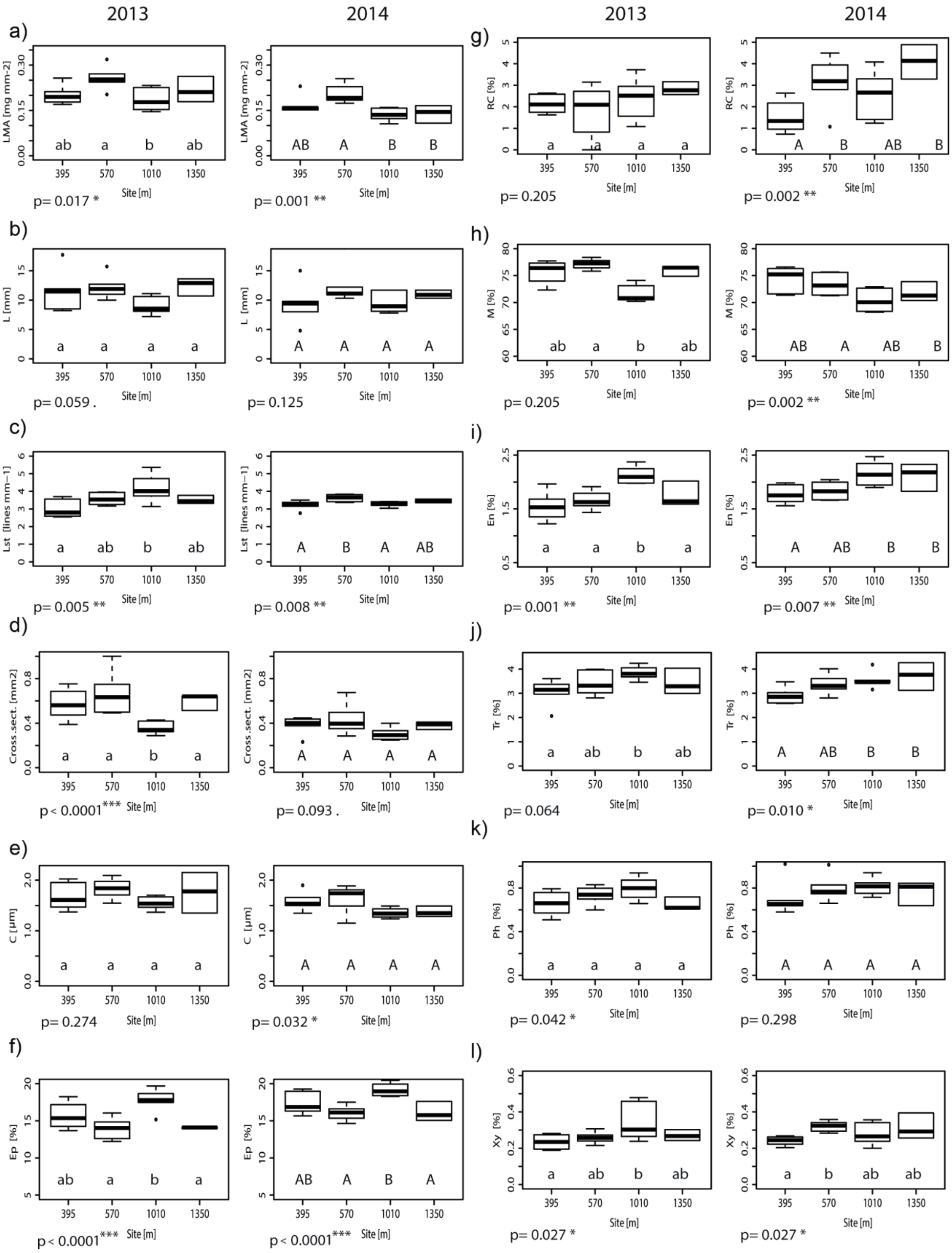


Figure 3-2 Variation along the gradient of the twelve selected variables for spruce (see Table 3-1) during two consecutive years. Mean (N=3) ± 1xSE are indicated Letters indicate the results of the post-hoc Tukey test after one-way Anova (p-value < 0.05), with site as factor and treatments T and TP pooled together to increase robustness (as no difference was found, see Supplementary Information Tables S2). When normality was not achieved, non-parametric tests were performed, namely for LMA and M.

3.4.3 Multivariate analysis of leaf trait responses

A redundancy analysis (RDA) using the morpho-anatomical data set under the constraints of climatic explanatory variables was performed separately for each species due to their difference in growth strategy. Because of contrasted weather conditions in 2013 and 2014, the analysis was kept separate for each year.

Gradient for beech leaves

The first canonical axis was highly significant in both years (p-values < 0.001 and < 0.01, in 2013 and 2014, respectively) while the second axis was less significant (p-values < 0.05 and < 0.01, in 2013 and 2014, respectively), explaining 35 % of total variance in 2013, and 27.6 % in 2014 (Figure 3-3).

Overall, we observed a high response of most variables to higher extreme temperatures, resulting in larger leaves with thicker cuticle and increased vein network, while having smaller stomata. In year 2013, we observed a clear separation along axis one between the lowest sites (395 and 570 m), with warmer and drier conditions, and the higher sites (1010 and 1350 m), with cooler and wetter conditions. This axis was mainly explained by the proportion of extreme warm days, T > 25 ºC (p-value < 0.01) and of cold days (p-value <0.05), as well as by the amount of rainfall (p-value < 0.05) and VPD > 1.5 kPa (marginally significant, p-value < 0.1). The leaf variables such as leaf area, parenchyma palisade, vein network and cuticle thickness were positively correlated to extreme warmer temperatures and high VPD, while epidermis thickness and stomata area were negatively correlated to these conditions. Moreover, the proportion of intercellular space, thickness of spongy parenchyma and stomata density were mainly related to the second axis, for which the amount of rainfall had the strongest weight, a pattern which was related to the extreme position of the intermediate lower site at 570 m.

The general picture of the biplot for 2014 was similar but the sites were less clearly separated as compared to 2013. While both the proportion of extreme warm and cold days as well as VPD were significant (p-values < 0.05, < 0.01 and < 0.05, respectively), the amount of rainfall was only marginally significant (p-value < 0.1). Some responses to higher temperatures found in 2013 were maintained in 2014 such as increasing cuticle thickness, vein-network and leaf area, but epidermis thickness and stomata area were no longer inversely related to the high temperature and VPD. Furthermore, parenchyma palisade was strongly related to cold temperature in 2014, as opposed to 2014.

Gradient for spruce needles

The RDA models for spruce had the first axis highly significant in both years (p-values < 0.001), but the second axis was significant only in 2014 (p-value < 0.01). Together, the first two axes explained 29.7% of variance and 33.8%, in 2013 and 2014, respectively (Figure 3-4).

In 2013, only the first axis was possible for interpretation, which is mainly explained by the proportion of extreme warm (p-value < 0.01) and cold days (marginally significant), as well as VPD (p-value < 0.05). This first axis isolated the site at 1010 m, while the other three sites were more intermingled, revealing similar responses. Most variables of needle tissues such as epidermis, endodermis, phloem, transfer and xylem were positively correlated to rainfall and cold temperature, and characterized mostly the site at 1010 m, while variables such as needle length, mesophyll, cross section, cuticle thickness and leaf mass area had an opposite response and related to higher temperatures and VPD's. The climatic data revealed a four consecutive-days period with minimum daily temperatures around 0ºC and a hail event few days after spruce sapling budburst in this site *(data not shown)*, which might have affected the young needles.

In 2014, the environmental variables which explained most the distribution were the VPD (p-value < 0.001) and temperature variables (p-values < 0.05). Needle variables were grouped in a similar way as in 2013, but since both axes were significant, a clearer pattern appeared. Variables such as xylem, resin ducts, transfer, frequency of stomata lines, needle length, endodermis and phloem were strongly related to the extreme cold conditions, while epidermis is now less related to the main gradient of temperature and could be linked to other not measured environmental variables.

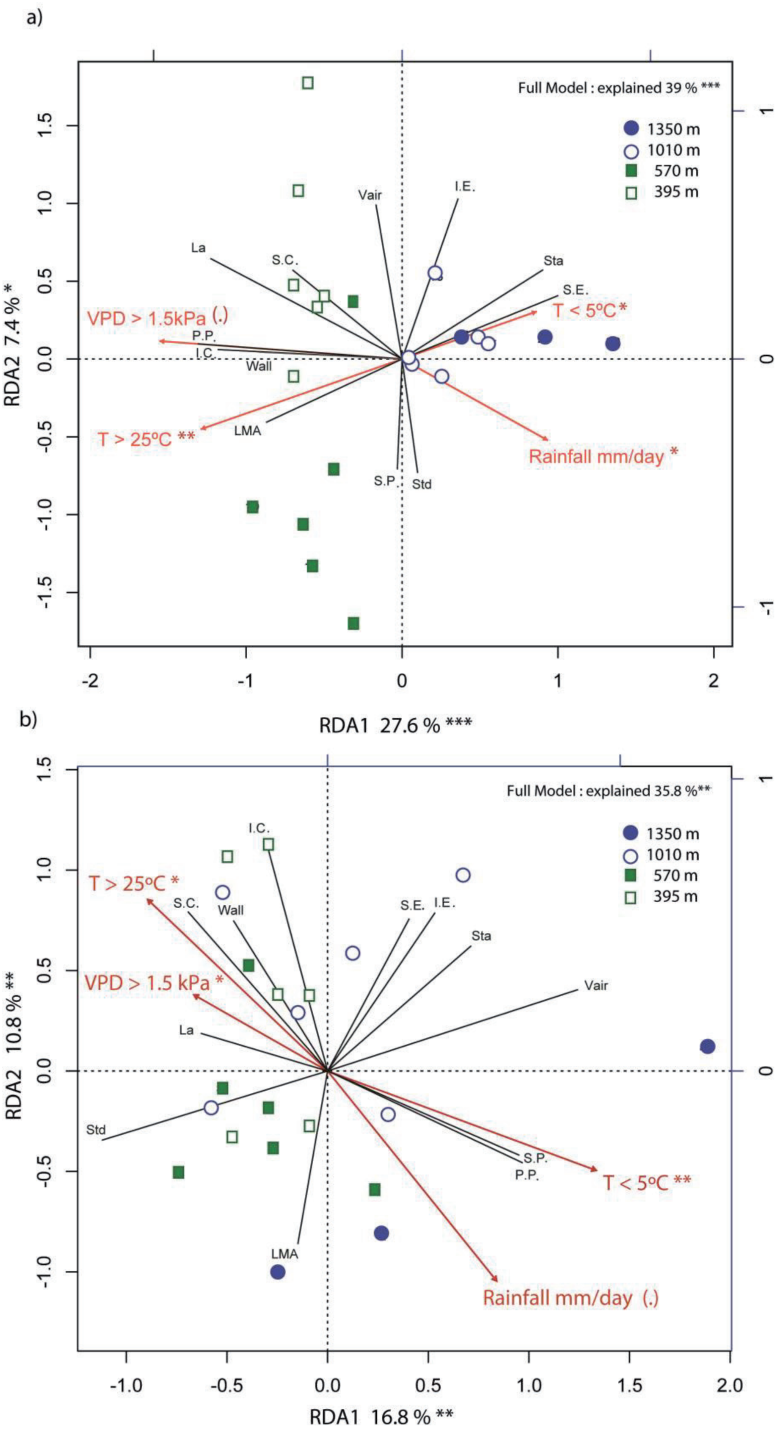


Figure 3-3 Redundancy analysis (RDA) of beech using twelve morpho-anatomical variables for years 2013 (upper graph) and 2014 (lower graph). Explanatory variables are temperature thresholds (number of days with temperature maxima above 25°C and number of days with temperature minima below 5°C), vapour pressure deficit (number of days with VPD > 1.5 kPa) and cumulative precipitation during the growing season (from budburst to August as the sampling date).

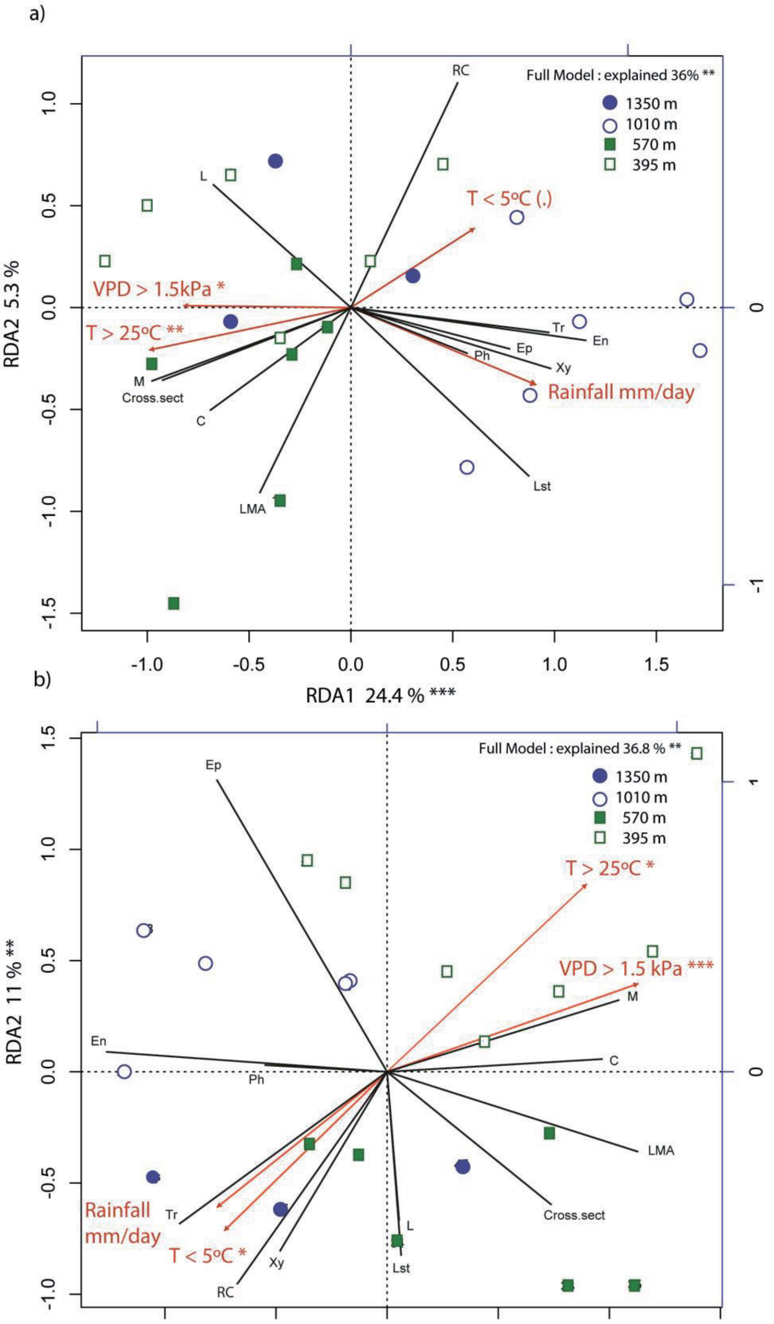


Figure 3-4 Redundancy analysis (RDA) of spruce using twelve morpho-anatomical variables for years 2013 a) and 2014 b). Explanatory variables are temperature thresholds (number of days with temperature maxima above 25°C and number of days with temperature minima below 5°C), vapour pressure deficit (number of days with VPD > 1.5 kPa) and cumulative precipitation during the growing season (from budburst to August).

3.4.4 Phenotypic plasticity index

On the level of individual leaf variables (same variables as in the previous analysis (Table 3-1), we could observe for both species a large variability of plasticity indexes, ranging roughly from 0.60 to less than 0.10 (Figure 3-5). For beech, the highest plasticity was observed for leaf area and leaf mass per area (Figure 3-5a) while for spruce needle cross-section and resin conducts showed the largest variation (Figure 3-5b). The overall phenotypic plasticity index of beech was 0.20 in 2013 and 0.14 in 2014 (Supplementary Information Figure S3-5), and for spruce the index was 0.23 and 0.22 for the respective years. The plasticity indexes were slightly higher in 2013, as compared to 2014, but the differences were not significant. However, we can observe that beech presented a different degree of plasticity between the macro- and micro-morphology in 2013, compared to a more stable plasticity between years for spruce.

3.5 Discussion

The results of the study confirmed the hypothesis that some leaf traits of spruce and beech developed more xeromorphic characteristics in response to warmer and drier conditions and that the phenotypic plasticity of the foliar traits studied were higher in beech than in spruce needles.

3.5.1 Tolerance range to warmer and drier conditions

Transplantation from the donor site downhill simulated gradually warmer and drier conditions, thus approximating three possible IPCC climate change scenarios (see Gavazov *et al.*, 2013). Overall, the climatic conditions suggest that saplings were exposed to similar warmer conditions at the two lowest sites, but were not water limited (Table 3-2), which is generally the main factor limiting tree growth at low altitudes (Way and Oren, 2010). Indeed, considering that the rainfall fulfilled the water requirements of beech, even at the lowest site, it can be assumed that this species was not water limited, nor was Norway spruce, which has a similar water requirement (Modrzyński and Eriksson, 2002). This suggests that in this study, the determinant factors were temperatures and VPD.

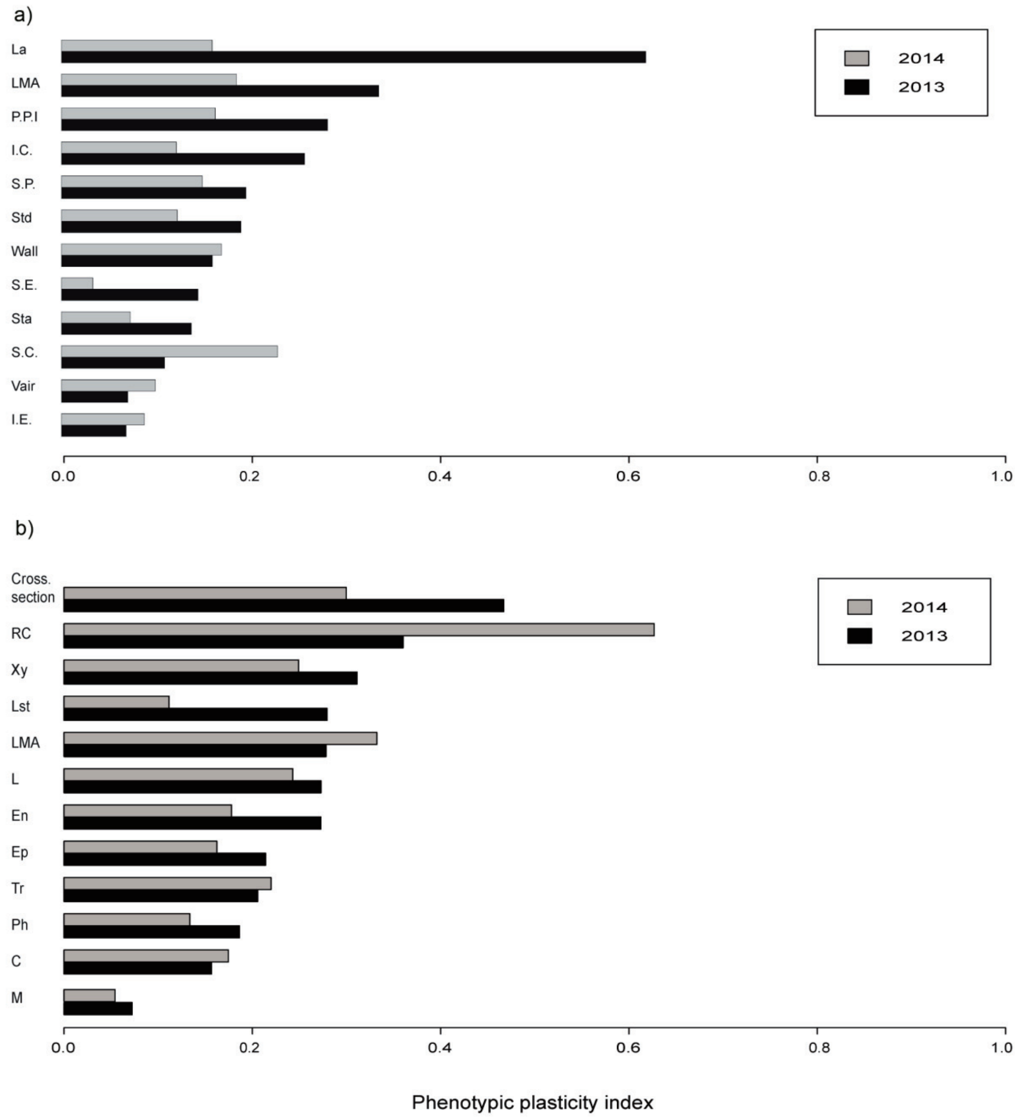


Figure 3-5 Phenotypic plasticity index of beech leaf variables (upper graph) and spruce needle variables (lower graph) for 2013 (black bars) and 2014 (grey bars). Index is calculated according to Valladares *et al.* 2000. Mean phenotypic plasticity for both species together was 0.21 in 2013 and 0.13 in 2014.

At the lowest site in our study, the months with highest mean temperature in 2013 were July and August, with mean values of 21.5ºC and 19.6ºC, respectively. These temperatures are above the optimal range established in literature for beech (Gelete, 2010). On the contrary, in 2014, highest mean monthly temperatures were 18.8ºC and 18.0ºC in June and July, respectively. Spruce has a lower thermal requirement than beech (Modrzyński, 2007) and therefore, as for beech, the saplings experienced temperatures above the optimal range. As the reduced precipitations at lower sites were not sufficient to expose the saplings to drought stress, changes in the morpho-anatomical traits due to warmer conditions might have been somewhat mitigated. Overall, if one considers VPD, as a proxy of the dryness of the air, it appears that the most stressful conditions were experienced during the summer 2013 at the two lowest sites. The different sensitivity to VPD between angiosperms and gymnosperms leads to a different tolerance of VPD to which the stomata remains open (Carnicer *et al.*, 2013). Moreover, it has been reported a higher stomata density of a range of tree species in response to increasing VPD (Stojnić *et al.*, 2015).

3.5.2 Beech shows higher leaf plasticity than spruce

The higher temperatures at the lower altitude increased the xeromorphy of some of the traits measured in beech leaves, such as the increase of cuticle thickness, vein network and also, but only in 2013, parenchyma palisade and leaf mass per area, concomitantly with decreased stomata area. In agreement with the literature, a well-developed palisade parenchyma, formed at the expense of spongy parenchyma, is considered as a characteristic of xeromorphic leaves (Bačić and Miličić, 1985). This trade-off between spongy and palisade parenchyma reduces the air space characteristic of the spongy parenchyma, and thereby also reducing transpiration. Smaller stomata are generally able to open and close more quickly, which in combination with a high stomatal density per unit area provides a greater capacity to rapidly increase stomatal conductance and optimize photosynthetic performance (Drake *et al.*, 2013) by reducing the water loss via gas exchange. Nevertheless, at the lowest site, the stomata density was the lowest, contradicting what is generally described in literature for xeromorphic features (Strobel and Sundberg, 1983), suggesting that the conditions at this site were not sufficiently stressful to present changes in this trait. If the measured climate gradient is considered, the second lowest site at 570 m a.s.l. might have experienced sometimes more extreme conditions than the lowest one in 2013, and indeed at this altitude stomata density was highest. Regarding other factors regulating the water evaporation of leaves, the increase of cuticle thickness, together with the reduction of stomata size, reduced the gas flow between the leaf and the air (Jenks, 2007), and consequently the water loss by transpiration of beech leaves developed in warmer conditions.

Contrary to what is commonly reported in literature as xeromorphic feature, beech leaves had a larger leaf area at lower altitudes (warmer and drier conditions) than at higher altitudes (cooler and wetter). Leaf flushing of beech occurs at lower altitudes around April-May when there is ample rainfall (see Table 3-2) in this region; thus, in our study the main climatic factor influencing the response of leaf expansion along the altitudinal gradient was air temperature, as the plants at this period were not at all water-limited. Therefore, the increase of temperatures at lower altitudes stimulated beech's leaf growth. Another study, performed in mature beech forests along an altitudinal gradient, also observed an exclusive effect of temperature in May on leaf size, for mature trees, while the number of leaves was affected by the mid-summer conditions, during which buds are formed for the following year (Meier and Leuschner, 2008). These authors therefore concluded that leaf area development in spring (April-May) and the impacts of summer droughts on matured leaves (July and August) were decoupled. They also described the risk of physiological failure later in summer as an unavoidable trade-off in the competitive strategy of beech to promote early leaf expansion and consequently tree growth. We related the leaf area with the annual volume increment of tree saplings and we observed a strong relationship between leaf area and increasing biomass in beech (Figure 3-6). As a consequence, beech saplings were able to increase their growth along the temperature gradient, most likely through larger leaves at the onset of spring, while they developed xeromorphic traits in foliage, later in the season, to cope with the higher evaporative demand in summer at the lower elevations. Spruce did not present as much plasticity as beech due to its inherent xeromorphic traits.

The phenotypic plasticity indexes of the different traits measured in both species reveal a general low degree of variation (c.a. 0.2), except for leaf area in beech and resin conducts in spruce (c.a. 0.6). However, this index enabled us to compare the plasticity among traits within species and highlights the higher response of traits of beech leaves to environmental conditions in 2013. This demonstrates that in case of marked environmental changes, beech is able to acclimate faster than spruce. Another possible point of view is a higher tolerance of spruce to the observed changing environmental conditions, probably linked to an inherent xeromorphy of needles.

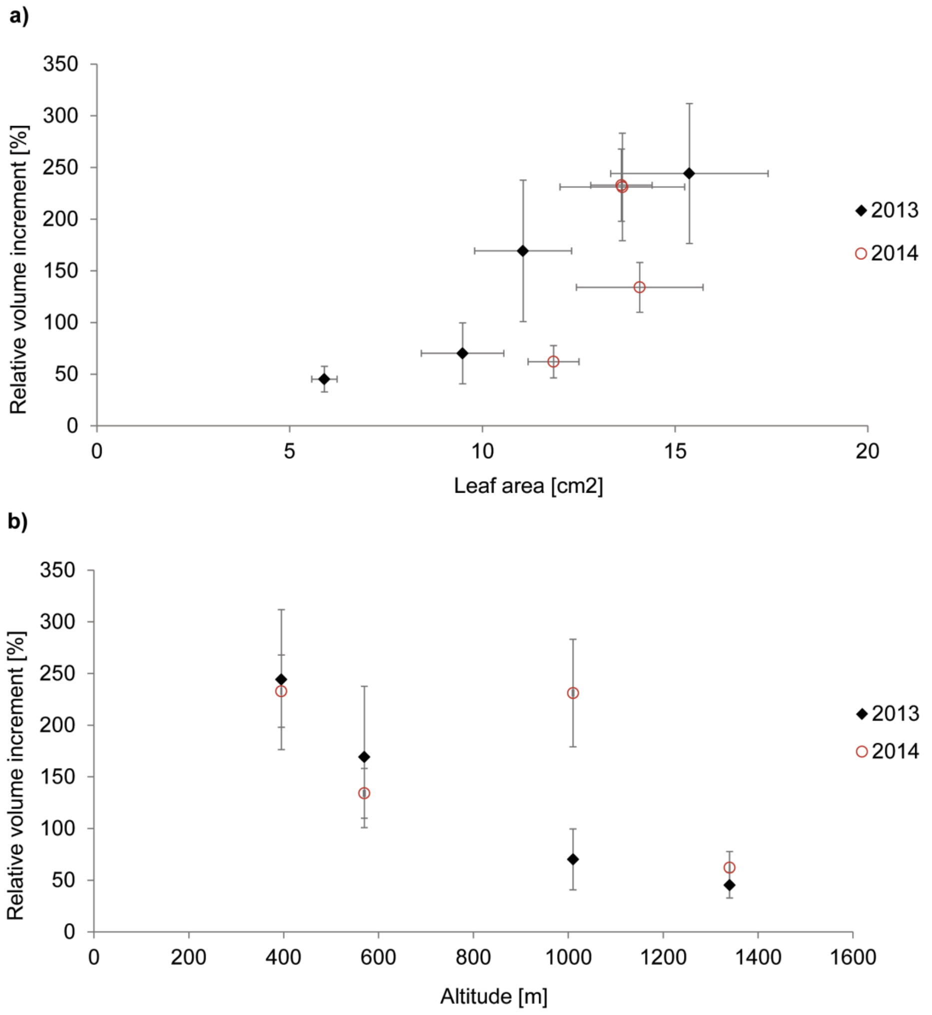


Figure 3-6 Relationship between annual volume increment of beech and leaf area a) and altitude b). Mean (N=3) ± 1xSE are indicated.

The equal mean temperature of 15.8ºC during the growing seasons at 395 m did not explain the difference in leaf responses of beech between the years 2013 and 2014, which indicates that the interannual climatic variability and its impact on tree physiology are not well assessed by mean temperature approach. The increase of 2.5 times the exposure of saplings to temperatures above 25ºC and the mean of ten maximal daily VPD values are better related to this interannual variabiliy. The higher plasticity found in beech is linked to the mesomorphic characteristics of leaves compared to the xeromorphic origin of needles. Due to the short-tem of this study, the climate variability approach was limited. We expect that leaves developed during a drier year with marked drought events would reveal a higher xeromorphy in the foliage of both species.

We confirm that the use of two contrasted years to study the phenotypic plasticity of foliar traits is sufficient to assess the acclimation abilities at leaf level of these species to these specific changing environmental conditions. However, we cannot predict the leaf morphological changes on the long-term but we demonstrate the potential plasticity of these species as a response to warmer and drier conditions and we prove the fast adjustment of leaves, which is the first step towards a potential adaptation. Finally, findings from this short-term experiment and findings from long term studies are not exclusive, but give together an insight on the mechanistic strategies of plants to cope with rapid environmental changes. This further understanding of the mechanisms underlying short-term foliar plasticity can serve as basis to design long-term experiments.

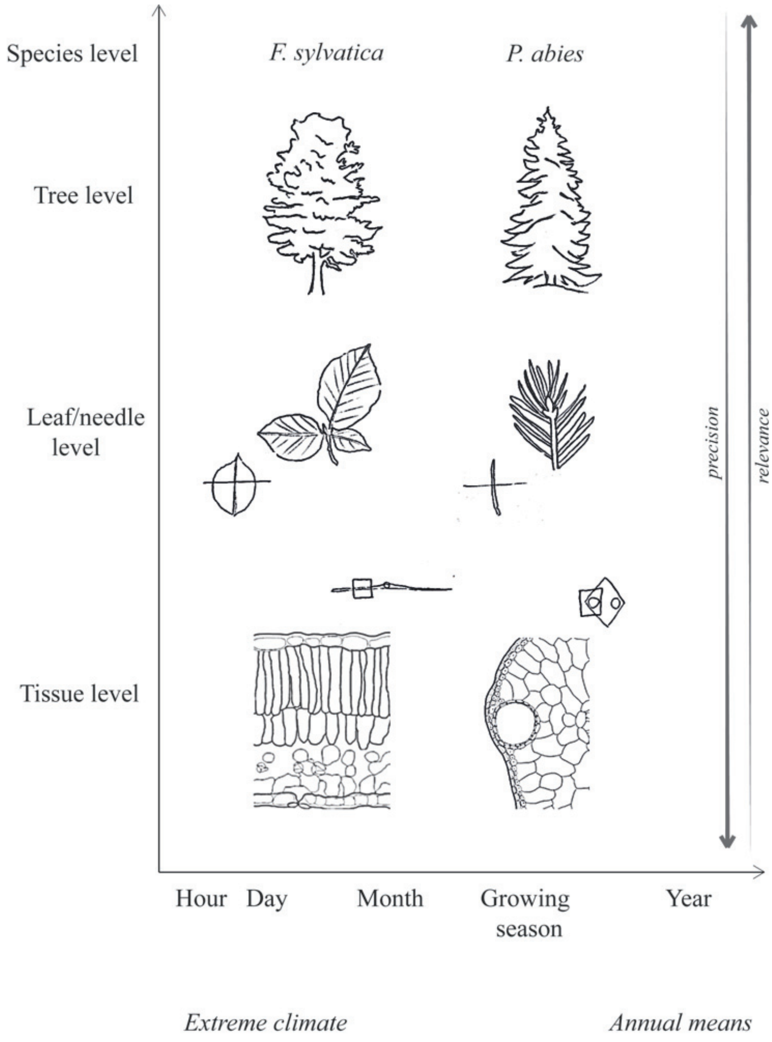
3.6 Acknowledgements

We are grateful to Terry Menard for guidance in laboratory analyzes, to Nadia Mudry for her assistance in the field and in the laboratory, and to Vincent Jassey for statistical advices. We also want to thank Thomas Spiegelberger for his help in designing the experiment, and to Federick Lenz, Pierre Mariotte and Tom Walker for reviewing of the paper. The work was supported by WHFF Wald- und Holzforschungsfonds (BAFU), by the Forschungsprogramm Wald und Klimawandel (BAFU-WSL) and by the Foundation Les Bois-Chamblard.

Contributions

AB and CS designed the experiment; PS and CS carried out the field work; PS did the morpho-anatomical analyses under the supervision of PV; RS supervised the statistical analyses; PS, AB and CS wrote the article with contributions from the other authors.

Chapter 4 Integrating tree responses in a multi-level assessment



Framework used to assess species-specific responses to warming at different structural and temporal scales (P. Sanginés, 2017).

> *"The First Draft of Anything is Shit"*
>
> *(Ernest Hemingway)*

The responses of ecological systems to climate change reflect the responses of the organisms that are within them. Therefore, forests functioning under climate change cannot be understood without knowledge of individual tree responses (Hansen *et al.*, 2001). Moreover, the individual scale is a desirable scale for characterizing ecophysiological behavior and responses of trees as it integrates the activities and processes that contributes to features such as survival, growth and reproductive success (Körner, 1994).

In the following chapter I aimed to assess by a multi-level approach, the mechanisms underlying the ecological response of spruce and beech saplings' to warming forcing. This will improve the understanding of these tree species capacities to respond to increasing temperatures by relating the structural and functional responses of beech and spruce to changing environmental conditions.

Article 3: **Mechanistic understanding of the ecological response of spruce and beech saplings' to warming: a multi-level assessment**

Sanginés de Cárcer, P., Signarbieux, Mariotte, P.,Wohlgemuth, T. and Buttler, A.

In preparation

4.1 Abstract

Forecasting climate change impacts and to which extent forests are able to cope with it remains today an important unsolved issue. Switzerland will face higher temperature increases than the global average, which will have strong impacts in mountain ecosystems. In the Swiss mountains, a rise in mean annual air temperature of 1.5ºC was observed during the 20th century with associated increases in the frequency of extreme warm summers and prolonged droughts. The major constraint in predicting vegetation responses to climate change resides in the complexity of interactions between plants and

the environment. This study advances the mechanistic understanding of the ecological functioning of spruce and beech regeneration under warmer and drier conditions. Native tree saplings from a cold and wet environment (1350 m a.s.l.) were transplanted downhill along an elevation gradient, resulting in a mean air temperature difference of 6^{o}C and a 30% rainfall reduction between the two extreme sites. We evaluated the growth efficiency using three-years monitoring of annual biomass increment. To further understand the potential difference in growth strategy, we determined the growing season length, the photosynthetic performance and morphological traits of the leaves. These high-resolution growth and physiology measurements provide data needed to assess the dynamic responses of trees to stress factors. Our results will serve to calibrate the spatial and dynamic WoodPam model, which has already been used to predict the evolution of landscapes and tree species in the Jura Mountains. Moreover, our findings will generally improve the parametrization of mechanistic models used to predict future species distributions.

4.2 Introduction

Understanding how climate and vegetation interact in order to define past, current and future vegetation distributions remains a principal challenge for plant ecologists. An approach to overcome this challenge is to model growth, mortality and reproduction rates of individual plants, and relate these to the abiotic and biotic environment (Scheiter *et al.*, 2013). A major constraint in predicting vegetation responses to climate change resides in the complexity of interactions between plants and the environment. One question is whether relationships between species localities and environmental conditions are sufficient for predicting future species distributions, or whether accurate predictions require a more mechanistic understanding of the processes underlying distributions (Kearney, 2006). Correlative models prediction species distributions estimate parameters phenomenologically by relating current distributions to environmental conditions, while mechanistic models explicitly capture hypothetical biological processes and derive their parameters from the phenotypes of organisms (estimated independently from current distributions). These parameters are then used to construct distributional models (Buckley *et al.*, 2010). Simply put, correlative approaches statistically link spatial data to species distribution records (Holdridge Life Zone model) while mechanistic models incorporate the links between the functional traits of organism and their environments (spatial distribution models) (Kearney and Porter, 2009). The principal assumption of correlative models is that processes setting range limits remain fixed in time and space, ignoring the dynamics of ongoing climatic changes (Williams and Jackson, 2007). Mechanistic models, however, rely on an understanding of the dominant processes that underlie survival and production to predict a geographic range and determine species' ecological niche through space and time (Kearney and Porter, 2009). The relevance of large spatial-temporal observations resides in the broad view given by the responses of forest ecosystems to climate change at the global scale. For instance, dynamic global vegetation models (DGVMs) provide valuable information on growth responses related to the interaction between vegetation and the atmosphere (Sass-Klaassen *et al.*, 2016). However, their ability to explain the variability of growth responses between and within species is limited, as they usually generalize tree species as plant functional types (Sass-Klaassen *et al.*, 2016).

Models are good predictors of species distributions but are sensitive to the accuracy of parametrization, which usually stems from empirical studies of tree responses to stressful conditions. However, models usually omit individual scale responses and thus cannot provide information on which species are vulnerable to which variables in the different habitats (Clark *et al.*, 2012). Therefore, an improvement of model parameterization by a mechanistic understanding of organisms' responses to climate change is needed. Furthermore, given that the response of an ecosystem to climate change is reflected in the responses of the organisms that composes it, responses of organisms at the individual level can begin a cascade of ecological change that may be observed at the ecosystem level (Hansen *et al.*, 2001). Moreover, Sass-Klassen *et al.* (2016) proposed the individual tree "as main source of information for understanding variability in growth responses as it has the potential to convey more details on specific tree responses to a given factor.

Stress can be defined as "an environmental factor that reduces the rate of some physiological process (e.g., growth or photosynthesis) below the maximum rate that the plant could otherwise sustain" (Lambers *et al.*, 2008). In this context, plants present a wide range of responses to stress, which scope from alterations in gene expressions and cellular metabolism to changes in growth rate and plant productivity (Shao *et al.*, 2008). In general terms, when an environmental factor has a negative effect on a plant process, an immediate stress response appears during a time that can span from seconds to days (Lambers *et al.*, 2008). In order to compensate this decline in performance, plants adjust their morphological and physiological properties through acclimation within the life time of an individual. As a result, the biochemical changes that occur induce changes in physiological processes, such as photosynthesis and growth rate, as well as changes in the morphology of organs. Eventually, a population becomes adapted to a specific environmental stress when the compensation for the decline in performance is achieved through genetic changes (Lambers *et al.*, 2008). Thus, tree responses can be discerned at three different levels: (1) growth and phenology (2) physiology (i.e. photosynthesis) and (3) morphology (Ahmad and Prasad, 2012).

The aim of this paper was to better understand the relationship between an individual tree and environmental climate changes at multi-organizational levels (tree, leaf, tissue). This high resolution growth and physiology monitoring provides with needed data to assess the dynamic responses of trees to stress factors (Sass-Klaassen *et al.*, 2016) in order to improve the parametrization of mechanistic models predicting future species distributions.

4.3 Materials and methods

4.3.1 Experiment design and set up

The experimental design consisted of a transplantation experiment along an elevational gradient of the two dominant tree species of the Swiss Jura Mountains, *Picea abies* (L.) Karst (Norway spruce) and *Fagus sylvatica* L. (common beech). The transect was determined by the project Mountland (Gavazov, 2013), in which two study sites were established based on IPCC climate change scenarios: Combe des Amburnex (N 46°52’, E 6°26’) as the control site at 1340 m a.s.l., and Les Bois Chamblard (N 46°47’, E 6°41’) at 395 m a.s.l. We obtain a notable climatic gradient with temperature increases of +5°C as well as a precipitation reduction of 50%. This type of experiment provides us with an approach of the impacts of future climate change by a “space-for-time/warming experiments” (Körner, 2003).

In May 2012, a common garden experiment of 60 saplings (30 spruce and 30beech) was established along the elevation gradient. Saplings, adapted to the environmental conditions at 1340 m., were collected in the surroundings of Combe des Amburnex (donor site) and transplanted to a lower elevation (395 m)

A generalized randomized block design was adopted, where block was matched with the transplantation altitude. The design of experiment included two blocks allocated one at each site, where the highest elevation served as a control site with an unchanged climate. Following a random selection procedure at each elevation, saplings were transplanted in 22 L pots into the ground and in rows with 0.3 x 0.9 m spacing to avoid light competition. A geotextile was placed at the bottom of the pots to avoid roots breaking through the recipient soil. Another geotextile was placed on the top to reduce soil water evaporation during the summer. In addition, within each block, treatments were randomly assigned to experimental units, which correspond to the saplings growing in the pots. An irrigation treatment (n=10 per species and recipient sites) was established and water was added weekly during the growing season, in accordance with the precipitation in the donor site.

Climatic variation is the baseline for our experiment, thus continuous monitoring of climate parameters (i.e. precipitation and air temperature) is required. For this matter, each site was equipped with wireless meteorological stations (Sensorscope Ag, EPFL).. In addition, sensor probes connected to Em50 data-loggers (Decagon Devices, Inc., USA) were installed at each elevation to record, at topsoil horizon and at one -minute interval, the soil temperature and the volumetric water content. These sensors were placed in a limited number of saplings in each site. Because of this, additional manual measurements (Fieldscout TDR-100-Spectrum Technologies,Inc. and Hanna Instruments-HI9850) were performed during the growing season once per week, for half of the replicates of each species and treatment.

4.3.2 Growth rate

To evaluate how changes in environmental conditions affect aboveground biomass an exhaustive monitoring of tree growth was carried out for all saplings during the growing season of 2013 and 2014 (before budburst and after senescence). In order to estimate the overall growth of each sapling, dasometric measurements of the stem and four main branches were recorded. For each individual, the four longest branches were previously selected and identified with colored cable to allow a continuous monitoring. Stem and branch diameters (basal and apical) were measured in mm using an electronic caliper with 0.01 mm of accuracy. The basal diameter of the stem corresponded to the average of two perpendicular measurements and was determined at approximately 1-2 cm from the base of the root collar. The basal diameter of a branch was recorded at the base. The apical diameter of the branches and the stem were measured below the dormant bud. Moreover, the stem and the branch length were determined with a measuring ruler, from the base to below the winter bud (accuracy of 0.1 cm). An allometric equation was developed to relate aboveground biomass with these above mentioned non-destructive measurements (i.e. stem basal diameter).

4.3.3 Phenology

Budburst

Phenological observations were carried out along the elevation gradient for all saplings during the 2013 growing season. In spring, the monitoring of the leaf emergence was followed every 2-3 days by the same observer. The development stages that were considered were based on Vitasse (2009). The stages considered for beech include: (0) dormant buds, (1) swollen and/or elongating buds, (2) budburst and (3) at least one leaf is fully unfolded. For spruce we considered: (0) dormant buds, (1) buds expanding with visible new green behind the transparent cupule and (2) needles unfolded. The budburst date was recorded when 50% of the buds, at individual level, had reached the stage.

Senescence

In autumn, leaf colouring and/or leaf fall were the criteria followed in order to assess the leaf senescence of beech. The phenophase of senescence was considered reached when 50% of the leaves of an individual were no longer functional, i.e. either coloured or fallen according to Vitasse (2009). Finally, the growing season length was determined as the difference between the day of the year of senescence and the day of the year of budburst.

4.3.4 Ecophysiology

In situ eco-physiological measures of photosynthesis (CO_2 assimilation rate, An), leaf stomatal conductance (gs), water status of the plant (water potential, ψ) and efficiency of photosystem II apparatus (chlorophyll a fluorescence), were performed in two campaigns during the growing season of 2013 and 2014. The first of each year took place at the beginning of the summer and the second one at the end of the summer. A novel aspect of these campaigns is the performance of these measurements at the same environmental conditions (i.e. same air temperature, same humidity and, most importantly, at the same partial pressure of CO_2). In order to achieve this, the measured saplings (3 replicates x species x treatment x site) were brought to the same intermediate location (Saint George, 1010 m a.s.l) and left there for a window of 24h for acclimation to the local conditions prior to the eco-physiological campaign. This enabled a real and strong comparison of the photosynthetic capacity between the saplings and treatments growing along the elevation gradient. Moreover, leaves and last generation shoot needles that were measured were also sampled in order to preform further laboratory analysis, which include N and C leaf content.

In the following, a detailed description of experimental protocols employed for each measurement is presented.

Leaf gas exchange

Leaf gas exchange measurements were performed between 11 a.m. and 3 p.m. on clear sunny days, when the photon flux density (PFD) reached values of around 1800 μmol m−2 s−1. These measurements include the stomatal conductance (gs, mmol H_2O m−2 s−1) and the CO_2 assimilation rate (An, μmol CO_2 m−2 s−1) which were determined using an open infrared gas analyzer (IRGA) system (CIRAS-2, PP-System, Hitchin, UK) equipped with a leaf clip. Two leaf clips were used, one for each species. A PLC6 universal leaf cuvette (18 mm diameter), equipped with light units (LED), was employed for beech leaves. A special conifer leaf cuvette (PLC5) was used for spruce needles using natural ambient light. As needles did not cover the whole cuvette (80 mm x 30 mm), the photosynthetic surface area was recalculated by means of a leaf area meter (LICOR-3100 area meter). For both leaf clips, the inside temperature was kept constant at 25 °C, whereas the relative humidity was always close to the ambient air. Moreover, an external CO_2 cartridge plugged into the CIRAS-2 allowed us to maintain a constant level of CO_2 (380 ± 5 ppm) in the cuvette during the measurements.

Chl a fluorescence

Chl a fluorescence was performed at the same environmental conditions and period of time as the leaf gas exchange with an additional predawn measurement. These were recorded using a portable pulse amplitude fluoremeter (PAM-2500, Heinz Walz GmbH, Effeltrich, Germany) where all associated parameters (ΦPSII, Fv/Fm) were determined and calculated according to Maxwell and Johnson (2000). The measure of chlorophyll a fluorescence served as an indicator of non-stomatal limitation of photosynthesis (Signarbieux and Feller, 2011).

Leaf water potential

In order to evaluate the water status of saplings, leaf water potential was measured at predawn and at midday using a Scholander pressure chamber (Scholander *et al.*, 1965). The minimum leaf water potential (ψm, MPa) was measured between 11 a.m. and 3 p.m, whereas pre-dawn leaf water potential was recorded between 4 a.m. and 6 a.m.

4.3.5 Leaf macro- and micro-morphology

In order to assess the presence or not of morphological and anatomical stress symptoms in leaves and needles, samples were collected at the beginning of August 2013, within the same individuals measured during the eco-physiological campaigns, following specific criteria (i.e. avoiding sylleptic branches). Two leafs were collected for each beech replicate and three generation needles shoots (2011, 2012 and 2013) for each replicate of spruce. Macro-morphological measurements (i.e. fresh and dry weight) were immediately performed in the laboratory after the sampling. Then, leaf discs of beech and mid-portion segments of spruce needles were fixed by infiltration with buffered 2.5% glutaraldehyde at pH 7.0 and stored at 4^{o}C until further processing. For microscopic examination, cross sections were obtained with a manual microtome and either directly observed under transmitted and fluorescence light or stained before microscopic observations. All sections were observed in a Leica microscope and images were processed using Adobe Photoshop Cs5 (version 12.0.0.0).

4.3.6 Statistical analysis

Using effect size (Eq.5) allowed to quantify the impact of changing environmental conditions on the different variables measured at individual tree (Table 4-1). All statistical analyses were performed in R 3.1.2 using the *mgcv* package.

$$Effsize[i] = \frac{x[i] - \overline{x_c}}{\sigma x_c}$$

Equation 3 Calculation effect size

where x is the variable considered for each observation [i], $\bar{x}_c$ and σx_c are the mean and standard deviation of the control population.

We divided by the standard deviation of the control to standardize the measurements. We use this instead of the total deviation because the control consists of a representative group of the population who has not been affected by the experimental intervention. The effect sizes were calculated for each explanatory variable and also the 95% intervals of confidence. Through a simple one way t-test we tested the significance of traits being different to zero. The resulting statistical analysis enabled to assess which of the explanatory variables were more or less affected by the warming treatment.

We performed all the measurements on the same triplicated saplings in each treatment, species and elevation during the two consecutive years (2013-2014).

Table 4-1 Measured traits and variables during the experimental period (2013-2014) on beech and spruce sapling and on the soil

Code	**Trait/variables definition**	**Time scale**
T mean	Mean temperature	Growing season
Biomass	Annual increment of aboveground biomass	End of growing season
Budburst	Date of budburst	Beginning of growing season
GSL	Growing season length	Growing season
N	Leaf nitrogen content	Mid-growing season measure
C:N	Leaf carbon nitrogen ratio	Mid-growing season measure
SLA	Specific leaf area	Mid-growing season measure
Surface	Leaf surface	Growing season
Cuticle	Thickness of the cuticle tissue	Mid-growing season measure
Epidermis	Thickness (beech) or area (spruce) of the epidermis tissue	Mid-growing season measure
St.density	Stomata density (beech) or frequency of stomata lines (spruce)	Mid-growing season measure
Mesophyll	Thickness (beech) or area (spruce) of the photosynthetic tissue	Mid-growing season measure
An	Photosynthetic net assimilation rate	Mid-growing season measure
Gs	Stomatal conductance	Mid-growing season measure
NBI	Nitrogen balance index of the leaf	Growing season
ψ predawn	Basal water potential	Mid-growing season measure
ψ midday	Midday water potential	Mid-growing season measure
Fv/Fm predawn	Chl fluorescence at predawn	Mid-growing season measure
Fv/Fm midday	Chl fluorescence at midday	Mid-growing season measure
ΦPSII	Efficiency of photosystem II	Mid-growing season measure
Soil VWC	Soil moisture	Growing season
Soil temperature	Soil temperature	Growing season
Soil respiration	Soil respiration	Mid-growing season measure

4.4 Results

4.4.1 Biomass allocation

The harvest of trees at the end of the study allowed us to assess the distribution of biomass in the different compartments (Figure 4-1). Overall we observed that beech allocates more biomass belowground than aboveground. For both warming treatments, there was an increase in the aboveground biomass compared to the control treatment, and higher in the irrigated treatment compared to the non-irrigated. Similar allocations above and belowground biomass were observed for spruce. Interestingly, there was a notable increase (3-5%) in leaf biomass in the saplings subjected to warming compared to the control treatment.

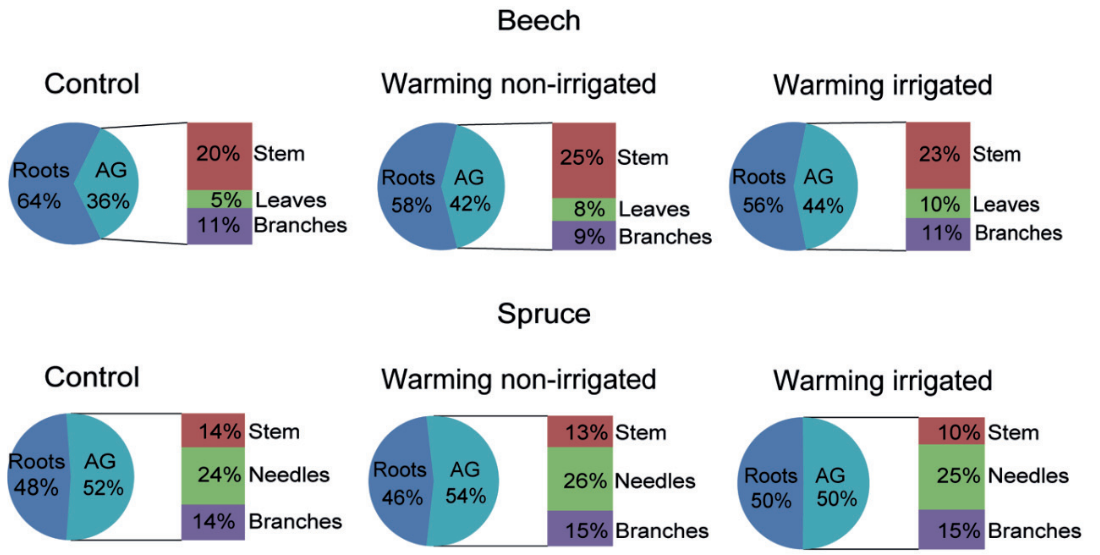


Figure 4-1 Biomass allocation (percentage of dry weight) of saplings (N=5) in each species and treatment at final harvest in autumn 2014 in control (1350 m) and warming treatments (395 m.)

The relative aboveground biomass increase is presented in Figure 4-2, where we found distinct interannual variability in tree growth. In 2013, a significant positive trend in aboveground increase was observed for the warming treatment in beech, while spruce presented a decreasing trend. Moreover, a significant difference between species was observed for the warming irrigated treatment. No significant differences were found between species and treatments in 2014.

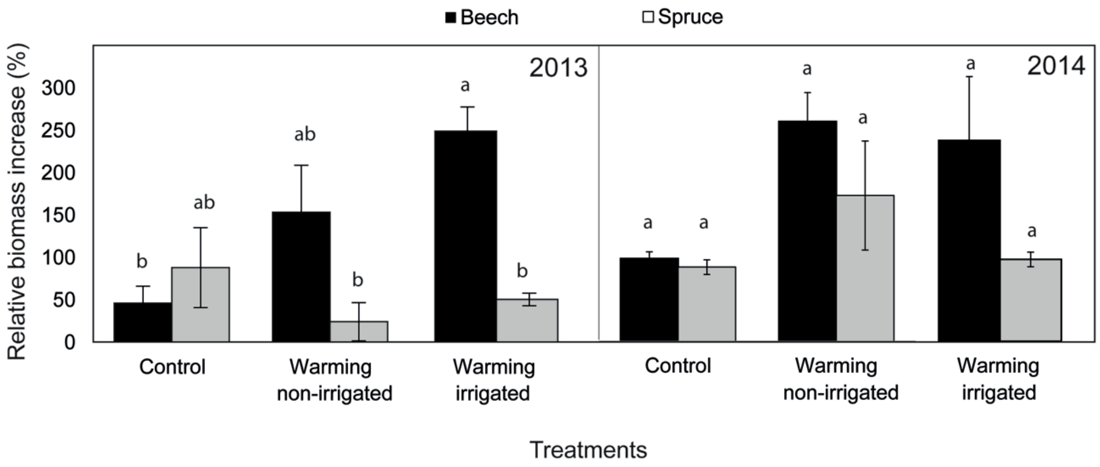


Figure 4-2 Annual relative aboveground biomass increases (n=3 ± SE) per species and treatments (control at 1350 m, warming non- irrigated and warming irrigated at 395 m). For the warming irrigated, trees had the same amount of rainfall then the control at highest elevation

(donor site). Letters represent the results of a Tukey multiple comparison of means test (95% family-wise confidence level) within each year between treatments and species.

4.4.2 Multiple comparisons

Standardized effect sizes (Figure 4-3) provided with an assessment of which explanatory variable was more affected by the warming treatments and which of the two species was more responsive (Novick *et al.*, 2016). Moreover, it enabled the comparison of the magnitude of these effects at multiple levels. Overall, beech responded more than spruce to warming (13 significant variables in beech versus 6 in spruce in 2013).

Growing season 2013

For beech, warming significantly ($P < 0.05$) increased biomass, growing season length (GSL), cuticle thickness, C:N leaf ratio, leaf surface and thickness of the mesophyll tissue, while having a negative effect on the timing of budburst, leaf nitrogen content, epidermis thickness, net CO2 assimilation rate and soil moisture. For spruce, warming increased growing season length and the epidermis thickness, while the opposite was observed for biomass increase, budburst timing and stomatal conductance.

Growing season 2014

Biomass increase, budburst timing, growing season length, cuticle thickness and soil moisture presented the same trends than 2013 for beech. Similarly occurred with spruce except for biomass increase which did was not significant different to zero.

Opposite trends were observed for the following beech variables leaf nitrogen content and epidermis thickness stomatal conductance. Leaf surface, C:N leaf ratio, mesophyll thickness and stomatal conductance were not significantly different from zero in 2013.

Spruce presented significant differences in variables measured in 2014 compared to 2013. Specific leaf area was lower while mesophyll thickness and leaf water potential presented a positive size effect. Stomata density and soil respiration did no change between the control and the warming site for neither of the species and for neither of the years.

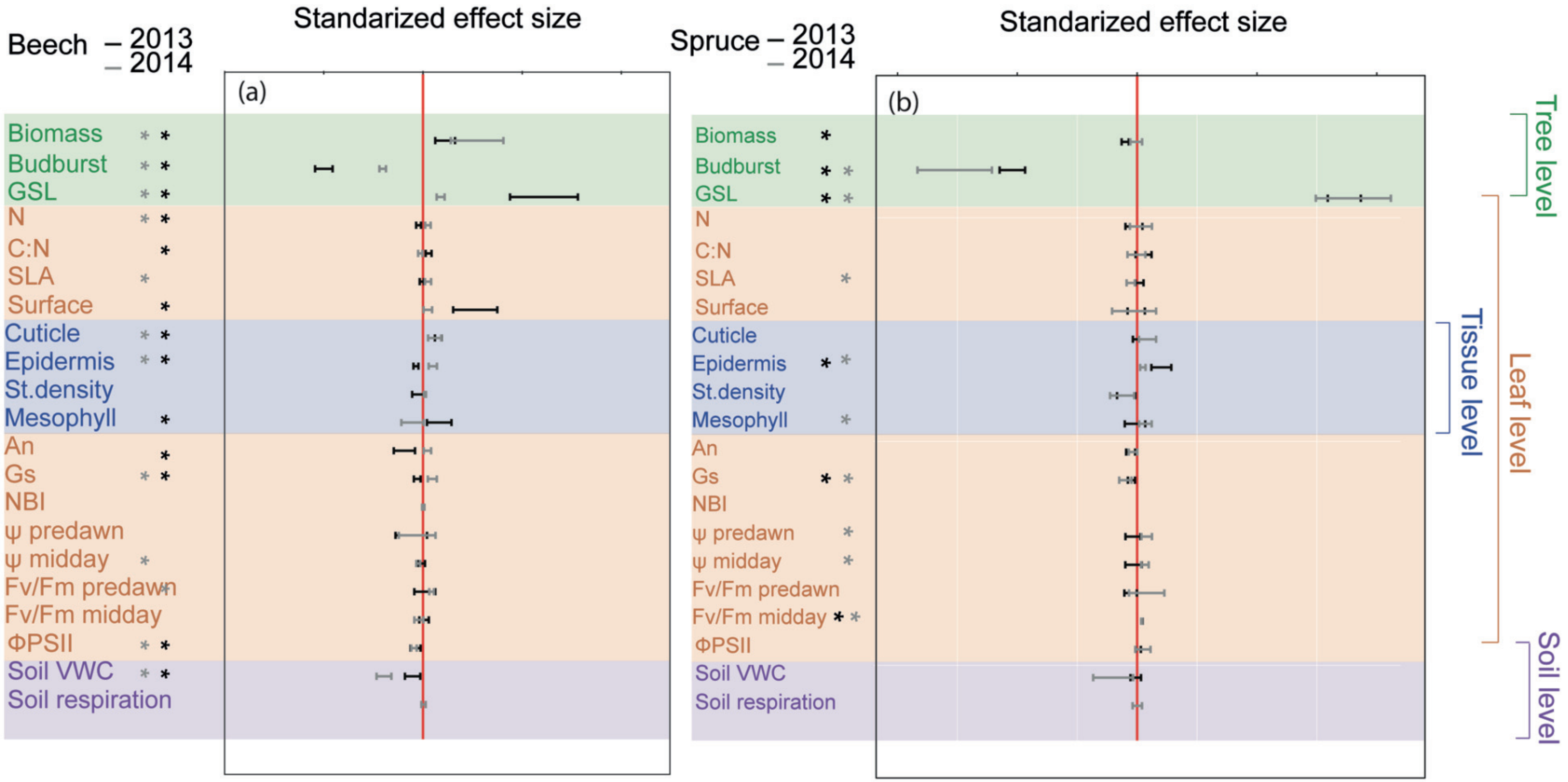


Figure 4-3 Standardized effect size ± interval of confidence of 95% ((a) beech and (b) spruce), where stars represent the significant effect size different to zero (red line). The effect size is measured as the difference of a given variable between the warming treatment and the control treatment and standardized by the standard deviation of the control treatment. The two warming treatments (irrigated and non-irrigated) were pooled together as no significant effect resulted from ANOVA test, so n=6.

4.5 Discussion

4.5.1 Tree growth-climate relationship

Tree growth rates revealed contrasting growth responses of beech and spruce to warming in 2013 while no difference in responses was observed in 2014. This suggests a high sensitivity of biomass increment to changing environmental changes and interannual climatic conditions. Beech saplings adapted to high elevation conditions experienced an enhanced growth with increasing temperatures. Studies have related increases in tree growth to warmer temperatures in longer growing seasons in locations provided with enough water supplies (Lindner *et al.*, 2014). Same increasing temperatures were applied to both species, which might suggest a lower tolerance of spruce to increasing temperatures and drier conditions. However, the average temperature increase of 6.3°°°C was the same for both growing seasons (2013 and 2014), while the reduction in precipitation was higher in 2014 than in 2013. No direct link was observed between the growth patterns and the mean climatic variables. Due to the drought sensitivity of these species, one could expect that warmer temperatures associated with a decrease in precipitation would decrease growth rates. However, spruce decline in growth rate during 2013 was not observed in 2014 where the precipitation was lower. It can be arguable that the precipitation rate does not reflect the water availability in the soil. In this study the weekly monitoring of volumetric water content in the soil revealed that no differences between control and warming treatments was observed for spruce. Contrarily, beech presented significant negative size effects in soil moisture for both years, which once again are not in agreement with the growth patterns.

Regarding evaporative demand of the air, significant differences were found in the amount of hours during which saplings were exposed to elevated VPD (Figure 4-4). It has been hypothesized that VPD may trigger stomatal closure to avoid an excess of water loss (Carnicer *et al.*, 2013) and that the degree of sensitivity of stomatal closure to elevated VPD is highly species-specific. This notable difference in amount of hours with elevated VPD is most likely to have a strong impact in the growth rates of these species.

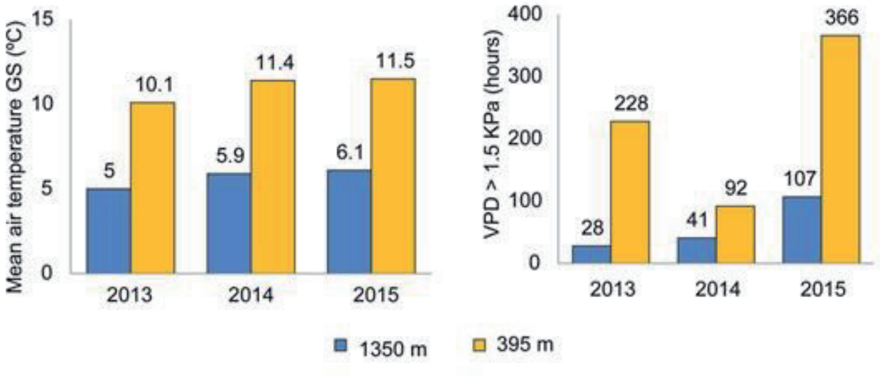


Figure 4-4 Comparison between average climatic variables (mean temperature; left panel) and extreme climatic variables (number of hours with VPD > 1.5 kPa; right panel) throughout the growing seasons (GS) 2013, 2014 and 2015, and between the two extreme sites.

4.5.2 Most responsive variables to changing environmental conditions

The selection of parameters included in a model for species distributions predictions requires a detailed understanding of the factors responding to changing environmental conditions and its relationship with growth rates. The standardized effect size of measured variables at tree, leaf and tissue level, together with soil measurements, enabled to pin point those with a higher degree of response. Tree level associated variables such as biomass increments; budburst and growing season lengths were the most responsive variables for both species. Therefore, the growing season length, defined as the period of time between budburst and budset, must be considered in the parametrization of models. Most models include spring phenology in their predictions but none take into account shifts in autumn phenology.

To conclude the multi-level assessment of species-specific responses to simulated climate change provided with useful information regarding the sensitivity of the explanatory variables to warming treatments. The overall higher response of beech suggests a higher phenotypic plasticity of this species to modify its leaf phenology and morphology and physiological processes in accordance to the changes in environmental conditions to maintain a certain level of growth performance. Finally, growth related variables were the most responsive together with variables related to uptake of carbon (e.g. leaf surface and proportion of photosynthetic tissue) and those to the control of water loss (e.g. cuticle and epidermis thickness).

4.6 Acknowledgements

The work was supported by WHFF Wald- und Holzforschungsfonds (BAFU), by the Forschungsprogramm Wald und Klimawandel (BAFU-WSL) and by the Foundation Les Bois-Chamblard..

Chapter 5 Climate change and phenology

Pictures from the upper row corresponds to the budburst stages of spruce needles[1] and the lower row corresponds to the senescence process of beech leaves[2]

P. Sanginés ([1]Aubonne 2014 and [2]Bois Chamblard 2013)

"Autumn is a second spring when every leaf is a flower"

(Albert Camus)

Phenology is defined as “the study of recurring plant and animal life cycles stages, especially their timing and relationships with weather and climate” (Schwartz, 2013). Phenology has a key role in the coordination of tree performance with climate conditions. The timing of budburst and leaf senescence determines the length of the vegetative period and thus the growth performance of individuals (Loustau *et al.*, 2005). Moreover, the coordination of the different phenophases with annual climate variations is a key factor leading to species adaptations to climatic constraints. Therefore, the capacity of species to alter their phenology in response to environmental changes will partly define their abilities to survive, or not, to future climate changes (Vitasse, 2009).

5.1 On-set spring phenology

Increasing temperatures, associated to climate change, will influence both dormancy release in winter (chilling temperatures) and the initiation of bud development in spring (forcing temperatures) (Harrington and Gould, 2015). In fact, warming may not necessary mean an advance budburst in spring (Chuine, 2010) as some species might need an increase in forcing hours to compensate the insufficient chilling requirement for bud dormancy release (Fu *et al.*, 2014a, 2014b). However, there is no consensus about i) how the decreasing in chilling hours will affect the timing of budburst, ii) at what range of chilling temperatures bud dormancy breaks occurs and iii) how dependent is spring phenology to the previous spring (Keenan and Richardson 2015). Moreover, many studies focus exclusively on one phenological stage, but do not evaluate the correlation between phenophases within the same cycle (Delpierre *et al.*, 2015).

In this section, I present unpublished results regarding the chilling and forcing requirements of spruce and beech saplings provided by a the Master’s Thesis of Rocio Andrey (2015). These results are important to further understand the phenological mechanisms of these species to warming. Additionally, in section 5.3 and on I present published results from a side project regarding the legacy effects of advanced or delayed budburst on the budset timing of beech leaves. My main contribution in this project was related to the experimental set up, and the definition of the methodology together with the field work during the growing season 2014. To a lesser extent, I participated in the analysis of results and the manuscript preparation.

5.1.1 Chilling and forcing requirements of spruce and beech saplings

Chilling and forcing hours were estimated using a parallel model, and a threshold of 5°C was established. The number of chilling hours (-1° < temperature ≤ 5°C) was calculated from the second of November until budburst date, while the forcing hours (temperature > 5°C) was calculated for the period between the second of February to budburst. Temperature data was collected at hour resolution by *in situ* meteo-stations (Senseroscope, S.A.) placed along the elevation gradient.

A strong exponential relationship (R^2 spruce=0.82 and R^2 beech=0.79) was found between forcing and chilling hours for both species (Figure 5-1). This indicates that the more number of chilling hours experienced by an individual the less forcing hours it requires for budburst. Furthermore, spruce saplings require more forcing hours for a given chilling requirement than beech. For instance, for a same amount of chilling hours of c.a. 1900, beech's forcing requirement is 1000 h while spruce requires a sum of 1200 h.

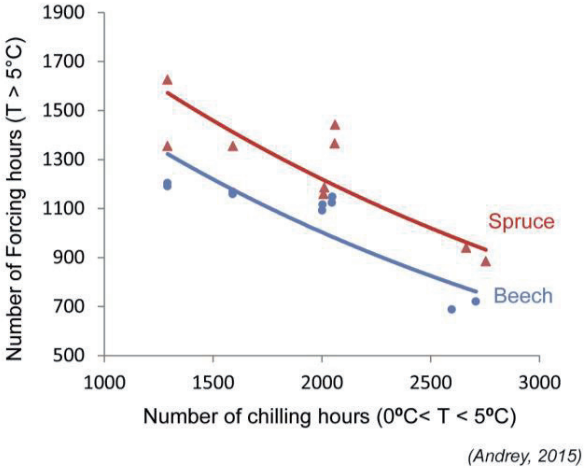


Figure 5-1 Relationship between the number of chilling hours and the number of forcing hours during the years 2014 and 2015 (Andrey, 2015)

This further knowledge, of the temperature effects on the timing of dormancy release and budburst, will help to evaluate the impacts of climate change on tree species (Søgaard *et al.*, 2008).

5.2 Off-set growing season

Autumn phenology also plays a key role in the determination of the growing period. It has recently been hypothesized that a delay on leaf senescence could also lead to a longer growing season (Menzel and Fabian, 1999; Vitasse *et al.*, 2011). In contrast to spring phenology, budset and leaf senescence in temperate trees have received fewer attention and the factors that trigger the process (i.e. photoperiod, content of nonstructural carbohydrates in leaves or decrease and temperatures during autumn) are still poorly understood (Estrella and Menzel, 2006).

In the current section, I present unpublished results linking leaf nitrogen content, measured with Dualex Dx4 (FORCE-A, Orsay, France), the net assimilation rate of CO2 (Ciras-2 PP-System) and leaf (Biesalski codes (1957)) during the senescence period of beech 2013. The Dualex 4 Scientific (Dx4) is a recent developed leaf-clip that measures leaf epidermal flavonoids (Flav) at 375 nm, using the Chl fluorescence screening method (Agati *et al.*, 2005), simultaneously to chlorophyll content in the same measured point. Moreover, I wanted to assess to what extent the device Dualex (Dx4) was accurate in measuring leaf nitrogen content. In total, six beech saplings were selected at 395 m of elevation to follow a continuous monitoring of the senescent process (October-November).

Measurements were performed weekly on two to four leaves per individual tree and further sampled for subsequent laboratory analyses. Gas exchange measurements were performed prior leaf sampling and corresponded to the average of three measurements of nitrogen balance index. All measurements were carried out in the center of the leaf to maintain consistency in the measurement protocol.

The follow-up of leaf senescence showed a strong correlation between nitrogen balance index (NBI) and the net assimilation rate (Figure 5-2). Similarly, a relationship between these variables and leaf coloring was also observed (Figure 5-3).

The results suggest that the Dualex is a useful device for monitoring nitrogen leaf content when leaves are still green. When leaves start to be senescent this device is less accurate giving a wide range of responses to a given content of nitrogen. Moreover, gas exchange measurements are usually carried out when leaves are mature, however, the exact moment of this stage is not clearly defined (can range from 2 weeks to 1.5. months). Therefore, the accurate timing for photosynthesis measurements is crucial for the correct evaluation of photosynthesis responses, and in particular, when comparing photosynthetic capacities between trees growing at different elevations (which present different budburst timing and leaf development). That is why I found that this device was very useful for assessing maturity of leaves prior photosynthetic measurements. As leaf nitrogen content increases through leaf development, this device indicates the moment in which [N] in the leaf has stopped increasing and remains stable (i.e. maturity stage of the leaf).

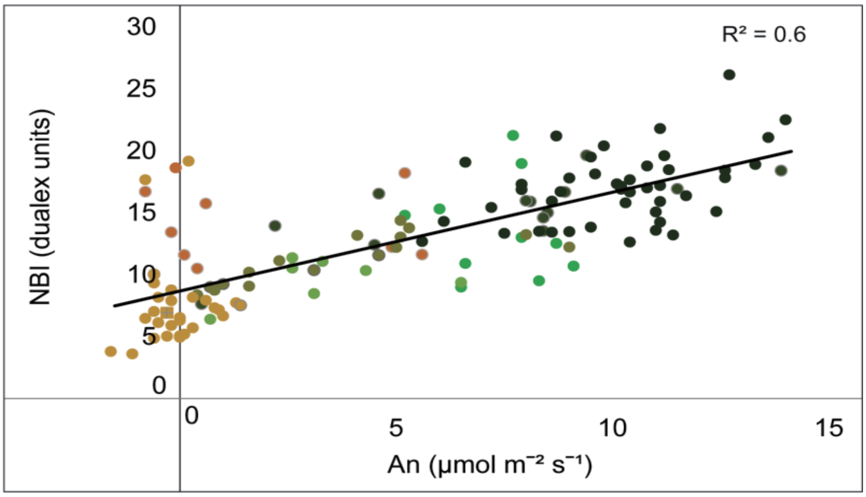


Figure 5-2 Relationship in beech leaves between the nitrogen balance index (NBI) and the CO2 assimilation rate (An). The measurements were made during the leaf senescence period in 2013. The coloring of leaves was measured using the Biesalski codes (1957) and correspondent colors are associated to each of the dots in the graph.

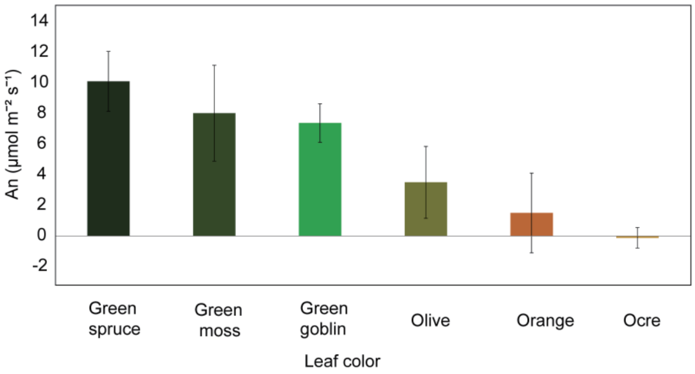


Figure 5-3 Correspondence of each color code with the assimilation rate (An) ± standard error

Article 4: **Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring**

Signarbieux, C., Toledano, E., Sanginés de Cárcer, P., Yongshuo, H.F., Schlaepfer, R., Buttler, A. and Vitasse, Y.

In press: Global Change Biology (2017)

5.3 Abstract

In temperate trees, the timings of plant growth onset and cessation affect biogeochemical cycles, water, and energy balance. Currently, phenological studies largely focus on specific phenophases and on their responses to warming. How differently spring phenology responds to the warming and cooling, and affects the subsequent phases, has not been yet investigated in trees. Here, we exposed saplings of *Fagus sylvatica* L. to warmer and cooler climate during the winter 2013–2014 by conducting a reciprocal transplant experiment between two elevations (1,340 vs. 371 m a.s.l., ca. 6°C difference) in the Swiss Jura mountains. To test the legacy effects of earlier or later budburst on the budset timing, saplings were moved back to their original elevation shortly after the occurrence of budburst in spring 2014. One degree decrease in air temperature in winter/spring resulted in a delay of 10.9 days in budburst dates, whereas one degree of warming advanced the date by 8.8 days. Interestingly, we also found an asymmetric effect of the warmer winter vs. cooler winter on the budset timing in late summer. Budset of saplings that experienced a cooler winter was delayed by 31 days compared to the control, whereas it was delayed by only 10 days in saplings that experienced a warmer winter. Budburst timing in 2015 was not significantly impacted by the artificial advance or delay of the budburst timing in 2014, indicating that the legacy effects of the different phenophases might be reset during each winter. Adapting phenological models to the whole annual phenological cycle, and considering the different response to cooling and warming, would improve predictions of tree phenology under future climate warming conditions.

5.4 Introduction

Phenology of temperate and boreal trees plays a crucial role in ecosystems structure and functioning (Fu *et al.*, 2016). An advance or a delay in the beginning and/or end of tree growth may considerably impact biogeochemical cycles and therefore feedback on the global earth's climate (Cleland, Chuine, Menzel, Mooney, and Schwartz, 2007; IPCC, 2014; Ma, Pitman, Lorenz, Kala, and Srbinovsky, 2016; Peñuelas, Rutishauser, and Filella, 2009; Richardson, Keenan, and Migliavacca, 2013; Wolf *et al.*, 2016). For instance, increasing temperatures have extended the growing season of temperate and boreal trees and resulted in a larger carbon uptake (Keenan, Gray, Friedl, and Toomey, 2014). Earlier leaf-out as a result of warmer spring may even compensate for the carbon loss occurring in more frequent dry summers (Wolf *et al.*, 2016). In addition, as phenological response to temperature and photoperiod was shown to highly differ among coexisting species, climate change will likely affect the competition among species (Vitasse *et al.*, 2011) and ultimately lead to shifts in species distributions (Chuine, 2010). Long-term phenological observations and experiments showed that spring tree phenology does not only depend on warm temperatures in spring because chilling temperatures during winter control the dormancy release, so that spring phenophases respond in a nonlinear manner to temperature increase (Fu, Zhao *et al.*, 2015; Morin, Roy, Sonie , and Chuine, 2010). While much attention has been paid to the effect of warming on trees, tree phenological responses to cooling have got little attention in the scientific literature (but see a recent study conducted on alpine meadows Li *et al.*, 2016). In particular, the advance of spring phenophases in response to global warming may put leaves in colder conditions that in turn may affect the subsequent phenophases. Understanding how plant phenology will respond to both warmer and cooler conditions is thus essential to predict the overall effect of ongoing climate change on plants.

Numerous studies have reported significant phenological shifts worldwide in response to ongoing climate warming (Fu, Piao, de Beeck, and Cong, 2014; Menzel and Fabian, 1999; Menzel, Sparks, Estrella, and Koch, 2006). Over the period 1980–2012, the dates of spring leaf phenology have advanced by 6–19 days in Europe and North America, respectively (Fu, Piao *et al.*, 2015). But warmer climate may not necessarily induce earlier leaf-out in spring because temperature has a dual role that affects both dormancy release in winter and the initiation of bud development in spring (Chuine, 2010; Harrington and Gould, 2015). It has been recently reported that warmer temperature recorded during the last two decades has resulted in an increase in forcing requirement for budburst of temperate trees in Europe, which is likely the result of insufficient chilling requirement for a full dormancy release or an increasing photoperiodic limitation (Fu, Zhao *et al.*, 2015). For example, for *Fagus sylvatica* L. (European beech), photoperiod plays an important role in regulating the timing of budburst, which then limits the extent of the phenological response to global warming (Basler and Körner,2014; Vitasse and Basler, 2013). The complex interaction between chilling, forcing temperatures, and photoperiod involved in bud dormancy is probably the reason why numerous questions remain open to predict future spring phenology. For instance, there is no agreement on how the expected decrease of chilling in winter will affect the budburst timing in the coming decades or which range of chilling temperatures is actually efficient to break the bud dormancy. In contrast to spring phenology, budset and leaf senescence in temperate trees have received fewer attention and the environmental cues that influence autumn senescence are still poorly understood (Gallinat, Primack, and Wagner, 2015; Panchen *et al.*, 2015). Photoperiod and temperature are considered as the main drivers of growth cessation and leaf senescence (Delpierre, Vitasse, Chuine, and Guillemot, 2016), but precipitation and nutrient availability may also play a non-negligible role (Liu *et al.*, 2016; Panchen *et al.*, 2015).

Most of the studies focus on a single phenophase and therefore do not consider any potential effect of one phenophase to the other phenophases occurring within the same growing season (Delpierre *et al.*, 2016) or even from 1 year to the next one, that is, the so-called carryover effect. Yet, growing evidences show that the different phenophases are not independent from each other (Delpierre *et al.*, 2016; Fu, Piao, Zhao *et al.*, 2014; Keenan and Richardson, 2015). Based on a manipulative warming experiment, Fu, Campioli, *et al.* (2014) found a carryover effect of spring over autumn phenology or even to the next year spring phenology. Considering that most experiments have been performed in climate chambers by manipulating temperature and photoperiod (Basler and Korner, 2012; Chung *et al.*, 2013; Fu, Campioli, Deckmyn, and Janssens, 2013; Fu, Piao, Zhao *et al.*, 2014; Sherry *et al.*, 2007), experiments conducted in natural conditions could provide precious insights to further explore these mechanisms and improve our understanding of the potential carryover effect of one phenophase to the next one.

In this study, we conducted a reciprocal transplant experiment between two elevations (1,340 m a.s.l. vs. 371 m a.s.l.) in the Swiss Jura mountains using saplings of *Fagus sylvatica* L. The upward and downward transplantation allowed us to simulate cooler or warmer temperatures during winter and early spring. Then, the saplings were moved back to their original elevations shortly after budburst in order to test the legacy effects of the budburst timing on the timing of budset in autumn and next year budburst. More specifically, we expect (i) an impact of warmer- and cooler-induced winter on autumn phenology through shifts in the beginning of growth onset, (ii) a stronger effect of a cooler winter than a warmer winter both on spring phenology and on the legacy effects for autumn phenology, and (iii) a carryover effect of autumn phenology on next year spring phenology.

5.5 Materials and methods

5.5.1 Study sites and experimental design

The experiment was conducted at two sites, at low and high elevation in the Swiss Jura mountains, in order to simulate warmer (transplant from the higher site to the lower site) and cooler (transplant from lower to higher site) conditions. The high-elevation site (Alpage des Amburnex: N46°540 , E6°230) was located at 1,340 m a.s.l. and has a mean annual rainfall of ca. 1,450 mm and a mean annual temperature of 5.5°C (2009–2015). The low-elevation site (Bois Chamblard: N46°270, E6°240) was situated at 371 m a.s.l. and has a mean annual rainfall of 1,180 mm with a mean annual temperature of 11.4°C (2009–2015). Hereafter, these two sites are referred to as “high site” and “low site” according to their elevation.

Figure 5-4 represents the configuration of the experiment at different time of the experiment that took place from autumn 2013 to spring 2015. In early September 2013, 60 six-year-old saplings of *Fagus sylvatica* L. (European beech) were collected in the vicinity of the two study sites, referred hereafter as the donor forests (Figure 5-4a), with 30 saplings from each donor forest. The saplings were selected for having similar height and morphology (diameter of 7–9 mm and height of 70–80 cm). When the plants were collected, we paid particular attention not to disturb the roots of the saplings by taking as much as possible untouched soil around the roots, in order to minimize transplantation stress. These saplings with their clods of about 30 cm diameter and 30 cm depth were then planted in pots (22 L, 35 cm of diameter and 40 cm depth) filled with their local soil. The transplant experiment (Figure 5-4b) was conducted on the week from the 23 to 27 of September 2013, as follows: At the low site, 15 of the 30 potted saplings served as a control for the cooling treatment and were kept at the low site in a protected common garden (low site common garden) during the whole experiment. They are referred hereafter as control cooling (CC). The other 15 saplings were moved to the high site in another protected common garden (high site common garden) to experience a cooler climate and are referred hereafter as cooling (C). Similarly, at the high site, 15 of the 30 potted saplings served as a control for the warming treatment and were kept at the high site common garden during the whole experiment. They are referred hereafter as control warming (CW). The other 15 individuals were moved to the low site common garden to experience a warmer climate

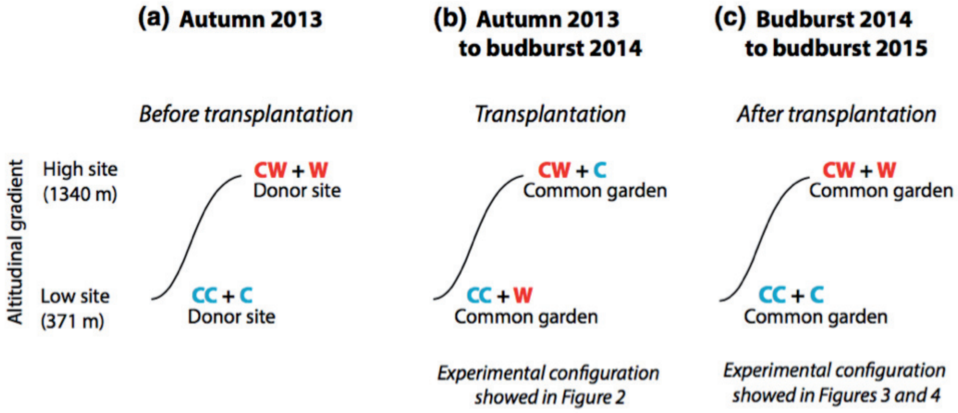


Figure 5-4 Experimental design of the transplant experiment before (a), during (b), and after the transplantation (c). The scheme represents the situation of the saplings of the different treatments at their different locations during the experiment. C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013–2014 (b) and then moved back to their original (donor) low-elevation site (c). CC refers to the control cooling treatment in which trees remained during the whole experiment at the low site. W refers to the warming treatment and represents the trees moved to low elevation during winter/spring 2013–2014 (b) and then moved back to their original site at high elevation (c). CW refers to the control warming treatment in which trees remained during the whole experiment at the high site

and are referred hereafter as warming (W). All pots were placed in an open area near the donor forest and buried into the ground, so that the top of the pots was about the soil surface. We monitored bud development in spring 2014, and approximately 2 weeks after budburst for each individual tree separately, they were transplanted back to their original site. The timing of 2 weeks allowed the leaves of each sapling to reach a minimum of maturation to increase their freezing resistance once back at its original site, especially important for the saplings going back to the high-elevation site. By that time, the tree reached the phenological stage 4 (fully unfolded leaf), but the shoot elongation was only at its beginning. At the end, all the potted trees were moved back to their original site from April 20th to May 9th and from May 29th to June 7th at the low- and high-elevation sites, respectively (Figure 5-4c). With this procedure, treatments and controls were under the same environmental conditions during the growing season 2014 and only differed in their budburst timing and previous winter conditions. It is also noteworthy that the elevational gradient allowed to induce contrasting temperature regime (mean difference of 4.2°C for the period from October 2013 to April 2014 between the two sites), while keeping similar day length between the two sites. Saplings only received water from natural rainfall. Except three individuals at the low site, all individuals survived during the whole experimental period up to spring 2015.

5.5.2 Phenological observations

Bud development was monitored during springs 2014 and 2015 using five distinct stages: dormant bud (0), swollen bud (1), budburst (2), leaves folded in a single plan (3), and at least one leaf fully unfolded (4), as commonly used for deciduous trees (e.g., Vitasse, Lenz, Hoch, and Körner, 2014). The observations were conducted on the apical bud of each sapling in each site by the same observer three times a week from March 24, 2014, until all saplings reached the stage 4. In summer/autumn 2014, while all transplanted saplings were back in their site of origin, we monitored budset on the shoot apical meristem three times a week using four different stages: ongoing leaf development (0), newly formed green and soft bud (1), small and brown bud (2), elongated (>1 cm), and brown bud (3). Budset timing was defined when a given sapling reached the stage 3, which was taken as a proxy for the cessation of the primary growth. Leaf coloration and leaf fall were also monitored, but we chose to use only budset data in this study as a proxy for growth cessation. The growing season length for each sapling was defined as the time in days between budburst and budset.

5.5.3 Climatic data

Relative air humidity, soil, and air temperature were recorded hourly in each site using EM50 data-loggers (Decagon Devices, Inc., Pullman, WA, USA). Air temperature sensors (VP3. Decagon Devices, Inc.) were placed at the average height of the saplings (i.e., 60 cm from the ground). Note that the high-elevation site was characterized by a snow cover up to ca. 1 m from the end of December to mid-March. Thus, saplings at the high site were covered by snow during some period in winter and may have experienced cooler temperatures than a few meters aloft. However, our temperature logger positioned at the tree canopy sapling captures the temperature as experienced by buds. Although numerous studies consider chilling temperatures to be comprised between 0 and +5°C, we considered temperatures between 1 and +5°C as efficient for chilling, because when the saplings of the high site were covered by snow, air temperature was slightly below 0°C and we assumed the temperature induced by snow to contribute to the chilling requirement. In order to assess the requirement of chilling and forcing temperatures for budburst, we calculated chilling hours as follows: We cumulated the chilling hours received from 1 November until budburst both in 2014 and in 2015. To evaluate the forcing temperature requirement to budburst, we calculated the number of hours above 5°C from 1st of February to the budburst date in both 2014 and 2015.

5.5.4 Growth and bud morphology

For each sapling, we measured the stem apical diameter (mm), the stem basal diameter at 3 cm from the ground (mm), and the stem length (cm). The four longest branches were labeled by colored strings, and their diameter and length were also measured. All growth parameters were assessed at the beginning and at the end of the growing season 2014. Additional measurements of the length and diameter of five buds were taken for each sapling including the apical and four surrounded buds at the end of the growing season 2014, as well as the total number of buds per saplings. The diameter of the buds was measured at one-third of the bud length from the base. All diameter and bud length measurements were made with a digital caliper (MarCal 16 EWR, accuracy 0.01 mm) while the stem and main branches length were measured with a tape (accuracy 0.1 cm).

We calculated the volume of the stem and branches assuming a truncated cone shape:

$$V_i = \pi \times \frac{D^2 + D \times d + d^2}{12} \text{ (mm}^3\text{)}$$

Equation 4 Volume estimation of stem and branches

where V is the volume of the stem or branch i, “D” is the basal diameter of the stem, and “d” is the apical diameter of the stem. The total volume of one individual sapling was then calculated as the sum of the volumes of the stem and the four main branches.

$$V_{tot} = V_{stem} + V_{branch1} + V_{branch2} + V_{branch3} + V_{branch4} \text{ (mm}^3\text{)}$$

Equation 5 Total volume for each individual sapling

The increase in the stem length and total volume was calculated as the difference between measurements conducted at the end (EGS) and at the beginning (BGS) of the growing season.

$$\Delta V = V_{EGS-2014} - V_{BGS-2014} \text{ (mm}^3\text{)}$$

Equation 6 Calculation of increase total volume

$$\Delta SL = SL_{EGS-2014} - SL_{BGS-2014} \text{ (mm}^3\text{)}$$

Equation 7 Calculation of increase stem length where “ΔV” is the total volume increase and “ΔSL” is the stem length increment.

5.5.5 Nonstructural carbohydrate (NSC) analysis

The NSCs are defined as low molecular weight sugars (glucose, fructose, and sucrose) plus starch. In November 2014, when leaves of all saplings were senescent (i.e., either colored or fallen), we collected one woody twig of 6 cm from each sapling (i.e., 58 samples) corresponding to the tissue produced in 2014. Within 2 hr after collection, the samples were heated at the laboratory in a microwave oven at 800 W for three times for 25 s, allowing to denature enzymes and stop microbial activity. Samples were then immediately dried to constant weight at 60°C. NSC was analyzed as described in Hoch, Popp, and Korner (2002) using an enzymatic digest technique with subsequent spectrophotometric glucose tests (Li, Hoch, and Korner, 2002). Samples were boiled in distilled water and after centrifugation, treated with invertase and isomerase to convert fructose and sucrose into glucose. Glucose was converted to gluconate-6phosphate using a Hexosekinase reaction kit (Sigma Diagnostics, St. Louis, Mo., USA). The insoluble material was kept at 40°C with the dialyzed crude enzyme clarase from Aspergillus oryzae to break down starch to glucose. Starch and sugar standards as well as laboratory standard of plant powder were used as controls for analyses. Finally, sugar, starch, and NSC concentrations (%) were calculated on a dry matter basis.

5.5.6 Data analysis

The experiment followed a one-factor design with repeated measurements. The studied factor consisted in four treatments: C = cooling, CC= control cooling, W = warming, and CW = control warming. The experimental unit was a pot with a single individual, which is also the observational unit. For the analysis of budburst, chilling, and forcing hours, each plant was observed twice, on year 1 (2014) and on year 2 (2015), while budset was observed only during year 1. Year is therefore a within factor and is considered in the analysis as repeated measurement. The design was slightly unbalanced due to some mortality in the warming treatment: treatments C and CC with n = 15 individuals, treatment CW with n = 12, and treatment W with n = 14 individuals. An analysis of variance was performed for testing the treatment effect (either cooling or warming) on budburst and budset timing, on chilling and forcing hours requirement, as well as on NSC content. Tukey tests adapted for repeated measurement designs were used to compare the statistical differences among the treatments. The relation between chilling and forcing was analyzed with linear regressions, both through the means of the different treatments ($n = 4$) over the 2 years 2014 and 2015 and through the individual sapling data. Growth and bud morphology parameters were analyzed using an analysis of variance at one factor for both treatments separately.

All analyses were performed using R 2.5.3 (R Core Team, 2013) using the R-package “easyanova” (Arnhold, 2013) to perform the ANOVA and calculate the Tukey tests.

5.6 Results

5.6.1 Budburst in spring 2014 after a cooling or warming winter

The mean budburst date in the cooling treatment (C; saplings transplanted to the high site) was significantly later than in the control treatment (CC; saplings kept at the low site), with a mean delay of 46 ± 2 days (Figure 5-5a). Likewise, the mean budburst date for saplings in the warming treatment (W; saplings transplanted to low site) in 2014 was significantly advanced (mean 37 ± 2 days) compared to the control warming (CW; saplings kept at the high site) (Figure 5-5a). No difference was detected in the budburst timing of saplings at the high-elevation site between the control warming and the cooling treatments, whereas at the low site budburst occurred 8 days earlier in the control cooling treatment than in the warming treatment ($p < .001$).

The magnitude of the impact of warming and cooling on the timing of budburst was significantly different. Giving the temperature difference of 4.2°C (see first section of Material and Methods) between the two sites during the reciprocal transplantation, that is, from October 2013 to April 2014, the cooling treatment showed a delay of 10.9 days per degree decrease in air temperature measured at sapling height, whereas the warming treatment showed an advance of 8.8 days per degree increase in air temperature (Figure 5-5a).

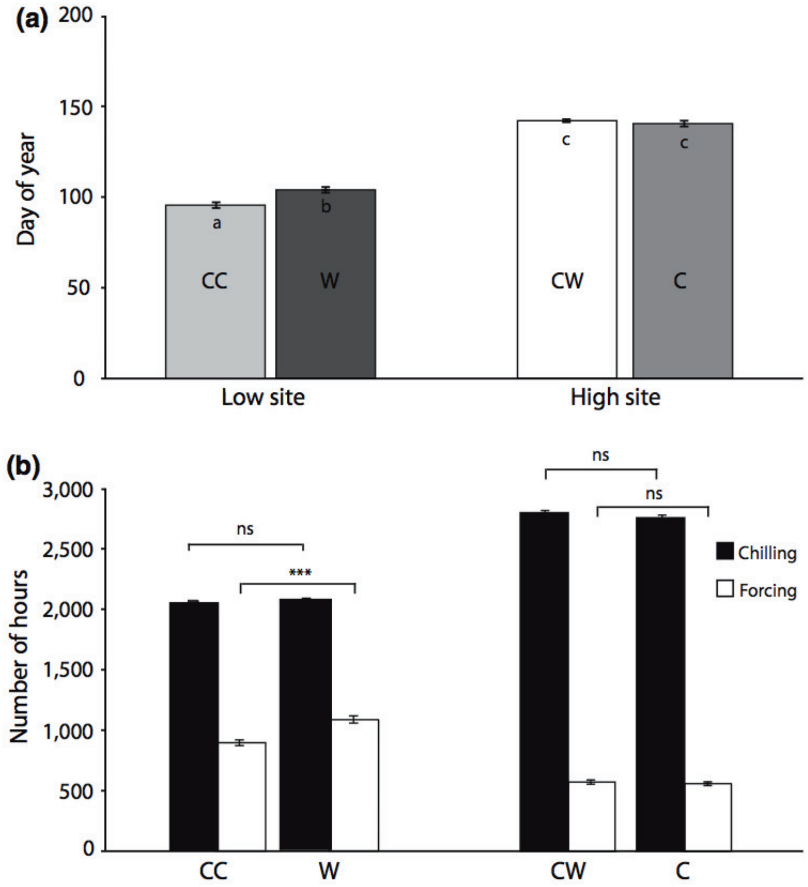


Figure 5-5 Budburst timing in 2014 (day of year) of European beech (a) and number of forcing hours and chilling hours required to budburst for each site (low and high elevation) and treatment (b). C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013–2014 and then moved back to their original site at low elevation. CC refers to the control cooling treatment in which trees remained during the whole experiment at the low site. W refers to the warming treatment and represents the trees moved to low elevation during winter/spring 2013–2014 and then moved back to their original site at high elevation. CW refers to the control warming treatment in which trees remained during the whole experiment at the high site. Bars represent mean values `1 SE for each treatment (n = 15). Different letters in the histograms (a) denote significant differences (at $p < .05$). The square brackets above the histograms (b) are used to compare chilling hours and forcing hours per treatment at the same site, respectively, with ns for nonsignificant difference and *** for $p < .001$

Although saplings under cooling control (CC) and warming treatments (W) at the low site experienced similar duration of chilling, that is, 2,051 and 2,055 hr, respectively, from 1st of November to budburst (Figure 5-5b), saplings under warming treatment required 189 additional forcing hours to budburst as compared to saplings under the cooling control ($p < .001$; Figure 5-5b). By contrast, saplings of the cooling treatment

(C) and warming control (WC) at the high site required almost the same duration of forcing hours to budburst, that is, 560 and 566 hr, respectively (Figure 5-5b).

5.6.2. Carryover effects of the timing of budburst on budset, and next year budburst

The cooling treatment in winter 2013–2014 induced a significant delay of 31 days in budset in autumn 2014 ($p < .001$; Figure 5-6a) while the warming treatment induced an advance of 10 days in budset compared to the control, but not significant (Figure 5-6b). Interestingly, we observed the same trend on the leaf senescence date as no effect of treatment, warming, or cooling was found on the time lag between budset to leaf senescence at each site (Table 5-1). Nevertheless, the two populations showed differences in their time lag, with 27–33 days for the control cooling (CC) and the cooling treatments (C), and 2–6 days for the control warming (CW) and the warming treatments (W), respectively. In detail, we found that 10-day delay in spring budburst induced by the cooling treatment was associated with 6.7-day delay in the timing of budset in autumn compared to the control, whereas no significant change of the budset timing was observed in the warming treatment with 10day advance in budburst associated with 2.7 days in the budset date (Figure 5-6b).

Table 5-1 Time lag expressed in days between the date of budset and the date of leaf senescence for control cooling (CC) and cooling (C) treatments at the low site and of control warming (CW) and warming (W) treatments at the high site in autumn 2014. Additionally to the budset, leaf coloration and leaf fall were also monitored and we considered individual sapling as senescent when 50% of its leaves were colored or fallen according to the methodology employed in Vitasse, Porte *et al.* (2009). Data are means ± SE with n = 10.

Site	Treatment	Time lag (budset to senescence)	
Low	C	33 ± 5.4	ns
	CC	27 ± 3.9	
High	W	6 ± 2.4	ns
	CW	2 ± 3.5	

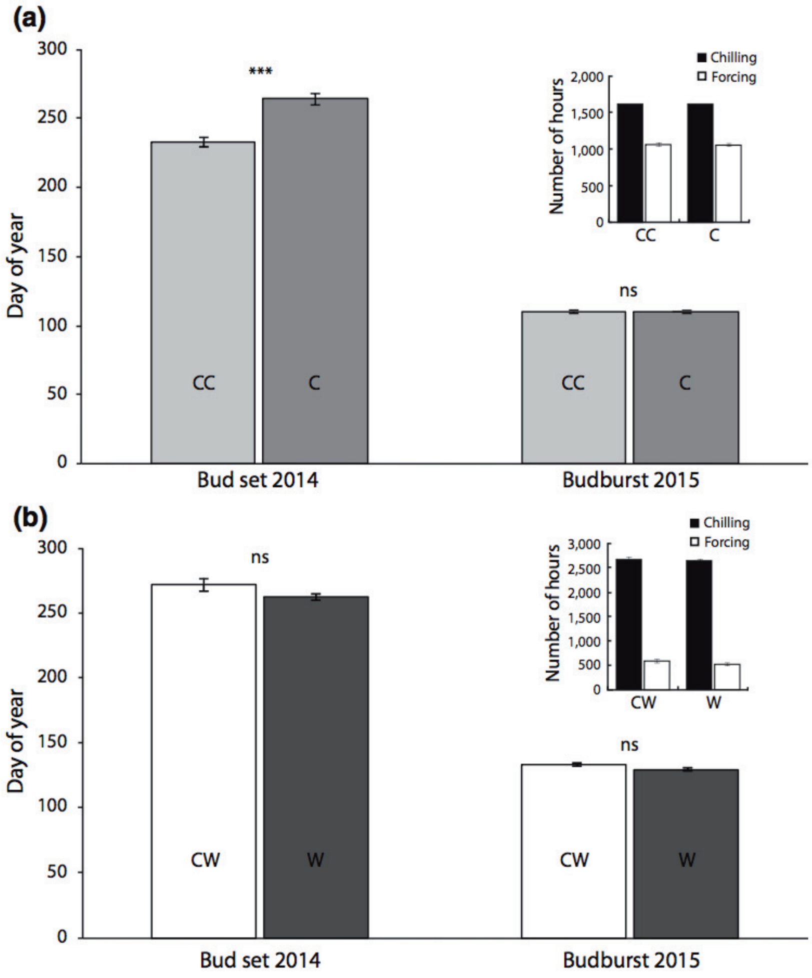


Figure 5-6 Budset timing in 2014 and budburst timing in 2015 (day of year) of control cooling (CC) and cooling (C) treatments at low elevation (a) and of control warming (CW) and warming (W) treatments at high elevation (b). Number of forcing and chilling hours required to budburst, respectively to their treatments, is indicated in the insets. Bars represent the mean values `± SE for each treatment (n = 12 for CW, n = 14 for W and n = 15 for CC and C). The stars denote a significant difference (at $p < .001$) between treatment at the different timings while ns is used for nonsignificant difference

In spring 2015, that is, 1 year after the saplings were moved back to their original sites, we did not find significant differences in the budburst date between the cooling treatment and its control (Figure 5-6a) or in their forcing requirement (1,041 and 1,053 hr, respectively; inset Figure 5-6a). Moreover, despite 4-day advance in the warming treatment (Figure 5-6b) that required 68 forcing hours less than the control (inset Figure 3b), the shifts, that is, both budburst timing and forcing requirement, were not significant ($p = .16$) between the warming and control treatments. Nonetheless, during that second spring, the first individuals that tended to budburst first were all from the warming treatment, as shown in Figure 5-7. Actually, frost damages were observed on the developing new leaves at the high-elevation site on May 11, 2015 (red arrow, Figure Figure 5-7), and at that time, among the damaged individuals, seven saplings of 14 from the warming treatment reached budburst, while only three over 12 reached the same stage for the control warming saplings (Figure 5-7). Before this specific date, we found that air temperature (blue line, Figure 5-7) dropped three times to freezing temperatures, up to 2.5°C. After this date when temperatures got warmer, all saplings from the warming treatment reached budburst by the 17th of May, while the rest of the saplings from the control warming treatment reached this stage by the 26th of May.

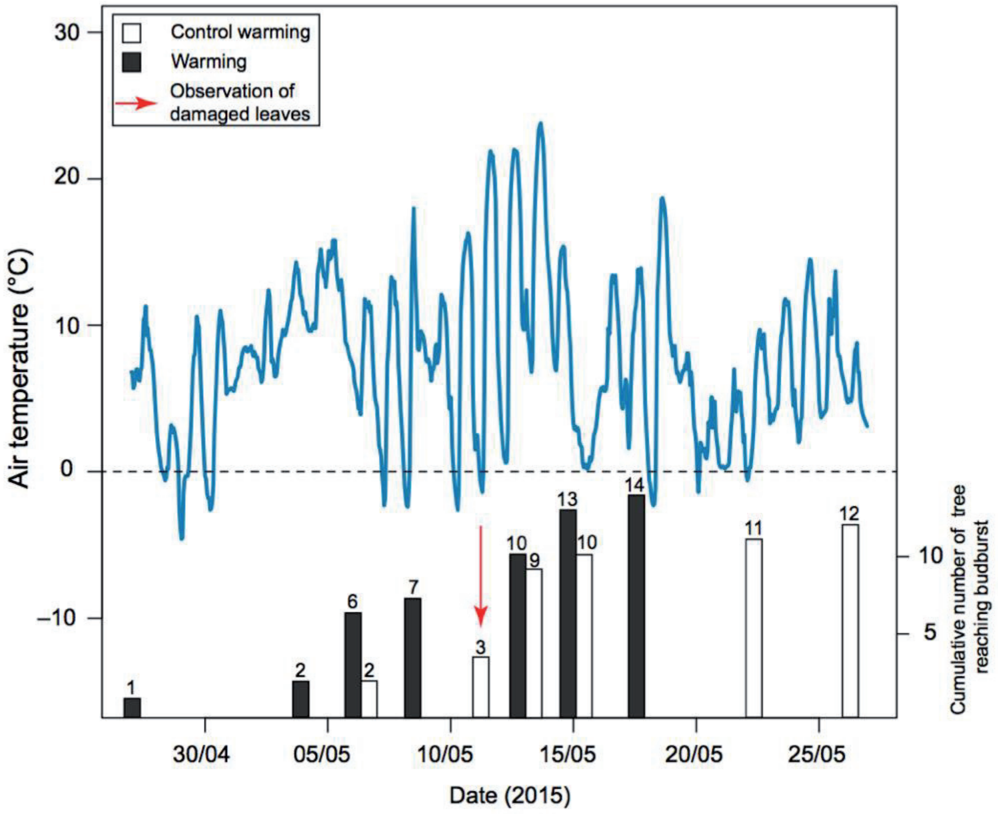


Figure 5-7 Air temperature (blue line) and cumulative number of trees (histograms) that have reached the budburst stage for the warming treatment and control warming in spring 2015 at the high site. The red arrow indicates when frost-damaged leaves (brownish young leaves) were observed in the field

Interestingly, for all saplings in each treatment over 2014 and 2015, the duration of forcing hours required to budburst was negatively correlated to the duration of chilling hours experienced by the saplings (R2 = .89, p < .001). The more chilling experienced by the saplings, the less forcing was required to budburst (Figure 5-8(

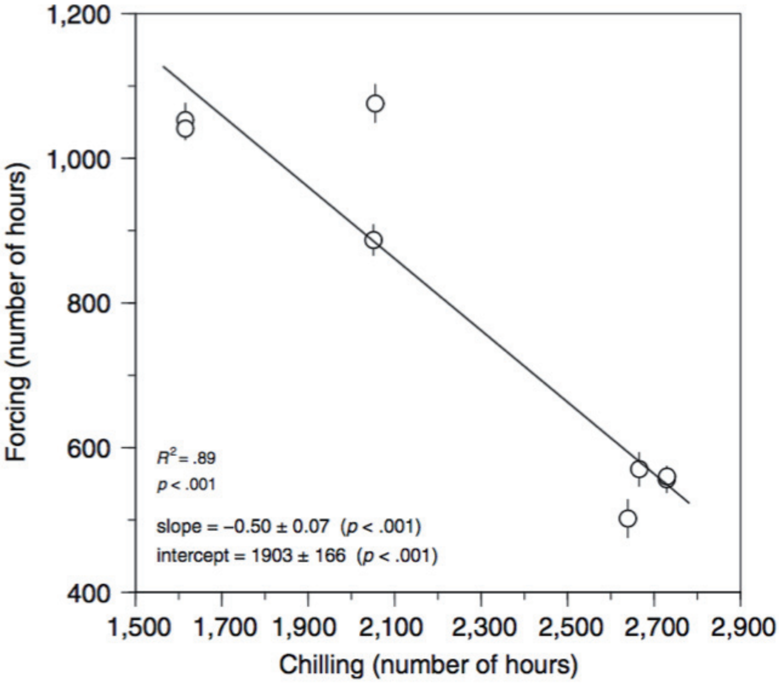
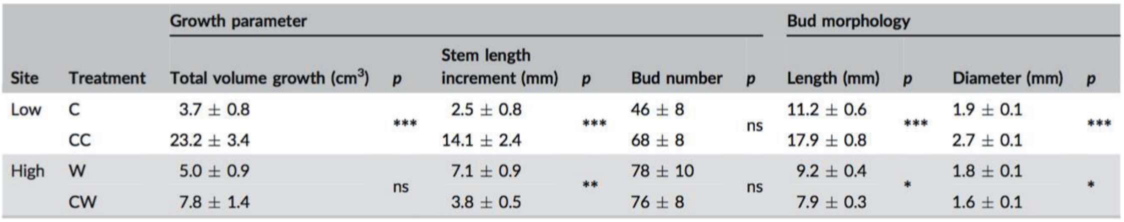


Figure 5-8 Relationship between the accumulated number of chilling hours received (for air temperature between 1 and +5°C) from the 1st November to budburst, and the number of forcing hours required to budburst, calculated as the sum of hours above 5°C from the 1st of February to budburst date for each treatment in both 2014 and 2015 (linear regression). Air temperature was recorded at the canopy level, i.e. around 60 cm above the ground

5.6.3 Carryover effect on growth, bud morphology, and non-structural carbohydrates (NSCs)

Stem length increment and total growth in volume during the growing season 2014 were found to be associated with the previous year's warming–cooling treatments (Table 5-2). Cooling treatment (C) had a negative impact on both parameters. Although saplings in the cooling treatment experienced the growing season at the low site (as they were moved back to low elevation shortly after budburst), the cooler winter and early spring conditions and delayed-induced budburst had significantly decreased their growth (i.e., volume growth and stem length increment; Table 5-2) compared to saplings of the cooling control (CC) (p < .001). At high elevation, the difference in growth between warming (W) and control warming (CW) was less marked (with 5.0 and 7.8 cm3 in volume growth for W and CW, respectively), but the stem length increased consistently (.01 < p < .001) between CW and W (with 3.8 and 7.1 mm, respectively). The number of buds was not statistically affected by both treatment warming and cooling compared to their respective controls. Interestingly, saplings from the cooling treatment (C) had smaller buds (p < .001), in length and diameter, after the end of the growing season, than those from the control treatment (CC). Conversely, saplings from the warming treatment (W) had longer and larger buds (.05 < p < .01), compared to their control (CW).

Table 5-2 Growth and bud morphology parameters measured at the beginning and at the end of the growing season 2014 for a given site and treatments. C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013–2014 and then moved back to their original site at low elevation, and CC refers to the control cooling treatment in which remained during the whole experiment at the low site. W refers to the warming treatment and represents the trees moved to low elevation during winter/spring 2013– 2014 and then moved back to their original site at high elevation, and CW refers to the control warming in which remained during the whole experiment at the high site. Data represent mean `1 SE with n = 10. The stars denote a significant difference (at p < .001) between treatments at the different timings while ns is used for nonsignificant difference.



		Growth parameter						Bud morphology			
Site	Treatment	Total volume growth (cm^3)	p	Stem length increment (mm)	p	Bud number	p	Length (mm)	p	Diameter (mm)	p
Low	C	3.7 ± 0.8	***	2.5 ± 0.8	***	46 ± 8	ns	11.2 ± 0.6	***	1.9 ± 0.1	***
	CC	23.2 ± 3.4		14.1 ± 2.4		68 ± 8		17.9 ± 0.8		2.7 ± 0.1	
High	W	5.0 ± 0.9	ns	7.1 ± 0.9	**	78 ± 10	ns	9.2 ± 0.4	*	1.8 ± 0.1	*
	CW	7.8 ± 1.4		3.8 ± 0.5		76 ± 8		7.9 ± 0.3		1.6 ± 0.1	

The NSC concentration did not show significant differences between warming–cooling treatments and their controls (Figure 5-9). Indeed, in spite of the substantial shift of budburst timing due to the cooling or warming treatments, the proportion of NSC stored in the twigs produced during the current growing season reached similar levels. The weak differences seem to root more in the origin of saplings (donor site) rather than the result of the treatments. Saplings coming from the lower site tended to have higher concentrations of total NSC and higher starch proportion compared to sugar. Only saplings from the warming treatment produced as much sugars as starch, showing a significant change in the proportion of sugar (p < .001) (Figure 5-7; Table S1), compared to the other treatments. Furthermore, no apparent relationships were observed between the total NSC content and the growing season length 2014 (GSL), as the population from the low site (CC and C), showing the higher NSC content, had a GSL comprised between 123 and 137 days ($p < .06$), while the population from the high site (CW and W) showed a GSL between 131 and 159 days ($p < .001$; Figure 5-7). Interestingly, the cooling and the warming treatment did not affect the GSL in the same way, that is, cooling reduced the GSL by 14 days whereas warming increased the GSL by 28 days.

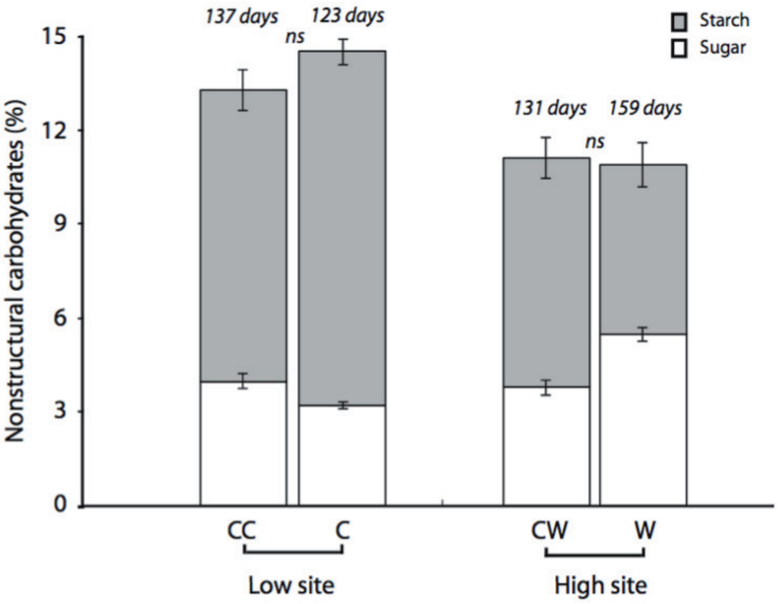


Figure 5-9 Percentage of nonstructural carbohydrates (sugar and starch) and growing season length 2014 (numbers above histograms) of control cooling (CC) and cooling (C) treatments at the low site and of control warming (CW) and warming (W) treatments at the high site. Bars represent the mean values ± SE (n = 10), for which significance level between treatments and their controls are indicated for the total nonstructural carbohydrates (sugar + starch content)

5.7 Discussion

Using an original transplant experiment from a ~1,000-m elevation gradient allowed us to induce natural warming and cooling to European beech saplings. We showed that spring budburst phenology has a significant but different response to warming and cooling. In particular, we found that beech trees had a greater budburst response to cooling than to warming, that is ~11-day delay vs. ~9day advance per degree cooling and warming, respectively. Interestingly, the induced advance or delay in the budburst due to the downward or upward transplantation significantly affects the budset timing in the following autumn, while the saplings were growing at their site of origin during the duration of the growing season. Earlier budburst correlated with earlier budset timing and vice versa. However, the magnitude of this legacy effect of spring phenology over autumn phenology differed between the warming and the cooling treatments with higher legacy effect found in the cooling treatment. Additionally, this asymmetric effect of warming and cooling is reflected on the growing season length (GSL), where cooling reduced the GSL by 14 days whereas warming increased GSL by 28 days. Our study provides evidence in natural conditions of the carryover effect of spring phenophases over following phenophases in a temperate tree.

5.7.1 Asymmetric budburst response to cooling and warming

Our study showed that one degree of temperature change induced a larger phenological shift in the cooling treatment than in the warming treatment. This result is consistent with the theory that warmer temperatures may reduce the duration of chilling to fully release winter endodormancy and therefore increase the duration of forcing required to budburst. Similarly, Fu *et al.* (2013) found no further advance in the leaf-out timing of oak and beech when temperature was artificially warmed by 6°C compared to the 5°C warming, in which earlier leaf-out was still observed. European beech is known to have a high chilling requirement for the dormancy release and to interact with photoperiod (Vitasse and Basler, 2013). The population from high elevation was exposed to a warming of about 6°C. We therefore suggest that spring phenology in the warming treatment has responded less than in the cooling treatment due to a lack of chilling exposure or too short photoperiod that has reduced the sensitivity of buds to respond to forcing temperatures (Basler and Körner, 2014). To our knowledge, only one study has shown an asymmetric phenological response to warming and cooling by moving plants in different climate conditions, but on alpine meadows (Li *et al.*, 2016). The authors showed that prolonged phenological stage, such as flowering, induced by transplantation to warmer locations, led to longer reproductive phases and activity period, whereas cooler conditions led to shorter vegetative and reproductive phases. Studies using natural climatic gradient "as experiments by nature" are particularly relevant as plant phenological responses to artificial warming in experiments were found to significantly differ from long-term series of observations (Wolkovich *et al.*, 2012).

Interestingly, we found that during the first spring 2014, saplings in warming treatment required 189 forcing hours more than the control cooling treatment, although they were under the same conditions during winter, and hence received the same duration of chilling hours. This difference in forcing requirement to budburst at the lowest site may be caused by a genetic differentiation between low- and high-elevation populations as it is often found for the timing of budburst (Vitasse, Delzon, Bresson, Michalet, and Kremer, 2009; Vitasse, Porte , Kremer, Michalet, and Delzon, 2009). Nevertheless, the difference between the two populations vanished at the high site and might be due to genes vs. environment interactions (Vitasse *et al.*, 2013; Vitasse, Lenz, Kollas et al, 2014). Indeed, Vitasse *et al.* (2013) found a higher differentiation in the timing of budburst between different populations of beech trees in their lower elevation common garden, and as in our study, this differentiation vanished or was reduced at the high-elevation common garden. It could be that under warmer conditions (low elevation), warm-adapted individuals for which dormancy is released can start their growth, while cold-adapted individuals get less chilling and therefore need more forcing requirement (Figure 5-8). Conversely, under colder climate (high elevation), even if some individuals are released from dormancy—that is, warm-adapted population—and have fulfilled their chilling requirement, temperatures are still too cold, so that when temperature gets finally warmer, all populations start at the same time. The fact that phenological processes are not only affected by climatic differences across space, but also by underlying geographic variations in plant genetics, due to long-term climatic adaptation, has been already described on a broader scale and larger number of temperate species (Liang, 2016). This author demonstrates that spring phenology of colder climate-adapted populations can be either advanced compared to warmer climate-adapted populations through lower thermal requirements, or delayed because of higher chilling demands for dormancy release. Our results may also be explained by a carryover effect from the previous growing season since warming saplings originate from a forest located at higher elevation.

5.7.2 One phenophase can affect subsequent phenophases

The induced delay or advance of budburst had a strong impact on the timing of budset, used here as a proxy for primary growth cessation. The carryover effect of spring phenology over budset timing and leaf senescence was different in the two treatments: A delay in budburst induced by a cooler winter had more effect on budset timing than an advance of budburst induced by a warmer winter. This, again, highlights that effects of cooler conditions on phenological events will not simply mirror effects of warmer conditions in the opposite direction (Li *et al.*, 2016). It is noteworthy that the time lag between budset and leaf senescence timing was not affected by either treatment but differed in duration between the two populations. This could be explained by genetic differentiation between low and high populations as high populations may have evolved to start senescence and subsequent cold acclimation earlier than low elevational populations (Arora, Rowland, and Tanino, 2003; Vitasse, Lenz, and Körner, 2014). Our findings confirm that spring phenophases could affect autumn phenophases, as recently found in experimental conditions (Delpierre *et al.*, 2016; Fu, Campioli *et al.*, 2014; Keenan and Richardson, 2015). The relationship between spring and budset or autumn phenology might be explained by the effect of NSC storage (Fu, Campioli *et al.*, 2014). In agreement with Fu, Campioli *et al.* (2014), no significant difference in the NSC concentration was found between the treatments and their respective controls. Saplings in control and treatment were under the same environmental conditions during the whole growing season but differ in their spring phenology (due to warmer- or cooler-induced winter conditions). Thus, irrespective of the growing season, beech trees maintain a minimum of NSC content. The dynamic of NSC storage on branchlet may be seen as a balance between promoting primary growth or accumulating reserves in case of stress. Noteworthy, plants from the cooling treatment have grown 84% less (total volume growth) than the control even though they have spent the majority of the growing season in the same conditions at low elevation but have finally accumulated slightly more reserves than saplings in the control. This result suggests that an artificial delay of budburst promotes the accumulation of sugars until reaching a minimum threshold before to allocate carbon to growth. Similarly, in the warming treatment, saplings did not have higher NSC content even though they leaf-out earlier and had extended their growing season and growth was therefore promoted (stem elongation increased by 186% in comparison with the control). In these favorable conditions, the minimum threshold of NSC accumulation must have been reached way earlier, leaving more time for growth. Our study therefore supports the hypothesis of a minimum threshold of NSC reserves to ensure tree survival (Nardini *et al.*, 2016), tree resilience against stress conditions (Hartmann and Trumbore, 2016), and enough reserves in the twigs for next spring phenology, which strongly rely on the breakdown of branchlet starch as the main carbon source for budburst and leaf development (Klein, Vitasse, and Hoch, 2016). The relation between NSC content and budset or senescence is unclear, but trees may have a specific requirement of NSC storage that would inhibit growth and promote budset, which might be slightly different among the two provenances (Herold, 1980). A possible explanation is that once the trees fulfil their NSC requirement (storage capacity), they start the senescence process, because an excess of NSC may inhibit photosynthesis (Gent and Seginer, 2012; Seginer and Gent, 2014). Further investigations about the dynamics of NSC accumulation during this period would be relevant to better understand to what extent autumn phenology plays a role in the carbon pools of trees (but see Klein and Hoch, 2015; Klein *et al.*, 2016).

We did not find a significant carryover effect on next year spring phenology in both cooling and warming treatments, on the contrary to what was observed in previous warming experiment (Fu, Campioli *et al.*, 2014), although a non-negligible advance in the number of trees that reached budburst was observed for the saplings that experienced a warmer winter the year before, compared to their control. It is likely that this advance disappeared because of a freezing event that occurred shortly after the first signs of budburst, buffering the potential differences between the treatments, and also indicating that despite a potential memory effect from a previous warm winter/spring the year before (2013/2014), the climatic variables of the previous months (2015) were the dominant drivers of spring phenology.

5.7.3 Relationship between chilling and forcing requirements

Our study also supports the negative relationship between the duration of chilling and the forcing requirements for budburst: the more chilling beech saplings experienced, the less forcing they required to budburst. Even if the number of chilling hours was quite high at the high site (around 2,700 hr), trees required a significant duration of forcing (around 550 hr) to budburst, which is supposed to be a minimum heat requirement when chilling exceeds requirement for a full dormancy break. At the low site, beech saplings required significantly more forcing hours to budburst (around 1,000 hr). The low number of chilling hours received compared to the plants placed at the high-elevation site (around 2,000 hr compared to 2,700 hr) together with the short photoperiod during early spring may have enhanced the forcing requirement to budburst, especially as this species has been shown to require long duration of chilling to fully break dormancy and its sensitivity to forcing temperatures is higher under longer photoperiod (Basler and Körner, 2014; Vitasse and Basler, 2013). This control of chilling and photoperiod has been seen as a mechanism to avoid a too early flushing when late winter temperatures get warmer and therefore limit the risk of young leaves to be exposed to late spring frost (Vitasse, Lenz, Hoch *et al.*, 2014). This is particularly important because emerging leaves are the most sensitive stage to freezing temperatures (Lenz, Hoch, Vitasse, and Körner, 2013).

5.7.4. Limitations of the study

Although the phenology of saplings may not represent the phenology of adult trees (Vitasse, 2013), assessing their response to climatic variability is relevant as young trees represent the next generation in mature forests. The carryover effect of one phenophase to the next ones might be less pronounced for saplings compared to mature trees for which other factors such as flowering and seed formation might play a regulating role. Furthermore, other limitations such as precipitation or soil nutrient availability that were not controlled in our experiment may have also affected the timing of budset.

The legacy effects of earlier or late budburst on next year spring phenology is not fully discarded as late spring frosts observed in May in our study may have buffered the possible differences as previously mentioned. Because our study was focused on the legacy effect of the timing of budburst on the next phenophases, our experimental design was not appropriate to test the legacy effect of autumn phenology on next year spring phenology. The timing of leaf senescence and/or budset may postpone the dormancy period and therefore influence spring phenology on the following year, possibly counterbalancing the effect of global warming (Heide, 2003). A similar experiment focusing on the legacy effect of the timing of growth cessation would therefore be relevant to complement our understanding of relationships between phenophases.

Our study focused on a single species which is dominant in Europe but very particular in terms of phenology compared to coexisting species (Vitasse and Basler, 2013). Investigating the legacy effect of spring phenology over autumn phenophases in an array of species would be crucial to know whether we can generalize our results to other species and to better understand the future phenological responses of trees to ongoing climate warming.

In conclusion, we found that budburst timing of beech trees responds more to cooling than to warming. Furthermore, the induced delay or advance of budburst had a strong impact on the timing of budset, used here as a proxy for primary growth cessation, with again a stronger legacy effect of a cooler winter than of a warmer winter. As shown using in situ long-term series of observations (Keenan *et al.*, 2014), we suggest that the potential delay in senescence processes due to global warming might be smaller than expected because of this positive relationship between spring budburst timing and autumn phenophases. Our study also supports that a carryover effect could either modify the temperature sensitivity of the buds, that is, changing the chilling and forcing requirements for dormancy break and initiation of bud development, respectively or postpone the different phenophases. Overall, our experimental study provides direct evidence that besides the abiotic factors, the internal biotic effects should be considered in phenological models to improve predictions of trees response to climate change and models' performance.

5.8 Acknowledgements

The work was supported by the research program "Waldund Holzforschungsfonds" (BAFU-WHFF), by the "Forschungsprogramm Wald und Klimawandel" (BAFU-WSL), and by the Foundation Les Bois Chamblard (EPFL). We would like to thank Dr. Gu€nter Hoch from the University of Basel for his precious help and advices on the analysis of the nonstructural carbohydrates. We are also grateful to R. Mijancos who helped in the various tasks during the field work and to V. Jassey for his support on the data analysis.

Chapter 6 Discussion

Beech or spruce? cartoon by Alejandra de Cárcer (2014)

"Finished is better than perfect"

Paola Sanginés de Cárcer (2017)

The main objective of this thesis was to determine the growth response of European beech and Norway spruce saplings subjected to experimental warming forcing and to explain these responses at different organizational levels (tree, leaf and tissue). Besides, some soil factors were also investigated. By using transplantation along an elevational gradient in the Jura Mountains, I created realistic climate conditions to simulate three potential future climatic scenarios from the IPCC (Gavazov *et al.*, 2014). The site at 1010 m was equivalent to the conditions predicted for the A1B scenario with a moderate increase of greenhouse gas emissions. The site at 570 m was the equivalent to the scenario A2 related to the case of a notable increase of greenhouse gas emissions. Finally, at 395 m the extreme scenario from A2 was simulated. This experimental set up made it possible to distinguish short-term responses (2012-2015) in the Framework of the Forest and Climate Change Program carried out by WSL and OFEV (Pluess *et al.*, 2016).

By exposing saplings to an average increase of 6.3°C and a reduction in 30% of precipitation at the lowest site throughout the study period, I observed a significant increase of tree growth for beech that was not observed for spruce (Chapter 2). Accelerated beech growth was in part explained by a lengthening of the growing season (Chapter 2). Despite spruce presenting also an advanced budburst compared to the control site, and thus a longer vegetative period, it did not show an increase in growth at the tree level. The investigation into the phenotypic plasticity of foliage traits and physiological processes, such as photosynthesis, revealed a higher degree of plasticity in beech compared to spruce (Chapter 3 and 4). In summary, beech presents the potential for acclimating to rapid climate change and has therefore a competitive advantage over spruce (Chapter 4), at least when considered on a short-term basis.

In this chapter, I discuss the results of the thesis in the context of the questions posed in chapter 1 and present them in the wider context of these species' responses to climate change. Finally, I propose avenues for future research that emerge from the findings of this work.

6.1 Main findings

The main results resulted from this thesis are summarized in the conceptual Figure 6-1.

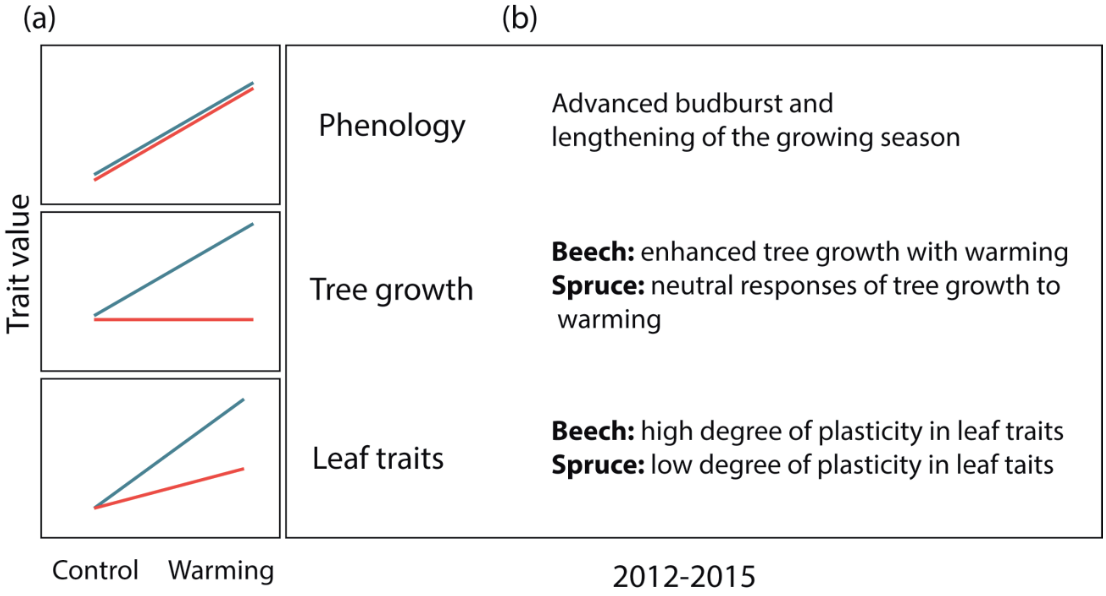


Figure 6-1 Conceptual Figure of CLIMARBRE findings in the three main topics approached in the thesis. Phenology (Chapter 2, 4 and 5), tree growth (Chapter 2 and 4) and leaf traits (Chapter 3 and 4).

6.1.1 Tree growth under simulated climate change

The productivity of a forest stand is directly linked to the growth and vitality of individual trees that composes it. In Chapter 2, key climatic factors limiting saplings' growth were presented. The observed growth performances of beech saplings confirm the results of National forest inventories, which already show a decrease in the contribution of spruce at low and mid elevations (Brändli, 2010) in favor to beech. In general, beech seems to take advantage of warmer conditions at lower altitudes to increase its growth. Additionally, models including extreme climatic variables improved and refined the assessment of the tree growth responses to simulated climate change, which is also corroborated by Zimmerman *et al.* (2009).

Results on phenology (Chapter 5) showed that increasing temperatures triggered budburst at lower elevations, leading to a lengthening of the growing season. This is corroborated by many studies (Menzel and Fabian, 1999; Piao *et al.*, 2007; Keenan, 2015; Signarbieux *et al.*, 2017). One would expect that with warming, growing seasons will become increasingly longer in temperate forests, and thus trees would be able to grow as much as the season lengthening. However, in chapter 2, I showed that a longer growing season could not fully account for species-specific growth responses to warming, which confirms Körner's hypothesis that a longer growing season enhances tree growth up to a certain limit (Körner, 2017).

Contrasting tree growth responses to warming between angiosperms and gymnosperms has been widely hypothesized (Carnicer *et al.*, 2013), and I demonstrate empirically in Chapter 2 that this is related to hydraulic safety margins. Thus, the contrasting growth response of beech and spruce has been shown in this thesis to be linked to different sensitivities to elevated vapor pressure deficits. Furthermore, I determined for the first time a threshold above which tree growth starts to decline for each species under no soil moisture limitation. This will have repercussions for the parametrization of predictions of species distributions in future climate change conditions.

6.1.2 Responses of foliage to simulated climate change

In chapter 3, I show that leaf traits are able to track the impact of environmental changes. Many authors have previously recognized leaf phenotypic plasticity as a key process by which plants cope with rapid climate changes (Nicotra *et al.*, 2010; Vitasse *et al.*, 2010; Stojnić *et al.*, 2015). By means of a plasticity index defined by Valladares *et al.* (2000), I compared the overall plasticity of both species and revealed a higher response of beech leaves than spruce needles to warmer and drier conditions. Beech leaves presented an increase in leaf xeromorphism through the increase of cuticle thickness, vein network and smaller stomata, which was associated with a higher leaf area. This antinomic response suggests that beech can grow in warmer conditions while coping with an increase of evaporative demand of the air during summer. This may eventually provide beech with a competitive advantage over spruce, which itself did not present as much plasticity as compared to beech. Moreover, an interannual variability in foliage responses revealed a fast acclimation of foliage to acclimate to yearly conditions.

It is not possible to predict long-term leaf morphological changes from the data presented here; however, I demonstrate the clear potential plasticity of these species by confirming the fast adjustment of foliage, which is the first step towards potential adaptation. Therefore, as suggested by Bradshaw (1965), plants are able to manage and respond to environmental changes through phenotypic plasticity in morpho-anatomical traits.

The coordination of the different phenophases with annual climate variations will be key factor leading to species adaptations to climatic constraints. In this study leaf phenological phases were found to be strongly related between each other and that cooling has a major impact in the timing of budburst than warming. An induced delay or advance of budburst was observed to have strong impacts on the timing of budset, and therefore the primary growth cessation. Overall, our experimental study provides direct evidence that, besides the abiotic factors, the internal biotic effects should be considered in phenological models to improve predictions of trees response to climate change and models' performance.

6.2 Research questions

In the context of the study, four different research questions were chosen to investigate the impact of simulated climate change in the performance (i.e. tree growth) of beech and spruce saplings. To which extent these questions were answered is presented in the following section.

Q1. Will the saplings of spruce and beech saplings acclimate to simulated climate change?

Very few studies exist that compare the acclimation abilities of species at different structural levels, from foliage tissue to tree biomass (chapter 4). I found evidence of acclimation in saplings subjected to moderate A1B climate change scenario (1010 m) up to moderate-extreme A2 scenario (570 and 395 m). I confirm the ability of both species to acclimate to the simulated climate change and that the degree of acclimation is highly species-specific.

Q2. Which is the main climatic driver influencing the performance of these species within our latitudes?

I reveal in chapter (2) that vapor pressure deficit is the main climatic factor influencing tree growth of these two species from different functional groups (evergreen conifer vs broadleaved deciduous). Moreover, I provide empirical evidence supporting the importance of including this variable in climate change assessments, which until now was largely ignored in modelling.

Soil moisture

Soil moisture is a key factor influencing tree growth limitation, so one could expect that trees adapted to a wet environment would suffer from water stress when transplanted to drier sites. However, tree saplings were not water limited in our study, at least for years 2013 and 2014 (supplementary information Chapter 2). In this section, evidence and a description of results are presented to support this statement. The influence of soil moisture on tree growth, and the different responses of tree growth related to site conditions, is crucial for comparing results among other studies.

Throughout the study a decrease of approximately 30% of precipitation was observed at the lowest site (395 m) compared to the donor site (1350 m) and was accompanied by an increase in temperature of approximately 6.3°C. Weekly measurements of soil volumetric water content (Figure 6-3 Supplementary) showed that saplings were not water limited at low elevations during the growing seasons 2013 and 2014. Assuming that at 1350 m of elevation trees are temperature limited rather than water limited, in Figure 6-3 a threshold of 20% of VWC was fixed as the minimum soil moisture observed in the control site. Values below this threshold cannot confirm a limitation in soil water content, but allow the comparison of soil moisture along the gradient, with 1350 m as the reference site with no water limitation. Overall, a soil moisture gradient between the higher sites (1350 m and 1010 m) and the lowest sties (395 m and 570 m) was revealed. This gradient was exaggerated during the growing season 2014, as the total precipitation during this period was generally lower than in 2013. Specifically, at the lowest site the amount of precipitation during the growing season 2014 was of 480 mm compared to 708 mm in 2013. Similar trends were observed at the control site, with 882 mm of rainfall in 2013 and 650 mm in 2014. Overall, I observed lower values of VWC along the gradient in 2014. This means that beech saplings growing at the two lower altitudes experienced values of VWC under this threshold, irrespective of the treatment considered (either irrigated or non-irrigated), while for spruce, the irrigated saplings at the lowest elevation experienced the lowest values of VWC. Linking soil moisture conditions to the corresponding growth performance of saplings observed in each year showed no relationship between soil moisture conditions and tree growth (Figures 6-2 and 6-3).

The growth patterns of beech showed that in 2013 and 2014 tree growth was higher at the lowest elevation compared to the control site. In the growing season 2015, beech saplings grew at the same rate along the gradient except at 1010 m. In contrast, spruce showed a decrease in growth rate at lower altitudes compared to the control site for years 2013 and 2015. Despite the lower soil moisture and lower amount of precipitation recorded during 2014, higher growth rates were observed at lower altitudes (Figure 6-2 blue rectangle). The growth patterns observed in 2013 and 2014 were mostly explained by elevated VPD under no water stress, demonstrated by GAMM modelling in Chapter 2.

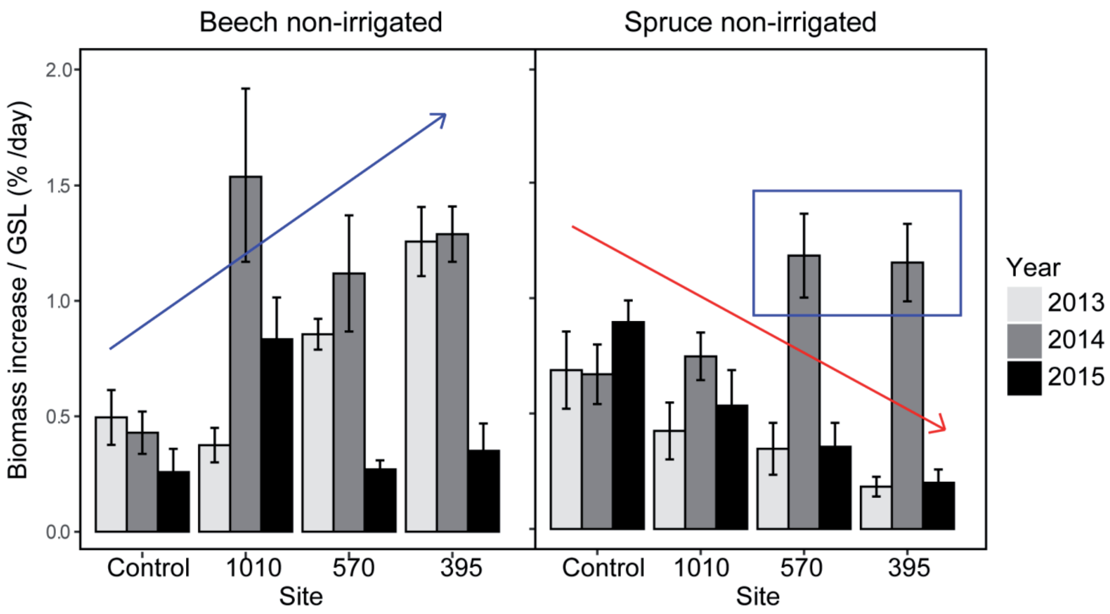


Figure 6-2 Supplementary: Temporal and spatial trends of biomass increase standardized by the growing season

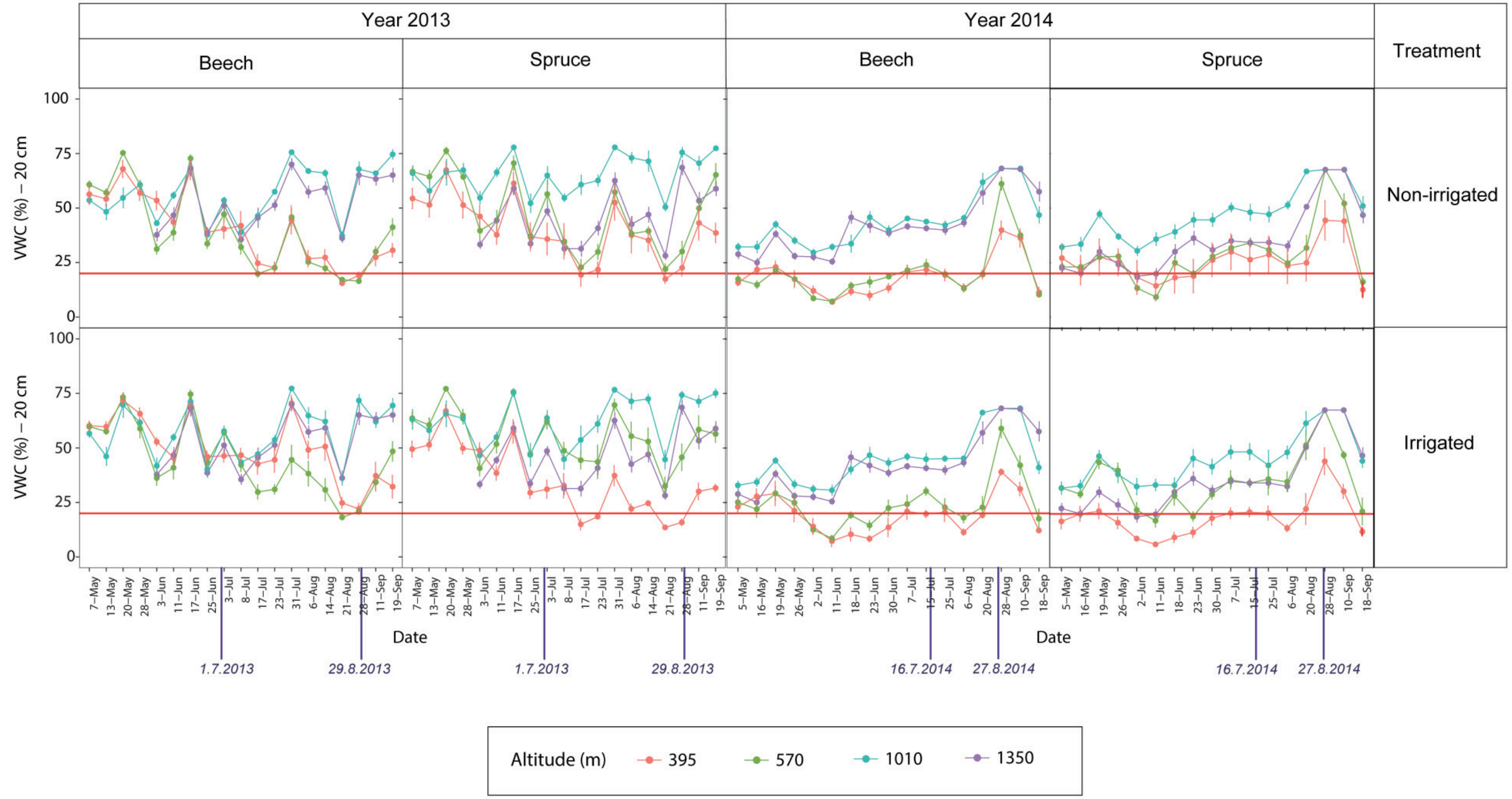


Figure 6-3 Supplementary: Variation of the volumetric water content (VWC %) measured weekly (years 2013 and 2014) by means of TDR at 20 cm depth along the elevation gradient. The specific dates correspond to ecophysiological measurements (i.e. stomatal conductance, predawn and midday leaf water potential) performed under same environmental conditions (see supplementary Table S1). The red line represents the threshold 20% below which the control site never fall below this value.

Therefore, within the region of study and during the study period, soil moisture was not the main limiting factor of tree growth, though it may have exacerbated the negative effects of other limiting factors.

Microbial activity in the soil

The composition and stability of ecosystems depend on the relationship between above and belowground compartments. For instance, the response of microbial activity to climate change has an effect in the turnover of nutrients (Wagg *et al.*, 2011). The symbiosis with mycorrhizal communities facilitates the access to limiting nutrients having a positive feedback in plant productivity (Whitfield, 2007). Therefore, any change in the composition of the microbial community can largely affect the biomass and nutritional status of plants, thus influencing their growth performance (Wardle *et al.*, 2004).

Microbial activity in the soil of the pots, where saplings were growing, was performed throughout the growing season 2014. These measurements allowed us to estimate the microbial activity through the CO_2 released during the decomposition of organic matter.

After two years of experimental warming forcing, no contrasted responses were observed between species (Figure 6-4). While spruce showed no response in soil respiration fluxes along the elevational gradient (c.a. 4 µmol CO2 m^{-2} s^{-1}), beech showed slightly lower respiration rates at the lowest elevation (c.a. 3 µmol CO2 m^{-2} s^{-1}) compared to the other sites (5-6 µmol CO2 m^{-2} s^{-1}). Moreover, within each site, inter-specific differences were not remarkable. Therefore, it seems that the microbial community has only been affected by simulated climate change in beech soil at the lowest elevation.

The microbial carbon in beech soil presented an increasing trend towards lower altitudes during the growing season (Figure 6-5). Microbial carbon in spruce soil, did not respond to the elevational gradient. However, the rate of microbial carbon was higher for both species during the growing season length compared to the state prior budburst and to the state after senescence. If we consider the ratio C/N (Figure 6-6), we observe an increase value towards lower altitudes for beech, which is not observed in spruce. These results suggest that the soil of both species, subjected to the same environmental changes, differ in response. Unfortunately, this study has a limit in soil analysis so we cannot go further in the withdraw of conclusions in this aspect.

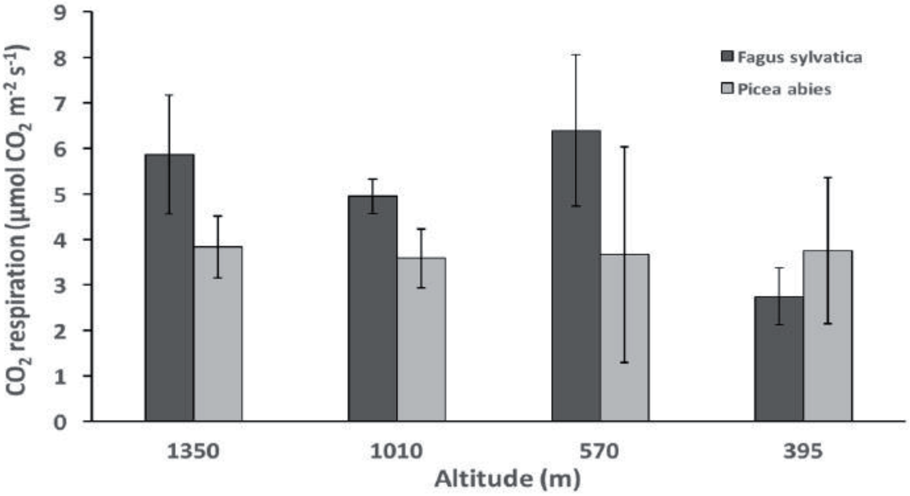


Figure 6-4 Soil respiration in pots of beech and spruce in the middle of the growing season 2014 All measurements were performed at the intermediate site at 1010 m when saplings were brought to the same environmental conditions during the ecophysiological campaigns (see chapter 4).

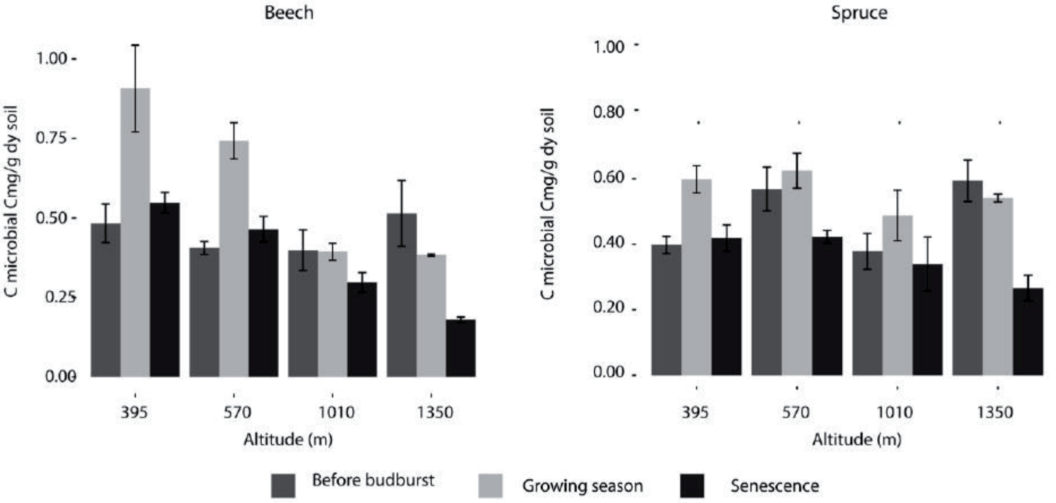


Figure 6-5 Temporal evolution of the microbial carbon during the growing season 2014. Soil cores were sampled three times, one before budburst, a second in the middle of the growing season and last during beech leaf senescence.

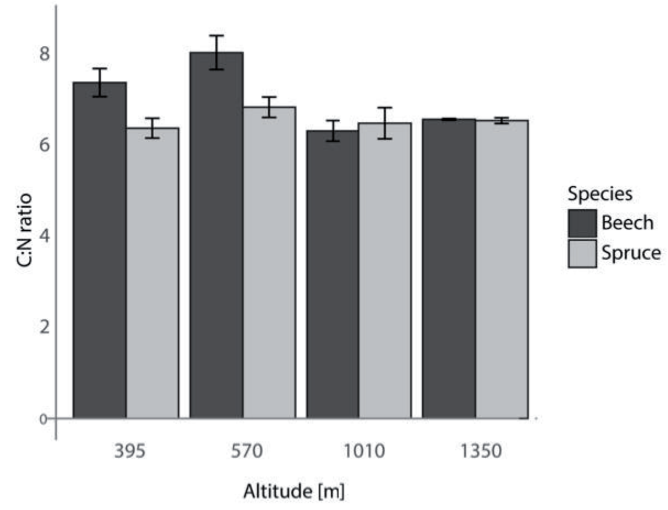


Figure 6-6 Microbial C:N ratio in soil in pots of both species along the elevation gradient

Other factors related to elevation gradient

In this study we mainly focused on the impact of changes in air temperature and precipitation in the tree growth of saplings. However, we acknowledge that there is a decrease in total atmospheric pressure and partial pressure of gases with altitude, as well as an increase in radiation under cloudless sky due to a decrease in atmospheric turbidity (Körner, 2007).

Atmospheric pressure

The observed decrease of partial pressure of CO2 with altitude does not occur in isolation but together with that of other gases, and its direct influence on plants may be diminished or enhanced by three factors as cited by Körner (2003): “(1) the oxygen partial pressure decreases as well, hence photorespiration, (2) “thinner” air allows CO2 molecules to diffuse faster through stomata and the intercellular spaces in the leaf and (3) the air temperature drops and so does leaf temperature, which counteracts (2) and enhances (1)”. So, whenever molecular gas diffusion comes into play, reduced temperature (slowing diffusion) is counteracting the effect of reduced pressure alone (increasing the rate of diffusion) (Körner, 2007). Therefore, we estimate that other factors, such as temperature regimes, have a superior effect on the leaf morphology than changes in partial pressure of gases. Moreover, studies about the leaf morphology using elevation gradient approaches generally do not take into account this parameter (Körner *et al.*, 1986; Joel *et al.*, 1994; Castro-Díez *et al.*, 1997; Li *et al.*, 2006; Kong *et al.*, 2014).

Solar radiation

Regarding solar radiation, the actual dose received by a plant will also depend on scattering elements such as clouds, which generally increase with altitude in mountain regions (Körner, 2007). Therefore, we assume that the increase in solar radiation at high elevations during the growing season is, to some extent, compensated with the associated increase in cloudiness. To support this assumption, we visually inspected the data solar radiation recorded by meteo-stations placed at our study sites (Figure 6-7) and observed similar July solar radiation averages and same trends along the spatial-temporal gradient.

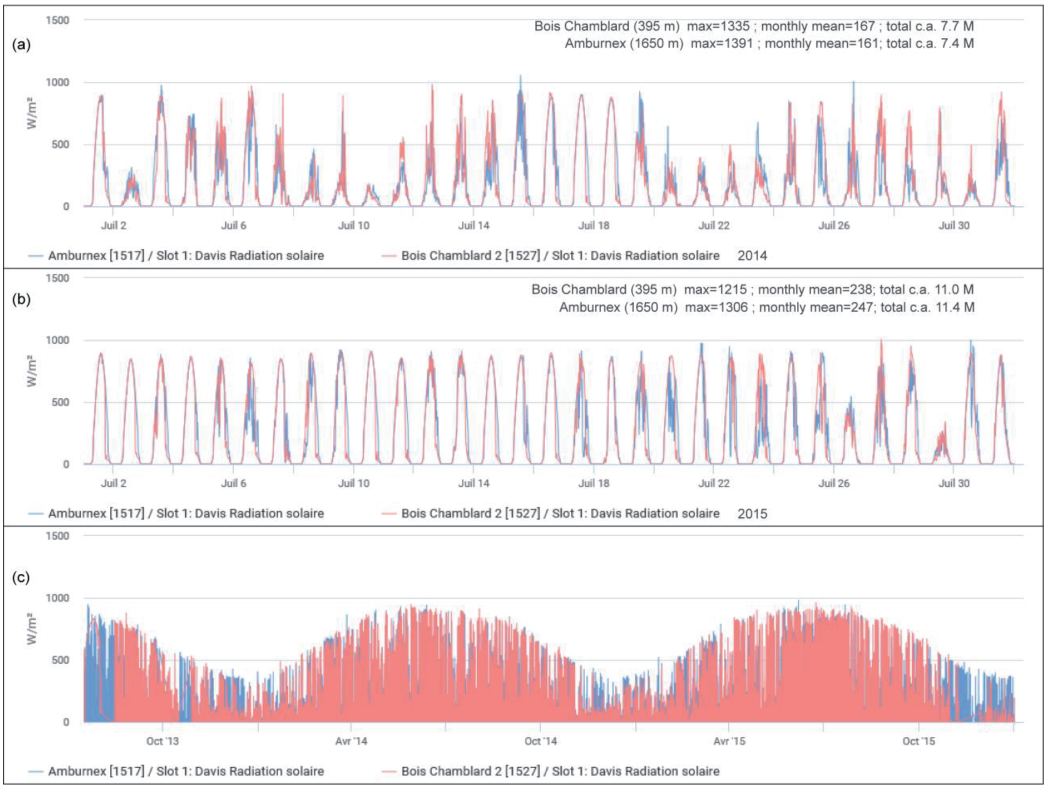


Figure 6-7 Solar radiation (W/m2) recorded at the highest (blue curve) and lowest elevation (red curve) for the month of July for years 2014 (a) and 2015 (b), and temporal and spatial trend of solar radiation throughout the study (c)

Q3. Will phenotypic plasticity (at different structural levels) lead to species-specific responses and, therefore, to different degrees of acclimation to the simulated climate change?

Findings from chapters 2, 3 and 4 revealed the species-specific responses of saplings subjected to simulated climate change. The higher degree of plasticity found in beech traits can only infer the better capacity of this species, relative to spruce, to modify its physiology under environmental changes in order to maintain a certain level of growth performance. However, it is important to consider that such higher plasticity also conveys a higher cost in resource-use for the species. Moreover, plasticity can be either adaptive or maladaptive or even neutral (Hendry, 2016). As such, my findings reveal a higher degree of plasticity of beech than spruce, which may provide this species with a short-term competitive advantage but cannot conclude about the future adaptability of these species to future climate changes. This topic remains beyond of the scope of this thesis. Nevertheless, given that phenotypic plasticity is the first step to adaptation (Vitasse, 2009), it can be inferred that acclimation abilities of beech have the potential to increase the fitness of future beech generations to warmer and drier conditions.

Q.4 Do extreme climatic variables improve the interpretation of species responses to climate change?

I demonstrate throughout this thesis (Chapters 2 and 3) that mean climatic values, such as mean annual temperature, are not sufficient to fully explain the tree responses to climate change. However, including consideration of climatic extremes improved the ecological interpretation of species responses to climate change (GAMM models, chapter 2). More generally, given that climate extremes are expected to become more regular under future climate change (IPCC, 2013), the use of extreme climatic variables, such as elevated vapor pressure deficit, will likely improve the assessment of the mechanistic response of any plant species to climate change.

6.3 Research limitations

6.3.1 Study duration

The problematic regarding the short-term investigation period in this study can be raised. Therefore, I must clarify that the main objective of this thesis was to characterize the short-term responses of overall tree growth and foliage features of beech and spruce saplings through phenotypic plasticity with regard of actual scenarios of climate change. Therefore, we transplanted saplings from one population per species growing in a cold environment at 1350 m a.s.l. in the southern Swiss Jura mountains to three recipient sites at lower elevations along an elevational gradient. I thus exposed sapling to warmer and drier climate conditions for 4 years (2012-2015), with contrasted weather during the vegetation season, using a space-for-time substitution approach (Körner, 2003). I confirm that the use of three contrasted years (2013, 2014 and 2015) to study the phenotypic plasticity is sufficient to assess the acclimation abilities of these species to these specific changing environmental conditions. I cannot predict the changes on the long-term, nor infer about the potential adaptability of this plasticity.However, I demonstrate the potential plasticity of these species as a response to warmer and drier conditions, which is the first step towards adaptation. Finally, findings from this short-term experiment and findings from long term studies are not exclusive, but give together an insight on the mechanistic strategies of plants to cope with rapid environmental changes. This further understanding of the mechanisms underlying short-term plasticity can serve as basis to design long-term experiments.

6.3.2 Pot experiment

I acknowledge that pot experiments are rather limited as they do not represent the “real” growth conditions of trees. In particular, the spatial and temporal variability of the environment is highly restricted in pots (Lanta and Lepš, 2006). However, this pot experiment allowed the transplantation of saplings together with their original soil in recipient sites without the influence of *in situ* soil conditions. Additionally, it allowed a higher number of replicates than an *in situ* transplantation directly in the site soil. I must clarify that the main objective of the study was to subject trees to changing temperature and precipitation regimes and not to changing soil characteristics. Moreover, pots allowed the removal of inter and intra species competition for nutrients and water. The main drawback, however, is the limitation of tree growth in fixe pot sizes. This confounding factor was reduced as possible my transplanting the pots to double size pots during autumn 2014.

6.4 Outlook

To which extent current tree saplings are able to cope with climate change was the main research question answered in this thesis. However, only a limited number of specific conditions, such as sufficient water supply and no inter-intra specific competition, were considered by this experimental design. Competition is known to modify species responses to climate change in mountain ecosystems (Alexander *et al.*, 2015). How beech and spruce saplings would respond in the context of such competition is thus a major research question needing future research. Moreover, extending the CLIMARBRE experimental design in both competitive and non-competitive environments would give real insights to infer what could happen to these species co-habiting under wooded-pastures.

The outcome of this thesis provides two new directions for future research. First, based on the carry-over experiment presented in chapter 5 and the findings of chapters 2-4, I propose a study whereby we replant the saplings growing along the elevational gradient at their original site of Amburnex (1350 m) (Figure 6-8). This new direction could answer questions such as "*how long does the acclimation of traits last after five years of experimental forcing warming*?" and "*how reversible are the phenotypic plasticity changes*?". Second, I suggest a continuation of the study on the legacy effect of advanced or delayed budburst on the budset timing. This would provide evidence regarding the carry over effects in the phenology of beech and spruce, while expanding and reaffirming the conclusions found for beech saplings in chapter 5.

6.5 Final conclusion

In order to define current and future vegetation distributions it is crucial to understand the climate-vegetation relationships. However, the main challenge plant ecologists need to face is the complexity of the interactions between plants and the environment.

Moreover, forest managers need decision-support tools in order to adapt forest managements within the uncertainty of climate change. Therefore there is a necessity to predict accurately species responses to environmental changes.

Throughout this thesis we showed that climatic extremes and the relationship between different phenological leaf stages (e.g. budset) must be taken into account in species distribution models to improve predictions of trees response to climate change. Moreover, these models need to be based in a good understanding of the mechanistic responses of species to changing environmental conditions. Therefore, experimental studies at multi-organizational levels (tree, leaf and tissue) provided with a better understanding of species-specific responses.

Finally, the results from this dissertation suggest that in the sylvopastoral ecosystems of the Jura, where spruce is today the dominant species, beech performance under global warming will provide these species with a competitive advantage over spruce. In the long run, this could change the dominance of species in Mountain landscapes and deeply transform the landscape, as shown by some landscape simulations (Buttler *et al.*, 2012, Peringer *et al.*, 2013). This landscape modeling has predicted the beginning of a replacement of spruce by beech in three sylvopasotral mountain ecosystems from 2100 for the IPCC B2 scenario, and a change of dominance from 2300, in particular due to the effect of strong droughts. However, this process will most likely be slow and could span several centuries, depending on the intensity of warming and the patterns of land use.

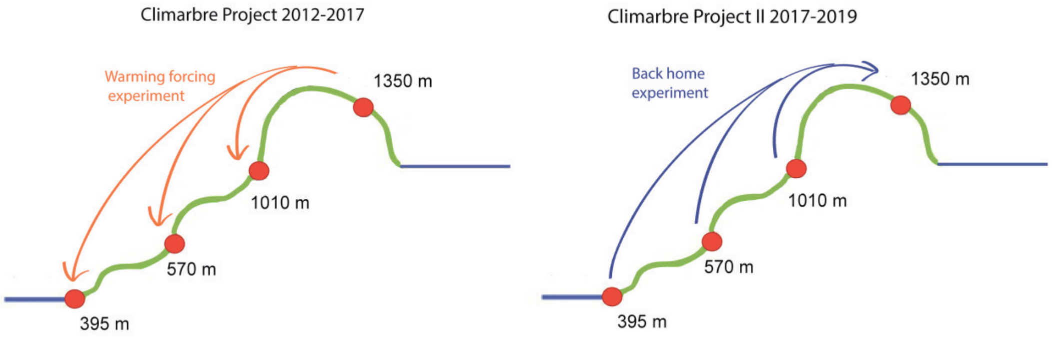


Figure 6-8 Back to home experimental design based on the previous warming forcing experiment of CLIMARBRE

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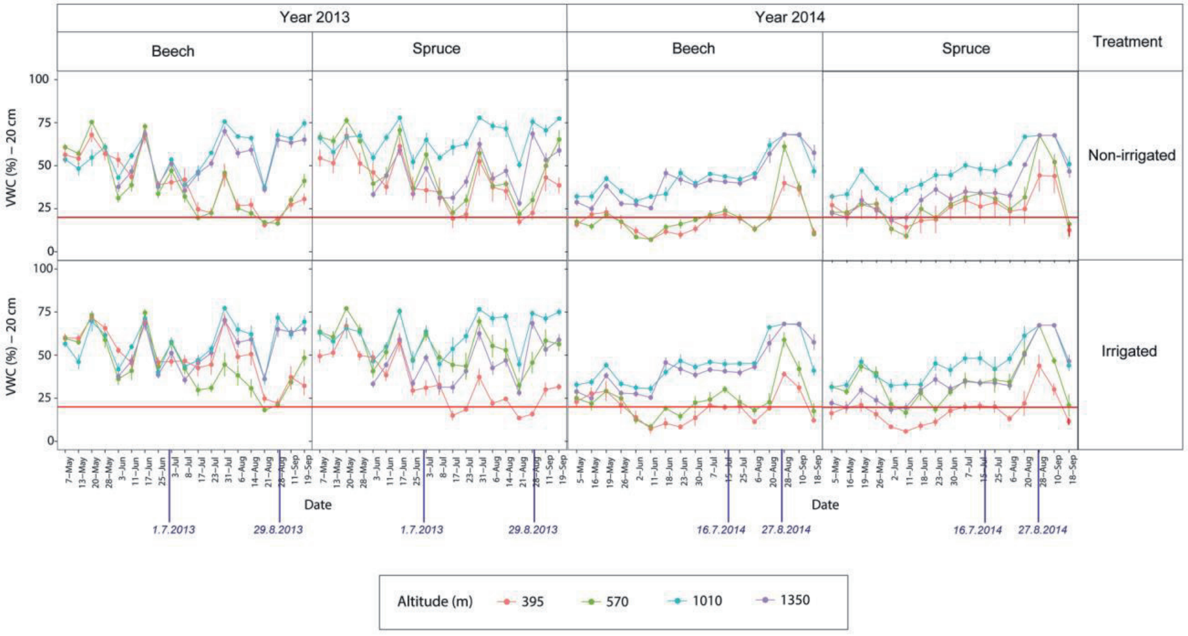
Supporting material

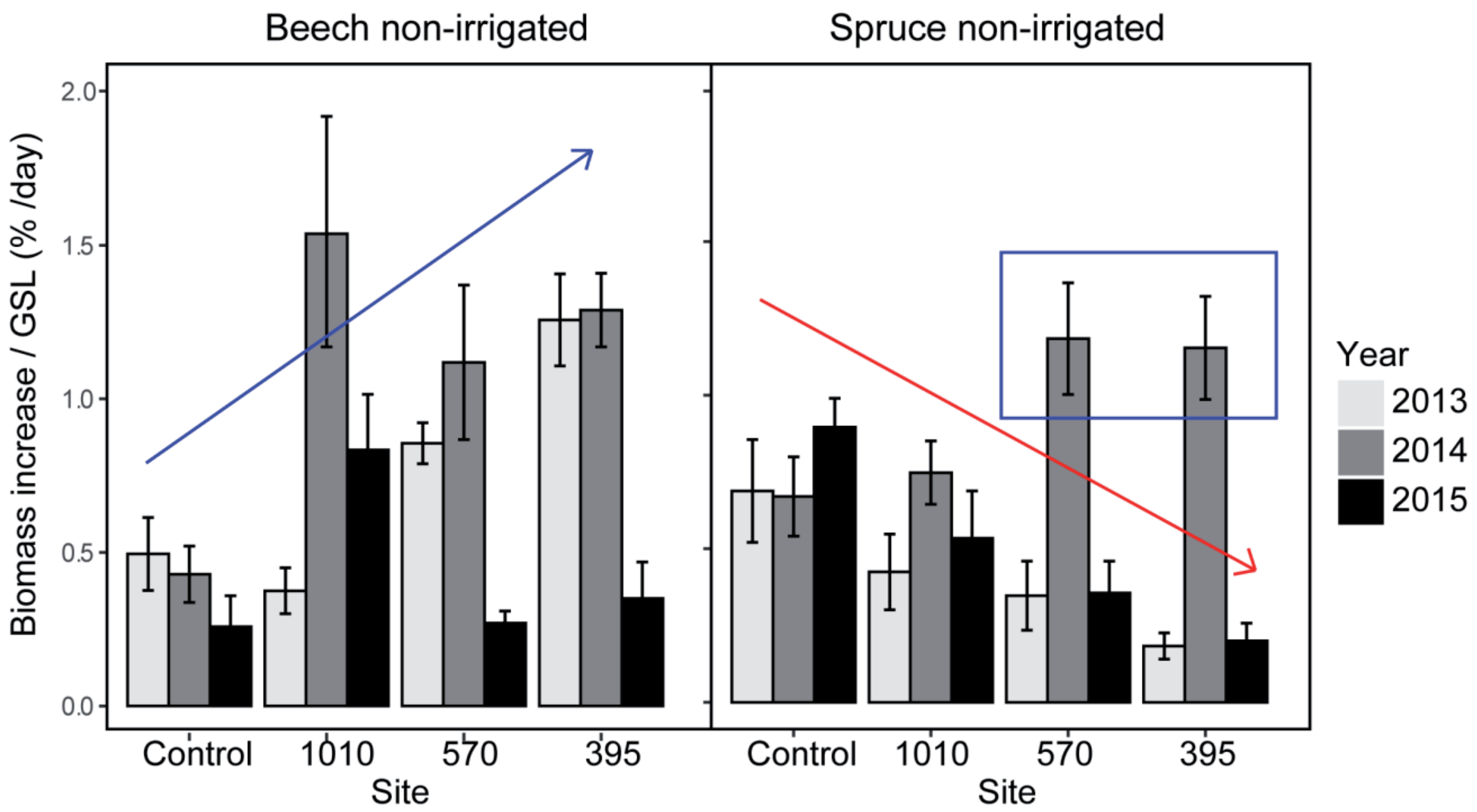
Amburnex landscape

P. Sanginés (2014)

Chapter 2 Supplementary information



Supplementary Figure S2-1 Variation of the volumetric water content (VWC %) measured weekly (years 2013 and 2014) by means of TDR probe (Time Domain Reflectometry; Field Scout, Spectrum Technologies, Inc.) at 20 cm depth along the elevation gradient. The specific dates correspond to ecophysiological measurements (i.e. stomatal conductance, predawn and midday leaf water potential) performed under same environmental conditions (see supplementary Table S1). Considering that at 1350 m trees are more temperature limited than water limited, we fixed 20 % of VWC at 20 cm as the minimum soil moisture observed in the control site (red line). We observed that beech saplings growing at the two lower altitudes experience values of VWC under this threshold no matter the treatment (either irrigated or non-irrigated), while for spruce the saplings irrigated at the lowest elevation experienced the lowest values of VWC

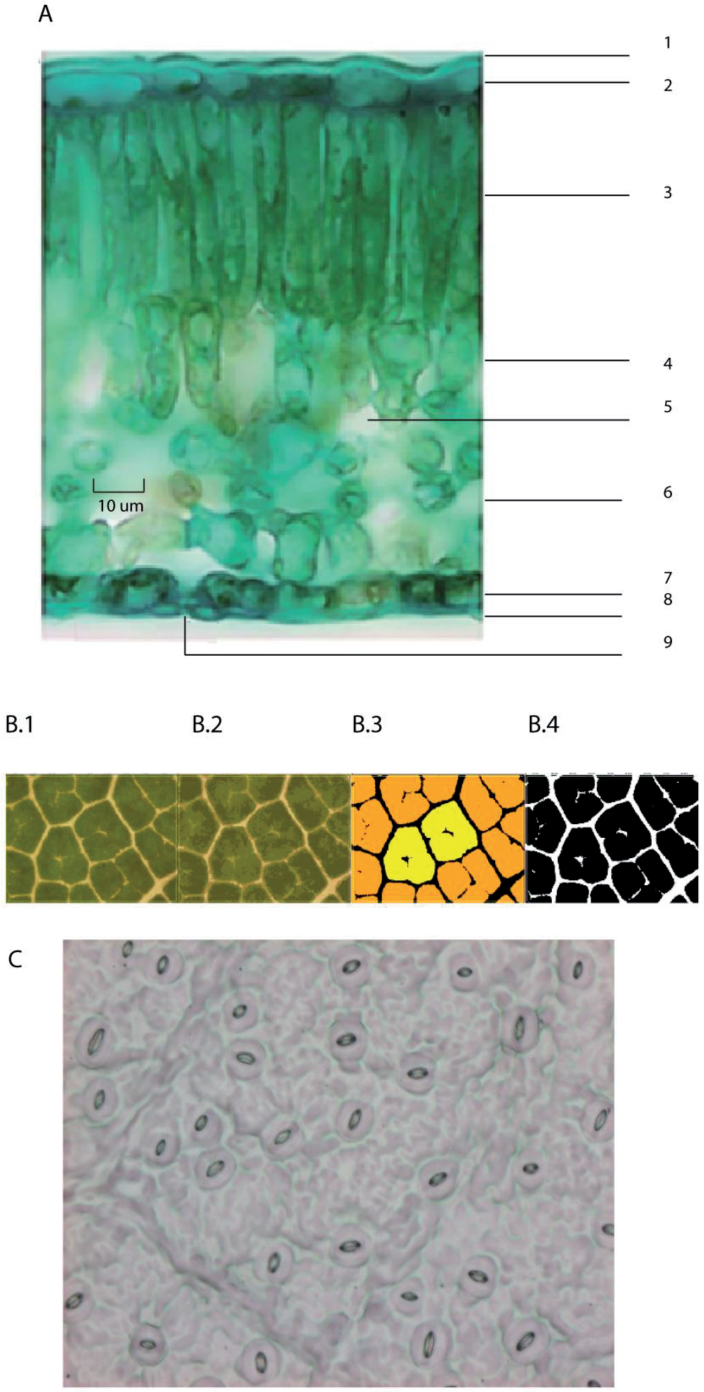


Supplementary Figure S2-2: Temporal and spatial trends of biomass increase standardized by the growing season

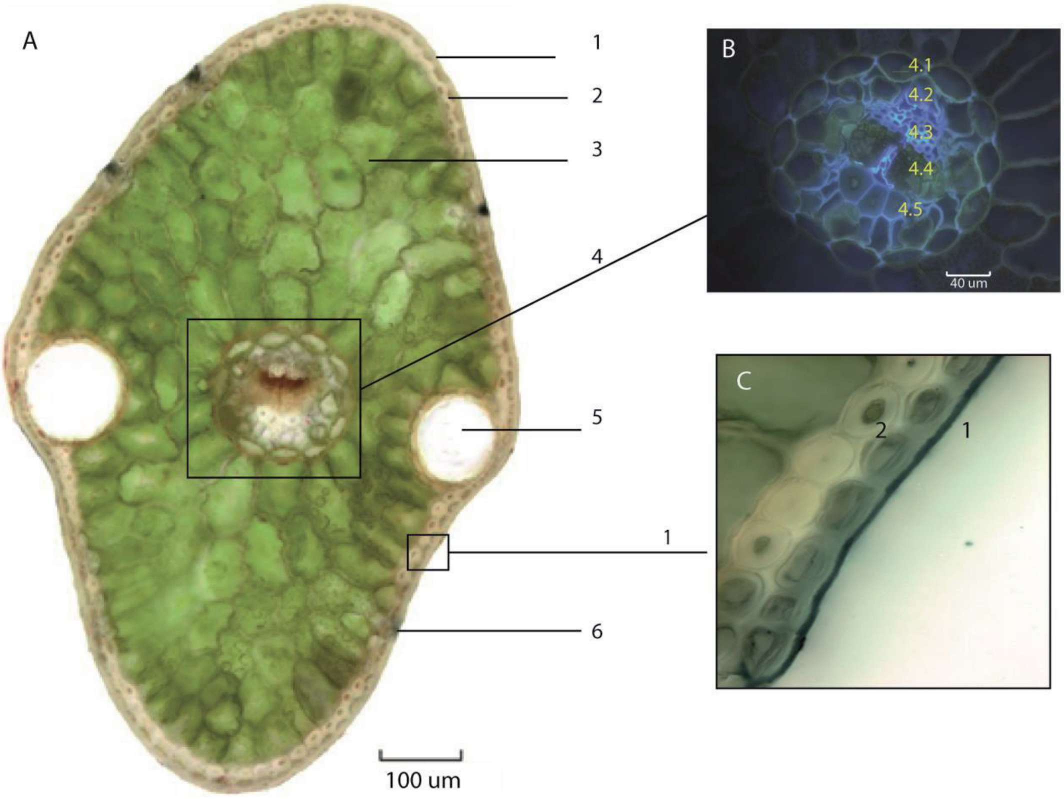
Table S2-1 Supplementary: ecophysiological measurements in leaves performed twice during the growing seasons 2013 and 2014 at each elevation (m). The mean values of predawn leaf water potential Ψp (MPa), midday leaf water potential Ψ_m (MPa) and leaf stomatal conductance (mmol g^{-1} s^{-1}) ± the standard error (SE) for both treatments non-irrigated (NI) and irrigated (I)

Elevation	Species	Leaf trait	Year 2013								Year 2014							
			1.7.2013				29.8.2013				16.7.2014				27.8.2014			
			NI	SE	I	SE	NI	SE	I	SE	NI	SE	I	SE	NI	SE	I	SE
1350	Beech	Ψp	-0.1	± 0.02			-0.2	± 0.09			-0.1	± 0.01			-0.2	± 0.03		
		Ψm	-1.5	± 0.00			-2.4	± 0.41			-1.9	± 0.13			-1.4	± 0.22		
		Gs	0.8	± 0.74			4.1	± 0.70			1.7	± 0.30			2.9	± 0.33		
	Spruce	Ψp	-0.3	± 0.03			-0.5	± 0.08			-0.3	± 0.04			-0.5	± 0.04		
		Ψm	-1.2	± 0.09			-1.6	± 0.18			-1.4	± 0.05			-1.1	± 0.20		
		Gs	0.9	± 0.12			1.1	± 0.30			1.5	± 0.22			1.8	± 0.18		
1010	Beech	Ψp	-0.3	± 0.05	-0.1	± 0.01	-0.1	± 0.03	-0.2	± 0.02	-0.1	± 0.00	-0.1	± 0.03	-0.2	± 0.05	-0.2	± 0.03
		Ψm	-1.2	± 0.27	-1.8	± 0.32	-2.4	± 0.07	-2.6	± 0.20	-2.3	± 0.15	-2.2	± 0.12	-1.4	± 0.46	-2.1	± 0.09
		Gs	3.2	± 0.40	3.9	± 0.25	6.7	± 0.57	5.8	± 1.13	2.5	± 1.02	3.7	± 0.90	7.0	± 0.78	6.6	± 1.79
	Spruce	Ψp	-0.3	± 0.05	-0.2	± 0.04	-0.5	± 0.08	-0.5	± 0.03	-0.2	± 0.04	-0.4	± 0.15	-0.4	± 0.08	-0.5	± 0.04
		Ψm	-1.1	± 0.09	-1.3	± 0.10	-1.8	± 0.06	-1.8	± 0.12	-1.7	± 0.09	-1.7	± 0.23	-1.3	± 0.03	-1.0	± 0.17
		Gs	1.1	± 0.13	1.3	± 0.09	1.2	± 0.23	1.4	± 0.16	1.7	± 0.44	1.9	± 0.15	1.0	± 0.44	1.4	± 0.26
570	Beech	Ψp	-0.1	± 0.06	-0.1	± 0.08	-0.7	± 0.16	-0.8	± 0.30	-0.1	± 0.01	-0.1	± 0.00	-0.2	± 0.02	-0.2	± 0.03
		Ψm	-2.1	± 0.13	-1.9	± 0.12	-2.8	± 0.12	-2.9	± 0.05	-2.3	± 0.01	-2.5	± 0.24	-1.5	± 0.20	-1.9	± 0.22
		Gs	3.8	± 1.06	3.0	± 0.22	1.6	± 0.35	3.2	± 1.20	2.5	± 0.16	3.1	± 0.94	3.9	± 0.44	3.6	± 0.38
	Spruce	Ψp	-0.2	± 0.09	-0.4	± 0.09	-0.4	± 0.05	-0.4	± 0.05	-0.2	± 0.03	-0.1	± 0.16	-0.4	± 0.01	-0.4	± 0.10
		Ψm	-1.5	± 0.19	-1.3	± 0.12	-1.9	± 0.13	-1.6	± 0.22	-1.7	± 0.18	-1.5	± 0.28	-1.1	± 0.16	-1.0	± 0.08
		Gs	1.0	± 0.10	0.9	± 0.06	0.6	± 0.09	0.6	± 0.19	1.2	± 0.19	1.2	± 0.42	1.1	± 0.17	1.2	± 0.16
395	Beech	Ψp	-0.3	± 0.10	-0.2	± 0.06	-0.5	± 0.37	-0.7	± 0.38	-0.1	± 0.01	-0.1	± 0.01	-0.2	± 0.04	-0.2	± 0.02
		Ψm	-1.9	± 0.09	-1.8	± 0.12	-3.0	± 0.31	-2.2	± 0.22	-2.5	± 0.18	-2.4	± 0.12	-2.5	± 0.17	-2.0	± 0.24
		Gs	3.4	± 0.65	3.5	± 0.73	2.5	± 0.67	2.9	± 0.64	4.3	± 0.45	1.8	± 0.18	5.2	± 0.30	4.8	± 1.03
	Spruce	Ψp	-0.2	± 0.09	-0.2	± 0.04	-0.6	± 0.00	-0.6	± 0.11	-0.2	± 0.04	-0.2	± 0.02	-0.4	± 0.07	-0.3	± 0.03
		Ψm	-1.2	± 0.03	-1.2	± 0.12	-1.7	± 0.25	-2.2	± 0.18	-1.6	± 0.03	-1.4	± 0.24	-0.9	± 0.14	-0.9	± 0.17
		Gs	1.2	± 0.28	1.3	± 0.12	0.6	± 0.15	0.6	± 0.31	1.1	± 0.46	0.9	± 0.44	1.3	± 0.38	1.1	± 0.20

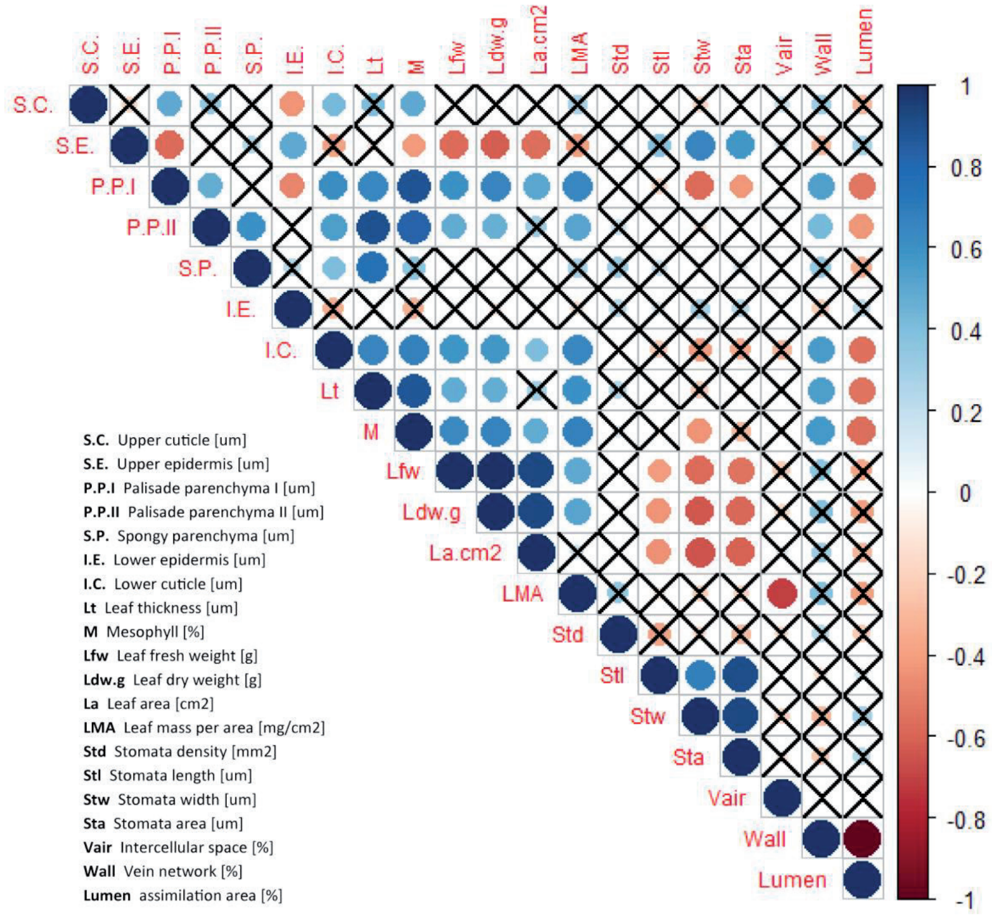
Chapter 3 Supplementary information



Supplementary Figure S3-1: Cross section of Fagus sylvatica L. broadleaf. The different tissues are labelled in Figure A) with the following numeration; upper cuticle (1), upper epidermis (2), palisade parenchyma I (3), palisade parenchyma II (4), intercellular space (5), spongy parenchyma (6), lower epidermis (7), lower cuticle (8), stomata (9). In Figure B the protocol of vein network estimation is presented with B.1 the original 5x image, B.2. selected colour class, B.3 colour group image with analyzed cells and B.4 binary image which is used to calculate the proportion of lumen and wall area. Figure C represents the process followed to measure the stomata area and stomata density after taking imprints of beech leaves.

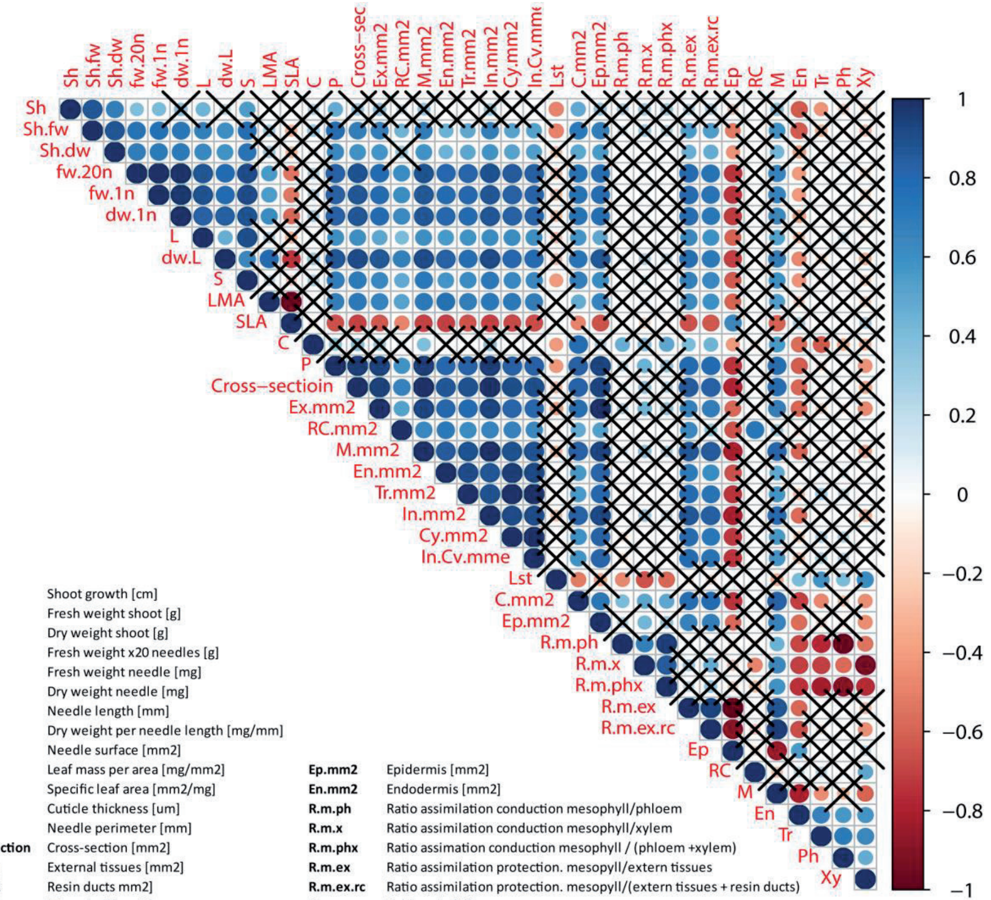


Supplementary Figure S3-2: Cross-section of Picea abies (L.) Karst needle. The different tissues are labelled with the following numeration; cuticle thickness (1), epidermis (2), mesophyll (3), vascular bundle (4), endodermis (4.1), transfer (4.2), xylem (4.3), phloem (4.4), fibers (4.5), resin duct (5) and stomata (6). A) is the representation of the cross-section of the needle taken at x10 magnification (the image is composed by 4-5 photographs merged with GUI software), B) shot of the vascular bundle taken under fluorescent light at x20 magnification, C) close up of cuticle thickness stained with Sudan-black and measured at x63 magnification.



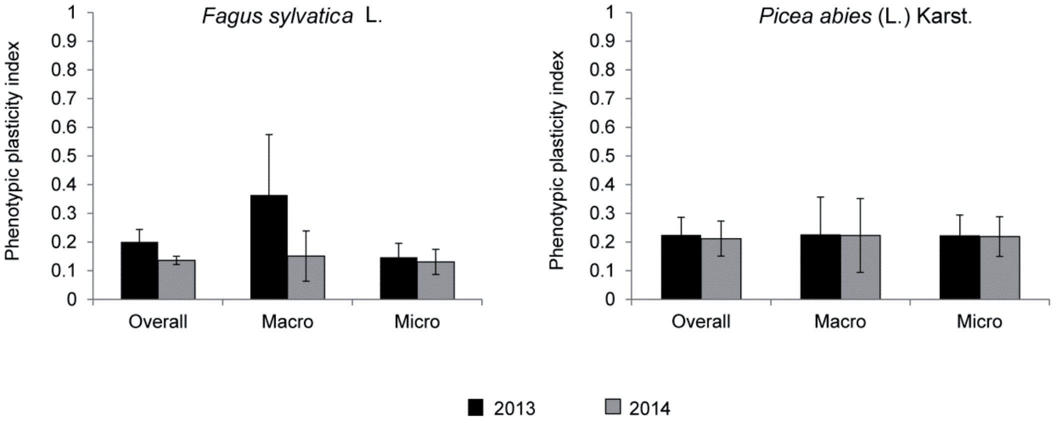
S.C.	Upper cuticle [um]
S.E.	Upper epidermis [um]
P.P.I	Palisade parenchyma I [um]
P.P.II	Palisade parenchyma II [um]
S.P.	Spongy parenchyma [um]
I.E.	Lower epidermis [um]
I.C.	Lower cuticle [um]
Lt	Leaf thickness [um]
M	Mesophyll [%]
Lfw	Leaf fresh weight [g]
Ldw.g	Leaf dry weight [g]
La	Leaf area [cm2]
LMA	Leaf mass per area [mg/cm2]
Std	Stomata density [mm2]
Stl	Stomata length [um]
Stw	Stomata width [um]
Sta	Stomata area [um]
Vair	Intercellular space [%]
Wall	Vein network [%]
Lumen	assimilation area [%]

Supplementary Figure S3-3: Pearson correlation matrix with twenty-two measured morpho-anatomical variables on beech leaves. Positive correlations are displayed in blue and negative correlations in red. Colour intensity and the size of the circle are proportional to the correlation coefficients. Correlation coefficients with p-value >0.05 are indicated with crosses.



Sh	Shoot growth [cm]		
Sh.fw	Fresh weight shoot [g]		
Sh.dw	Dry weight shoot [g]		
fw.20n	Fresh weight x20 needles [g]		
fw.1n	Fresh weight needle [mg]		
dw.1n	Dry weight needle [mg]		
L	Needle length [mm]		
dw.L	Dry weight per needle length [mg/mm]		
S	Needle surface [mm2]		
LMA	Leaf mass per area [mg/mm2]	**Ep.mm2**	Epidermis [mm2]
SLA	Specific leaf area [mm2/mg]	**En.mm2**	Endodermis [mm2]
C	Cuticle thickness [um]	**R.m.ph**	Ratio assimilation conduction mesophyll/phloem
P	Needle perimeter [mm]	**R.m.x**	Ratio assimilation conduction mesophyll/xylem
Cross-section	Cross-section [mm2]	**R.m.phx**	Ratio assimation conduction mesophyll / (phloem +xylem)
Ex.mm2	External tissues [mm2]	**R.m.ex**	Ratio assimilation protection. mesopyll/extern tissues
RC.mm2	Resin ducts mm2]	**R.m.ex.rc**	Ratio assimilation protection. mesopyll/(extern tissues + resin ducts)
M.mm2	Mesophyll [mm2]	**Ep**	Epidermis [%]
En.mm2	Endodermis [mm2]	**RC**	Resin ducts [%]
Tr.mm2	Transfer [mm2]	**M**	Mesophyll [%]
In.mm2	Inner tissues [mm2]	**En**	Endodermis [%]
Cy.mm2	Cylinder tissues [mm2]	**Tr**	Transfer [%]
In.Cy.mm2	Inner cylinder tissues [mm2]	**Ph**	Phloem [%]
Lst	Frequency of stomata lines [lines mm-1]	**Xy**	Xylem [%]
C.mm2	Cuticle surface [mm2]	**Tr.cal**	Transfer calculated [%]

Supplementary Figure S3-4: Pearson correlation matrix with forty measured morpho-anatomical variables on spruce needles. Positive correlations are displayed in blue and negative correlations in red. Colour intensity and the size of the circle are proportional to the correlation coefficients. Correlation coefficients with p-value >0.05 are indicated with a cross.



Supplementary Figure S3-5: Phenotypic plasticity index for each group of traits for both species per year; overall plasticity calculated as the mean of all the traits included in both categories, Macro plasticity includes only the traits related to macromorphology and Micro includes those related to micromorphology. The composition of each group for each species is presented in the *Table 1*. Index is calculated according to *Valladares et al.* (2000).

Supplementary Table S3-1 : F-values from ANOVA repeated measures of morpho-anatomical variables of beech leaves measured along the altitudinal gradient in two consecutive growing seasons (2013 and 2014). Degrees of freedom for the error is Df=16.

			Source of variation						
Variable	Acronym	Units	Site (S) df=3	Watering treatment (T) df=1	Year (Y) df=1	S x T df=3	S x Y df=3	T x Y df=1	R^2
Leaf mass per area	LMA	mg cm^{-2}	**7.27****	0.11	**32.2*****	0.05	**9.74*****	0.02	0.58
Leaf area	La	cm^2	**7.22****	0.00	**9.4****	0.07	2.80	0.02	0.58
Intercellular space	Vair	%	2.14	0.42	**64.1*****	0.05	1.11	0.03	0.30
Vein network	Wall	%	**6.87****	1.43	**78.2*****	2.870	0.85	0.06	0.66
Stomata density	Std	mm^{-2}	1.4	3.36	3.30	0.46	1.17	0.00	0.36
Stomata area	Sta	µm^2	2.99	0.43	1.17	1.80	0.92	0.56	0.48
Upper cuticle	S.C.	µm	**4.03***	0.56	**210.7*****	0.48	1.21	0.87	0.47
Upper epidermis	S.E.	µm	1.87	2.59	**298.3*****	0.36	3.17	0.02	0.37
Palisade parenchyma (I + II)	P.P.	µm	**4.25***	0.04	**27.8*****	2.96	**16.3*****	0.038	0.58
Spongy parenchyma	S.P.	µm	1.10	1.27	**72.7*****	0.31	**5.94****	0.20	0.26
Lower epidermis	I.E.	µm	0.93	0.19	**668.5 *****	0.92	**4.73 ***	3.48	0.65
Lower cuticle	I.C.	µm	**8.03****	2.65	**396.8*****	0.77	**6.04****	2.59	0.17

Signif. Codes: 0 “***” 0.001 “**” 0.01 “*” 0.05 “ “

Chapter 5 Supplementary information

Table S5-1. Non-structural carbohydrates (NSC), sugar and starch content (%) in twigs of beech saplings in autumn 2014. C refers to the cooling treatment and represent the trees moved to high elevation during winter/spring 2013-2014 and then moved back to their original site at low elevation, and CC refers to the control treatment in which remained during the whole experiment at the low site. W refers to the warming treatment and represent the trees moved to low elevation during winter/spring 2013-2014 and then moved back to their original site at high elevation, and CW refers to the control warming in which remained during the whole experiment at the high site. Data represent means ± 1 SE with n = 10).

Site	Treatment	Sugar (%)	Starch (%)	NSC (%)
Low	C	3.2 ± 0.1	11.3 ± 0.4	14.6 ± 0.5
	CC	3.9 ± 0.3	9.3 ± 0.7	13.3 ± 0.6
High	W	5.5 ± 0.2	5.4 ± 0.7	10.9 ± 0.6
	CW	3.8 ± 0.2	7.3 ± 0.7	11.1 ± 0.8

Appendix

CHANGEMENT CLIMATIQUE

Comment réagissent le hêtre et l'épicéa?

Des chercheurs de l'Institut de recherches WSL et de l'Ecole polytechnique fédérale de Lausanne (EPFL) s'intéressent à l'avenir de nos forêts. Ils veulent savoir comment pousseront le hêtre et l'épicéa dans les forêts du Jura dans un futur climat plus chaud. Regard sur un projet de recherche en cours.

Texte et photos: Maria Paula Sanginés De Cárcer, Rita Bütler, Constant Signarbieux*

Depuis 1970, la température moyenne en Suisse s'est accrue de 1,5° C. D'ici 2100, elle va encore croître de 2 à 7° C en été, et des étés secs comme en 2003 deviendront beaucoup plus fréquents. De plus, les précipitations estivales seront moindres, tandis qu'elles devraient augmenter en hiver. Les changements climatiques en cours sont déjà bien visibles. Par exemple, en Valais, le pin sylvestre souffre d'une forte mortalité et son rajeunissement est entravé, entre autres, par la sécheresse et le manque d'eau accrus; il est en train d'être remplacé par le chêne pubescent jusqu'à une altitude de 1200 m. D'autre part, depuis la deuxième moitié du XX^{e} siècle, la période de végétation s'est prolongée de 2,7 jours tous les dix ans en Suisse. Dès lors, une question s'impose: la forêt peut-elle réagir si rapidement à de tels changements et comment?

Climarbre dans le Jura vaudois

Le projet Climarbre étudie depuis quatre ans les capacités d'acclimatation du hêtre et de l'épicéa en phase de régénération dans le Jura vaudois. L'acclimatation est le fait, pour un organisme vivant, de s'adapter à un changement durable de son environnement. A la différence de l'adaptation évolutive, l'acclimatation se déroule sur une période courte, au plus égale à la durée de vie de la plante.

Celle-ci peut réagir par différents mécanismes au stress climatique. Tout d'abord, il est possible qu'elle accélère ou ralentisse sa croissance ou qu'elle modifie sa phénologie, par exemple par un débourrement précoce des feuilles. De plus, elle peut modifier son efficacité photosynthétique. Finalement, des changements de la taille des feuilles ou de la structure de leurs tissus sont possibles. La capacité d'acclimatation d'une espèce est déterminante pour son succès de survie dans un environnement modifié. Dans les hauteurs du Jura vaudois, où l'homme a favorisé l'épicéa au détriment du hêtre depuis des siècles, le gestionnaire forestier se pose aujourd'hui à juste titre la question de l'avenir qui est réservé à ces deux essences (fig. 3).

Mais comment peut-on étudier la réaction des plantes aux changements climatiques? Une méthode bien connue en écologie est la transplantation de plantes issues d'un environnement froid et humide dans un environnement plus chaud et plus sec, suivie par toute une batterie de mesures, que ce soit au niveau climatique ou en relation avec la réaction des individus transplantés (fig. 2). Une telle expérience de transplantation permet de simuler des conditions environnementales plus chaudes et plus sèches prédites en Suisse et d'observer la réaction des plantes sur un laps de temps relativement court.

Fig. 1: Maria Paula Sanginés de Cárcer, doctorante au laboratoire ECOS de l'Ecole polytechnique fédérale de Lausanne, mesurant la photosynthèse des arbres.

Différence de croissance

Dans le projet Climarbre, 200 jeunes hêtres et épicéas d'environ 4 à six 6 ans provenance de la Combe des Amburnex (1340 m) ont été transplantés à Saint-George (1010 m), à l'Arboretum d'Aubonne (570 m) et au Bois Chamblard (395 m) dans une expérience contrôlée. Entre les deux sites extrêmes, une différence de température de 6° C et une réduction de la pluviométrie de 30% ont été mesurées pendant la saison de végétation.

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* Maria Paula Sanginés de Cárcer est doctorante en écophysiologie au laboratoire ECOS à l'EPFL. Rita Bütler est responsable de l'interface recherche-pratique pour la Suisse romande au WSL et responsable pour la biodiversité en forêt à la Direction générale de l'environnement du canton de Vaud. Constant Signarbieux est écophysiologiste et collaborateur scientifique au laboratoire ECOS à l'EPFL.

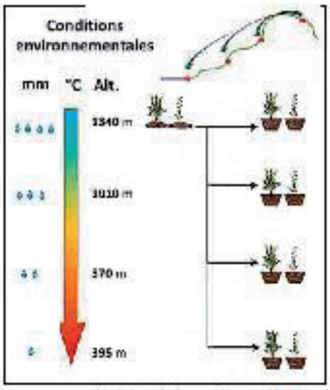

Fig. 2: Transplantation le long d'un gradient altitudinal dans le Jura suisse schématisant la diminution des précipitations (en mm), l'augmentation des températures moyennes (en °C) et l'altitude des différents sites receveurs.

Comment ces plantes ont-elles réagi au changement de leur environnement? Le résultat le plus frappant est la différence de croissance entre les deux espèces. Après quatre années, alors que les deux essences présentent une croissance similaire à 1340 m en multipliant leur biomasse par 1,5, le hêtre a, lui, multiplié sa biomasse par 9 à 395 m, alors que l'épicéa maintient une croissance constante le long du gradient altitudinal (fig. 4, au centre). Les deux essences ont été capables d'augmenter leur durée de saison de végétation en avançant le débourrement de leurs feuilles et aiguilles: celui-ci a lieu aux alentours de la mi-juin à 1340 m, mais déjà au début de mai à 395 m, l'épicéa démarrant environ 15 jours après le hêtre (mesuré lorsque la moitié des feuilles de chaque arbre étaient sorties). L'efficacité photosynthétique n'explique pas les différences observées au niveau de la croissance, car elle apparaît plus faible à basse altitude que dans les hauts du Jura pour les deux essences (fig. 1). Un premier élément de réponse pourrait se trouver au niveau des feuilles. En effet, les hêtres à basse altitude ont développé des feuilles deux à trois fois plus grandes que leurs congénères restés aux Amburnex.

Le hêtre en tête

Si les deux espèces ont réagi au stress climatique par un débourrement précoce du feuillage, le hêtre a néanmoins su beaucoup mieux exploiter les ressources supplémentaires en termes de croissance. Il apparaît donc clairement que le hêtre serait plus à même de tirer profit du climat modifié dans la phase de rajeunissement. Dès lors, est-ce que le hêtre supplantera en grande partie l'épicéa dans les hauteurs du Jura suisse?

Il est encore trop tôt pour donner une réponse définitive. Il faut tout de même signaler que ces observations concernent des provenances des hauteurs de la chaîne du Jura suisse et que les réponses observées ne sont pas généralisables à une plus grande échelle géographique.

L'expérience est toujours en cours afin de mieux identifier les mécanismes conférant une meilleure acclimatation du hêtre au réchauffement du climat. La réponse se trouve peut-être dans le sol, où les micro-organismes associés aux racines des hêtres pourraient mieux réagir que ceux de l'épicéa en libérant plus de nutriments pour la croissance de la plante. Affaire à suivre !

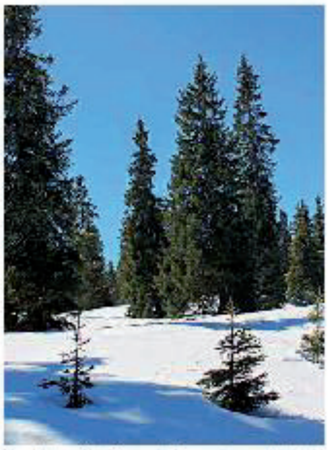

Fig. 3: Quel est l'avenir de ces jeunes épicéas de la chaîne du Jura suisse?

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Fig. 4: Rocio Andrey Mijancos, étudiante en master à l'EPFL, mesurant la biomasse des arbres. Après quatre ans d'expérience, on voit la différence de taille des arbrisseaux à haute altitude (à gauche, site contrôle), et à basse altitude près du lac Léman (au centre, un épicéa, à droite, un hêtre).

Figure A-1 Site Bois Chamblard 395 m

Figure A-2 Site Arboretum d'Aubonne

Figure A-3 Site Saint George 1010 m

Figure A-4 Site Amburnex 1350 m

Curriculum Vitae

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Personal Data

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Work Experience

July 2013 - Present	PhD candidate in Civil and Environmental Engineering, École polytechnique fédérale de Lausanne (EPFL)
May 2013-Mar 2017	Eidg. Forschungsanstalt für Wald, Schnee und Landschaft- (WSL), Lausanne. Research in ecological response of beech and spruce saplings to climate change along an altitudinal gradient in the Swiss Jura Mountains
Sep 2015-Jan 2016	Professor Assistant. Masters course “Ecologie numerique” (EPFL)
Sep 2013-Jan 2015	Professor Assistant. Masters course “Concepts of ecological engineering” (EPFL)
Oct 2012-Apr 2013	Laboratory Assistant. Thermal Engine Laboratory (ETSII). Universidad Politécnica de Madrid (UPM). Research in pyrolysis of biomass in a rotary kiln and characterization of solid products
Nov 2011-May 2012	Assistant at the Department of Applied Linguistics (ETSIM-UPM)
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Dec 2008-Sep 2010	Póker Croupier, Casino Gran Madrid

EDUCATION

July 2013 –Present	PhD candidate in Civil and Environmental Engineering (EPFL), supervisor A. Buttler, co-supervisor C. Signarbieux
Oct 2012-Apr 2013	Master Thesis (grade: Excellent 10/10). Thesis: “Thermal pre-treatment of olive stone by means of torrefaction: solid characterization and optimization” Supervisors: Rubén Laina (ETSIM-UPM) and Guillermo San Miguel (ETSII-UPM)
Sep 2010 June 2011	Exchange year at EPFL
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High school	Lycée français de Caracas, Center for Innovation and Development of Distance Education (CIDEAD)
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PUBLICATIONS

Sanginés de Cárcer P, Signarbieux C, Schlaepfer R, Buttler A, Vollenweider P (2017) Responses of antinomic foliar traits to experimental climate forcing in beech and spruce saplings. *Environmental and Experimental Botany*, 128–140.

Signarbieux C, Toledano E, Sanginés de Cárcer P, Yongshuo HF, Schlaepfer R, Buttler A, Vitasse Y (2017) Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring. *Global Change Biology*.

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Sanginés P, Domínguez MP, Sánchez F, San Miguel G (2015) Slow pyrolysis of olive stones in a rotary kiln: Chemical and energy characterization of solid, gas, and condensable products. *Journal of Renewable and Sustainable Energy*, 7.

CONFERENCE TALK

Sanginés P, Signarbieux C, Vollenweider P, Buttler A, "European beech shows higher phenotypic plasticity than Norway spruce under simulated climate change" British Ecological Society Annual Meeting 2015, 13 - 16 December, EICC, Edinburgh

AWARDS

Sep 2007-2011 Spanish Government Scholarship for under-graduated studies

Sep 2011-2012 Collaboration grant from UPM

Sep 2010-2011 Scholarship Erasmus Exchange program

ADDITIONAL INFORMATION

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Hobbies: Dance, language learning, tennis, ski, Irish music