ANATOMICAL, PHYSIOLOGICAL AND MOLECULAR RESPONSES OF EUROPEAN BEECH (FAGUS SYLVATICA L.) TO DROUGHT

Dissertation

In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy (PhD)
of the Faculty of Forest Sciences and Forest Ecology
Georg-August-University of Göttingen, Germany

Submitted by

Ngoc Quynh Nguyen

Born in Yen Bai, Viet Nam

Göttingen, February 2016

Referee: **Prof. Dr. Andrea Polle**

Co-referee: **Prof. Dr. Konstantin Krutovsky**

Date of examination: February 29th 2016

Table of Contents

Table of Contents **1**
List of Abbreviations **4**
Summary **8**
Zusammenfassung **12**
Chapter 1: General introduction **17**
1.1. Global climate change **17**
1.2. European beech forests in Germany **19**
1.3. Plant responses to drought stress **20**
1.4. Responses of European beech (*Fagus sylvatica* L.) to drought stress **21**
1.5. Scope of the present study **22**
1.6. References **23**
Chapter 2: Anatomical responses of mature beech trees along a gradient of precipitation **27**
2.1. Introduction **27**
2.2. Materials and Methods **29**
2.2.1. Study locations 29
2.2.2. Sampling 31
2.2.3. Wood anatomical analyses 31
2.2.4. Carbon and nitrogen measurements 32
2.2.5. Statistical analysis 33
2.3. Results **34**
2.3.1. Growth along a precipitation gradient 34
2.3.2. Anatomical characteristics of beech trees along a precipitation gradient 36
2.3.3. Variation of anatomical features within the year rings 40
2.3.4. Nitrogen and carbon in wood 43
2.4. Discussion **45**
2.5. References **48**
2.6. Declaration **52**
Chapter 3: Intra-specific variations in expression of stress-related genes in beech progenies are stronger than drought-induced responses **53**
3.1. Introduction **53**
3.2. Material and Methods **56**
3.2.1. Field sites and plant material 56

3.2.2. Experimental treatments and harvests 57
3.2.3. Relative electrolyte leakage (REL) 58
3.2.4. RNA extraction and analysis of gene expression 58
3.2.5. DNA extraction and microsatellite analysis 59
3.2.6. Data analysis 60
3.3. Results 61
3.3.1. Genetic structure of beech stands along a precipitation gradient 61
3.3.2. Stress responses of beech progenies from a precipitation gradient 63
3.3.3. ABA-related gene expression in beech progenies from a precipitation gradient 65
3.3.4. Stress-related gene expression in progenies from a precipitation gradient 66
3.3.5. Multivariate analysis of progeny- and drought-related performance of beech progenies 68
3.4. Discussion 71
3.4.1. Beech populations exhibit strong differentiation of stress-related gene transcription, but not of neutral genetic markers 71
3.4.2. Stress-related genes exhibit seasonal changes and differ in drought responsiveness 72
3.5. References 75
3.6. Declaration 81
Chapter 4: Drought avoidance and drought tolerance: evidence for intraspecific variation in juvenile beech (Fagus sylvatica L.) 87
4.1. Introduction 87
4.2. Materials and Methods 89
4.2.1. Plant material 89
4.2.2. Drought treatment and harvests 90
4.2.3. Physiological Measurements 91
4.2.4. Total RNA isolation and gene expression analysis 92
4.2.4.1. RNA extraction from frozen beech leaves 92
4.2.4.2. Evaluation of RNA concentration and purity 93
4.2.4.3. DNase treatment 94
4.2.4.5. Synthesis of first strand complementary DNA (cDNA) 94
4.2.4.6 Quantitative real time PCR (qRT-PCR) 94
4.2.5. Data analyses 95
4.3. Results 95
4.3.1. Juvenile beech dry matter remains unaffected by drought 95
4.3.2. The plant water status under drought treatment varies with the provenance 96

4.3.3. Minor injury to PSII performance under drought treatment 100
4.3.4. Stomatal responses to drought vary with the provenance 100
4.3.5. OST1 gene expression 102
4.4. Discussion 103
4.4.1. Beech provenances differ in drought resistance strategies 103
4.4.2. Fitness of beech provenances in relation to drought avoidance and drought tolerance 104
4.5. References 107
4.6. Declaration 112
Chapter 5: Conclusion and Outlook **..... 113**
5.1. Conclusion 113
5.2. Outlook 114
Declaration of originality and certificate of the authorship 116
Acknowledgements 118

List of Abbreviations

a^{-1}	per year
a.m	Before midday
a.s.l	Above sea level
ABA	Abscisic Acid
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
bp	Base pairs of nucleotides
C	a) Carbon b) Conductivity
C: N	The ratio of carbon to nitrogen
cDNA	Complementary DNA
CL	Calvörde loam
CS	Calvörde sand
Ct	cycle threshold
CTAB	Cetyltrimethylammoniumbromide
ddH_2O	Double distilled water
DM	Dry mass
DNA	Desoxyribo Nucleic Acid
dNTPs	Desoxy-nucleootide triphosphate
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed sequence tag
et al.	Et alia (Latin) = and others
F_0	Initial fluorescence
FAE	Formaldehyde: Acetic Acid : Ethanol
FF	Frequency of fibre
FLA	Fibre lumen area

F_m	Maximum fluorescence yield
FW	Fresh weight
G	Cumulative basal area
g	a) Grams (unit of weight) b) As unit of centrifugal force
GL	Göhrde loam
GS	Göhrde sand
g_s	Stomatal conductance
h	Hours
H_2O	Water
ha	Hectar
HCl	Hydrochloric acid
HP	High-precipitation
IP	Intermediate-precipitation
KCl	Potassium chloride
KLIFF	Klimafolgenforschung in Niedersachsen or Climate impact and adaption research in Lower Saxony
$KMnO_4$	Potassium permanganate
L	Litre
LP	Low-precipitation
m	Meter
M	Molar
MANOVA	Multivariate analysis of variance
$MgCl_2$	Magnesium chloride
min	Minute
ml	Mililitre
mm	Milimeter
mol	Mole
MOPS	3-(N-Morpholino) propane sulfonic acid
MPa	Megapascal
N	Nitrogen

NaCl	Sodium chloride
NH_3	Ammonia
^oC	Degree Celsius
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PCWA	Percentage of cell wall area
pmol	Pico mole, 10^{-12}mol
PSII	Photosystem II
PVP	polyvinylpyrrolidinone K30
qRT-PCR	Quantitative real – time PCR
RA	Ray parenchyma
REL	Relative Electrolyte Leakage
RNA	Ribonucleic acid
RNase	Ribonuclease
ROS	Reactive oxigen species
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
RWC	Relative water content
s	Seconds (unit of time)
SDS	Sodium dodecyl sulfate
SE	Standard error
SLA	Specific leaf area
SSTE	Sodium chloride SDS Tris HCl EDTA
SWC	Soil water content
TAE	Tris Acetate EDTA
Taq	*Thermus aquaticum*
TDFW	The wall between two adjacent fibre cells
TDR	Time domain reflectometry
T_m	Melting temperature of primer

Tris	Tris-(hydroxymenthyl)-aminomethane
U	Unit
UL	Unterlüss loam
US	Unterlüss sand
UV	Ultra violet
V	Voltage
v/v	Volume/volume
VLA	Vessel lumen area
w/v	Weight/volume
$\delta^{13}C$	The ratio difference of 13C to 12C
Φ_{PSII}	Maximum photochemical efficiency of PSII
Ψ	Predawn water potential

Summary

European beech (*Fagus sylvatica* L.) is a dominant forest tree species of high economic and ecological value in Central Europe. The natural distribution range of the species across Central Europe is determined by water ability. Extreme weather events with severe drought and drought periods are predicted to occur more frequently in the future. In the forest sector, water supply probably becomes a limiting factor in extended areas. It is, thus, necessary to evaluate the potential ability of beech to acclimate or adapt to water limitation.

The responses of beech to water shortage could be archived in wood anatomical properties and might be evaluated by analysing these properties. Moreover, water limitation may negatively affect carbon and nitrogen contents of beech wood. Soil humidity is an important factor influencing ^{13}C variations in tree rings, since water limitation can induce stomatal closure and thus increase the $\delta^{13}C$ of the incorporated carbon. Therefore, C, N content and $\delta^{13}C$ signatures in beech wood samples were analyzed to investigate effects of water shortage on beech wood properties.

A key pathway for drought acclimation involves abscisic acid (ABA) signaling to recruit drought defense responses and which result in stomatal closure, thereby, regulating plant water consumption. Another feature of drought stress is an increased production of reactive oxygen species. Therefore, activation of protective enzymes, especially of antioxidative defenses, is important to combat the oxidative degradation of vulnerable structures such as cell membranes. To address the plasticity and adaptation of beech in response to drought, expression levels of ABA- and stress-related genes were chosen for analyzing. In addition, leaf area and membrane integrity were determined as indicators of the responses of beech to drought stress.

Plant species have different strategies to cope with water stress: avoidance or tolerance. The basic mechanism of either strategy involves isohydric or anisohydric stomatal regulation. Isohydric plants close stomata before any changes occur in plant water status, whereas anisohydric species show a slow stomatal reaction in response to a decrease in the water potential. Soil water content, leaf predawn water potential, relative water content, chlorophyll fluorescence and stomatal conductance were characterized as good candidates to test these strategies. Furthermore, expression of *OST1* (open stomata 1), a protein kinase that links the guard cell reaction to the ABA signaling network was investigated.

In this study, the responses of seedlings, saplings and mature trees of European beech to drought have been investigated. The following hypotheses were tested:

Beech trees from drier habitats possesses changes in the xylem anatomy that enables them to cope with low precipitation.

Dry climate negatively affects carbon and nitrogen contents of beech wood.

Beech progenies from dry sites exhibit constitutively higher expression levels of ABA- and stress-related genes and are therefore less drought responsive than progenies from moist sites.

Beech originating from a low-precipitation climate show a stronger drought avoidance and beech from mesic habitats adopt a stronger drought tolerance strategy than those originating from dry habitats when exposed to decreasing soil water availability.

To test these hypotheses, three experiments were set up and conducted with either mature beech trees along a precipitation gradient or beech seedlings exposed to experimental manipulation of the soil water level.

A field study was carried out in three locations differing in the long-term annual precipitation. Wood increment, xylem anatomical properties as well as C, N content and $\delta^{13}C$ signatures was investigated. A strong reduction of annual increment of beech trees was found from moist sites to dry sites. Thus, water availability of study sites might be one of the limiting factors of wood increment of beech trees. Beech trees from dry sites showed changes anatomical traits that enable them to cope better with low precipitation climate. To compensate for narrower vessel lumen areas, beech trees stocking in the dry site had higher vessel frequencies. These anatomical changes probably enable beech trees balance between water uptake efficiency and avoidance of embolism in beech stems. Moreover, this mechanism probably helped the plants to maintain the water status of beech trees under dry condition, and to maintain C and N content in beech wood. This finding suggests that beech trees on the dry site may have a drought avoidance strategy to cope with low water availability in nature. Anatomical features varied significantly along the growing season. In early wood, anatomical parameters did not exhibit remarkable changes among sites. In latewood and transition wood regions, vessel lumen area decreased strongly and vessel frequency increased significantly. In late wood of beech trees stocking on the dry sites, thicker walls and narrower fibre lumina were found. In addition, decreased $\delta^{13}C$ values of beech trees living in the driest indicate higher water

use efficiency in the late growing season. The comparison of beech trees at the wet and the dry sites suggests that water availability caused anatomical changes. However, other factors as genetic factors may also contribute to better adaptedness of on dry sites to low precipitation.

To investigate the expression of genes related to ABA and stress in response to drought stress, a common garden experiment was conducted. The natural regeneration from five beech stands along a precipitation gradient was used in this experiment. The responses of well-watered and drought-stressed saplings to drought stress were measured throughout summer at an early, mid- and late season time points. Expression levels of ABA- and stress-related genes were determined. To link gene expression with plant performance we determined progeny-and drought-related effects on leaf area and membrane integrity in the absence and presence of acute oxidative stress. Drought stress resulted in decreased leaf area compared with well-watered saplings. Progenies from the wetter site, generally, showed larger leaf areas than those from the drier sites. Relative electrolyte leakage was changed by drought stress and increased toward the end of the growing season. Expression levels of ABA- and stress - related genes was strongly affected by drought stress except glutamine amido transferase *(GAT)*. In addition, expression levels of genes (nine-cis-epoxy-dioxygenase (*NCED*), protein phosphatase 2C (*PP2C*), early responsive to dehydration (*ERD*), ascorbate peroxidase (*APX*), superoxide dismutase (*Cu/ZnSOD*), aldehyde dehydrogenase (*ALDH*), glutamine amido transferase (*GAT*) was higher in the progenies from moist than in those from drier sites. Seasonal analyses of the transcriptional regulation of genes for drought signaling and defense uncovered intraspecific differences in constitutive expression and drought responsiveness. The progeny-related differences were stronger than the stress responses suggesting that selection for drought adaptation may already take place in local beech populations.

To investigate whether there is intraspecific variation in the drought resistance mechanisms, three beech provenances from a low, intermediate-, and high-precipitation climate (designated as LP, IP, and HP) were subjected to progressive drought. Soil and plant water status, the maximum quantum yield of photosystem II, and stomatal conductance of control and drought-treated seedlings were regularly measured. Moreover, transcript levels of *OST1* were determined. The data support that the within-species drought responses of beech can also vary between isohydric or anisohydric stomatal behavior. The beech provenance LP exhibited an isohydric phenotype because the plants showed more rapid stomatal closure and maintained higher leaf relative water content and predawn water potentials than those from mesic

conditions. Thereby, the population from the dry habitat clearly displayed a drought avoidance strategy. In contrast, the HP progenies showed a slow decline in stomatal conductance, but a stronger decrease in the predawn water potential upon water limitation. There was no drought influence on plant growth biomass allocation throughout drought treatment. Beech exhibited intraspecific variation in drought resistance strategies characterized by anisohydric or isohydric behavior. It suggests that the anisohydric functional type of beech is better endowed to cope with the predicted climate extremes than the isohydric type because it possess a drought tolerance strategy.

The results of this present study show that low precipitation climate and drought affect the anatomical, physiological and molecular responses of beech trees. Beech trees exhibited quite high intraspecific variation in drought resistance strategies with drought avoidance and drought tolerance strategies.

Zusammenfassung

Die europäische Buche (*Fagus sylvatica*, L.) ist eine dominante Waldbaumart von hohem ökonomischen und ökologischem Wert in Zentraleuropa. Die natürliche Verbreitung der Spezies in Zentraleuropa ist abhängig von der Wasserverfügbarkeit. In der Zukunft wird vermehrt von extremen Wetterbedingungen wie Hitzewellen und extreme Trockenheit ausgegangen. In ausgedehnten Bereichen des Waldes wird die Wasserversorgung wahrscheinlich ein limitierender Faktor. Daher ist es notwendig, die potentielle Fähigkeit der Buchen, sich bei Wasserlimitierung zu akklimatisieren oder anzupassen, zu bewerten.

Die Reaktionen der Buche auf Wasserknappheit könnten in anatomischen Eigenschaften des Holzes abgespeichert sein und durch die Analyse dieser Eigenschaften bewertet werden. Darüber hinaus könnte die Wasserlimitierung die Kohlenstoff- und Stickstoffgehalte im Buchenholz negativ beeinflussen. Die Bodenfeuchte ist ein wichtiger Faktor, der den ^{13}C-Gehalt in den Baumringen beeinflußt. Da Wasserknappheit den Verschluß der Stomata induzieren kann, wird das $\delta^{13}C$ des eingebauten Kohlenstoffes erhöht. Daher wurden C- und N-Gehalte und $\delta^{13}C$ Signaturen in Buchenholzproben analysiert, um die Auswirkungen von Wasserknappheit auf Buchenholzeigenschaften zu untersuchen.

Eine Schlüsselrolle für die Akklimatisierung an Trockenheit spielt Abscisinsäure (ABA), wodurch Abwehrreaktionen hervorgerufen werden, die zum Verschluß der Stomata führen und somit den Wasserverbrauch der Pflanzen regulieren. Ein weiteres Merkmal von Trockenstress ist eine erhöhte Produktion von reaktiven Sauerstoffspezies. Daher ist die Aktivierung von Schutzenzymen, insbesondere der antioxidativen Abwehr, wichtig bei der Bekämpfung des oxidativen Abbaus anfälliger Strukturen wie etwa der Zellmembranen. Um die Plastizität und Anpassung der Buche in Reaktion auf Trockenheit zu untersuchen, wurde die Expression von ABA- und stressverwandten Genen für die Analyse ausgewählt. Darüber hinaus wurden die Blattflächen und die Membranintegrität als Indikatoren für Reaktionen der Buche auf Trockenstress bestimmt.

Pflanzen haben unterschiedliche Strategien, um Trockenstress zu bewältigen: Vermeidung oder Toleranz. Der Grundmechanismus beider Strategien beinhaltet die isohydrische oder anisohydrische Regulation der Stomata. Isohydrische Pflanzen schließen ihre Stomata noch bevor sich der Wasserstatus in der Pflanze verändert, wohingegen anisohydrische

Spezies eine langsame stomatale Reaktion als Antwort auf ein geringeres Wasserpotential zeigen. Der Wassergehalt des Bodens, in den Blättern vor Sonnenaufgang (Predawn Water Potential), der relative Wassergehalt, Chlorophyllfluoreszenz und stomatäre Leitfähigkeit wurden als gute Merkmale charakterisiert, um diese Strategien zu testen. Des Weiteren wurde die Expression von *OST1* (open stomata 1), einer Proteinkinase, die zur Schließzellenreaktion des ABA-Signalnetzwerkes führt, untersucht.

In dieser Studie wurden die Reaktionen von Sämlingen und jungen, sowie ausgewachsenen europäischen Buchen auf Trockenheit untersucht. Die folgenden Hypothesen wurden getestet:

- Buchenpopulationen aus trockeneren Lebensräumen weisen Veränderungen in der Anatomie des Xylems auf, um mit geringem Niederschlag umzugehen.
- Trockenes Klima wirkt sich negativ auf den Kohlenstoff und den Stickstoffgehalt in Buchenholz aus.
- Buchennachkommen von trockenen Standorten zeigen eine konstitutiv erhöhte Expression von ABA-und stressinduzierten Genen und reagieren somit weniger auf Trockenheit als Nachkommen von feuchteren Standorten.
- Buchen, die aus niederschlagsarmen klimatischen Bedingungen stammen, zeigen eine stärkere Trockenheitsvermeidung. Buchen aus mesischen Habitaten bilden eine stärkere Toleranz gegenüber Trockenheit aus, als solche aus trockenen Habitaten, wenn sie einer abnehmenden Wasserverfügbarkeit in der Erde ausgesetzt sind.

Um diese Hypothesen zu testen, wurden drei Experimente durchgeführt. Einerseits mit adulten Buchen entlang eines Niederschlagsgradienten, andererseits mit Buchensetzlingen, die experimentell veränderten Bodenwassergehalten ausgesetzt waren.

Ein Freilandexperiment wurde in drei verschiedenen Gebieten durchgeführt, die ähnliche Bodeneigenschaften aufwiesen, sich aber hinsichtlich der jährlichen Niederschlagsrate unterschieden. Holzzuwachs, die anatomischen Eigenschaften des Xylems, sowie C- und N-Gehalt und die $\delta^{13}C$ Signaturen wurden untersucht. Es wurde eine starke Reduzierung des jährlichen Zuwachses bei Buchen von feuchten hin zu trockenen Standorten gefunden. So könnte die Verfügbarkeit von Wasser in den Untersuchungsgebieten einer der begrenzenden Faktoren des Holzzuwachses bei Buchen sein. Buchen auf trockenen Standorten zeigten Veränderungen anatomischer Merkmale, die ihnen ermöglichten, besser mit geringen

Niederschlägen umzugehen. Um schmale Gefäßlumen auszugleichen, zeigten Buchen an trockenen Standorten mehr Gefäße. Diese anatomischen Veränderungen ermöglichen Buchen wahrscheinlich die Balance zwischen Wasseraufnahmeeffizienz und der Vermeidung von Embolien im Buchenstamm zu halten. Darüber hinaus trägt dieser Mechanismus wahrscheinlich dazu bei, das Wasserpotential und den C- und N-Gehalt im Holz der Buchen unter trockenen Bedingungen zu erhalten. Dieses Ergebnis deutet darauf hin, dass Buchen von trockenen Standorten eine Vermeidungsstrategie gegen Trockenheit haben, um mit geringer Wasserverfügbarkeit in der Natur umgehen zu können. Anatomische Merkmale variierten während der Vegetationsperiode signifikant. Zwischen den Standorten wiesen die anatomischen Parameter im Frühholz keine bemerkenswerten Veränderungen auf. Im Spät- und Übergangsholz war die Fläche der Gefäßlumen stark vermindert und die Anzahl der Gefäße signifikant erhöht. Im Spätholz der Buchen auf trockenen Standorten wurden dickere Wände und schmalere Faserlumina gefunden. Darüber hinaus zeigten verringerte $\delta^{13}C$ Werte bei den Buchen auf den trockensten Standorten eine höhere Wassernutzungseffizienz am Ende der Wachstumsperiode. Der Vergleich der Bäume von feuchten und von trockenen Standorten wies darauf hin, dass die Verfügbarkeit von Wasser anatomische Veränderungen beeinflußte. Jedoch können auch andere Faktoren als die genetischen zu einer besseren Anpassung der Buchen auf den trockenen Standorten an geringe Niederschläge beitragen.

Um die Expression von Genen im Zusammenhang mit ABA und Stress als Reaktion auf Trockenstress zu untersuchen, wurde ein Gartenexperiment durchgeführt. Für dieses Experiment wurden die Nachkommenschaften von fünf Buchenbeständen entlang eines Niederschlagsgradienten verwendet. Die Reaktionen von gut bewässerten und trockengestressten Keimlingen gegenüber Trockenstress wurden während des Sommers zu einem frühen, mittleren und späten Zeitpunkt gemessen. Die Expressionsniveaus von ABA- und stressbezogenen Genen wurde ermittelt. Um die Genexpression mit der Leistungsfähigkeit der Pflanzen vergleichen zu können, wurden herkunfts- und dürrebedingte Auswirkungen auf die Blattfläche und Membranintegrität in Abwesenheit und Anwesenheit von akutem oxidativen Stress untersucht. Trockenstress führte zu einer verringerten Blattfläche verglichen mit gut gewässerten Setzlingen. Die Nachkommen von feuchteren Standorten zeigten allgemein größere Blattflächen als die von trockenen Standorten. Der relative Verlust von Elektrolyten wurde durch Trockenstress verändert und erhöhte sich gegen Ende der Vegetationsperiode. Die Expressionsniveaus von ABA- und stressverwandten Genen wurden stark von Trockenstress beeinflußt. Eine Ausnahme bildet die Glutamin Amidotransferase (*GAT*). Zusätzlich waren die

Expressionsniveaus der Gene Nine cis-Epoxy Dioxygenase (*NCED*), Proteinphosphatase 2C (*PP2C*), Early Responsive to Dehydration (*ERD*), Ascorbat-Peroxidase (*APX*), Superoxid Dismutase (*Cu / Zn-SOD*), Aldehyde Dehydrogenase (*ALDH*), Glutamin Amido-Transferase (*GAT*) höher in den Nachkommen von feuchten Standorten verglichen mit trockeneren Standorten. Saisonale Analysen der transkriptomalen Regulation von Genen für die Signalisierung und Abwehr von Trockenheit zeigten intraspezifische Unterschiede in der konstitutiven Expression und Reaktionsfähigkeit bei Trockenheit. Die herkunftsbedingten Unterschiede waren größer als die Stressreaktionen, was darauf hindeutet, dass die Selektion für eine Anpassung an Trockeheit bereits in lokalen Buchenpopulationen stattfindet.

Um zu untersuchen, ob es intraspezifische Unterschiede bei den Resistenzmechanismen gegen Trockenheit gibt, wurden drei Buchenherkünfte, aus einem niedrigen, einem mittleren und einem hohen Niederschlagsklima (als LP, IP und HP bezeichnet), zunehmender Trockenheit ausgesetzt. Der Wassergehalt in Boden und Pflanzen, die maximale Quantenausbeute des Photosystems II und die stomatäre Leitfähigkeit der Kontrollen und der trockenheitsbehandelten Setzlinge wurden regelmäßig gemessen. Außerdem wurden die Transkriptionsniveaus von *OST1* bestimmt. Die Daten weisen darauf hin, dass die innerartlichen Reaktionen auf Trockenheit bei Buchen auch zwischen isohydrischem und anisohydrischem Verhalten der Stomata variieren können. Die Buchenherkunft LP zeigte einen isohydrischen Phänotyp, da die Pflanzen einen schnelleren Verschluß der Stomata, einen höheren relativen Wassergehalt, sowie einen höheren Wassergehalt in den Blättern vor Sonnenaufgang (Predawn Water Potential) zeigten als Buchennachkommen aus mesischen Bedingungen. Dadurch wies die Population aus dem trockenen Habitat eine deutliche Vermeidungsstrategie bei Trockenheit auf. Im Gegensatz dazu zeigten die HP Nachkommen bei Wasserlimitierung einen langsamen Abfall der stomatären Leitfähigkeit, jedoch eine stärkere Abnahme des Predawn Water Potential. Es gab keinen Einfluss durch Trockenheit auf das Pflanzenwachstum oder die Biomasseallokation während der Trockenheitsbehandlung. Buchen zeigten intraspezifische Unterschiede bei den Resistenzstrategien gegen Trockenheit, gekennzeichnet durch anisohydrisches oder isohydrisches Verhalten. Das legt nahe, dass der anisohydrische Funktionstyp bei Buchen besser geeignet ist, um mit den vorhergesagten Klimaextremen umzugehen, als die isohydrische Typ, da er eine Trockentoleranz-Strategie aufweist.

Die Ergebnisse der vorliegenden Studie zeigen, dass Klimate mit geringen Niederschlägen und Trockenheit die Anatomie, Physiologie und molekulare Reaktionen von

Buchen beeinflussen. Buchen zeigten recht hohe intraspezifische Unterschiede bei den Strategien zur Trockenheitsresistenz, mit Strategien zur Trockenheitsvermeidung und Trockentoleranz.

Chapter 1: General introduction

1.1. Global climate change

Due to global warming, global surface temperature increased about 0.85ºC in the period from 1880 to 2012 (IPCC 2014). The period from 1983 to 2012 was considered as the warmest 30-year period of the last 1400 years in the Northern Hemisphere (EAA 2012). The global temperature was forecasted to increase from 0.3 to 4.8ºC by the end of this century depending on different scenarios (IPCC 2014) (Figure 1.1). The exact prediction of extreme climatic events is currently impossible. However, extreme weather and climate events, such as hot summer days, summer drought will probably or very probably occur more frequently during the 21st century (IPCC 2001).

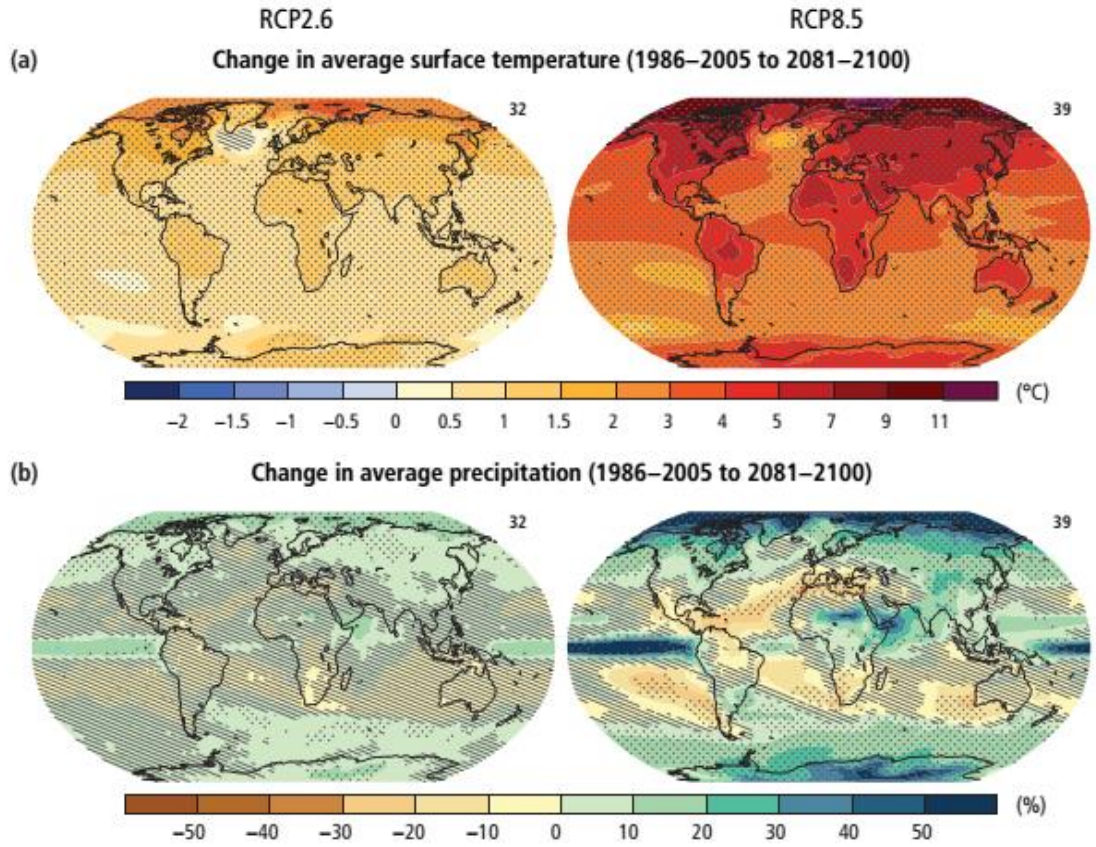


Figure 1.1: Change in average surface temperature (a) and change in precipitation (b) based on multi-model mean projections for 2081-2100 relative 1986-2005 under RCP2.6 (left) and RCP8.5 (right) scenarios (taken from IPCC 2014).

On the European continent, a remarkable increase of temperature was observed (EAA 2012). Some studies indicated that the average temperature of Europe increased already by 0.95°C (Brohan et al. 2006) and 2.0°C in the south of Germany and the Alps (Mayer et al. 2005) in the last century. In 2003, Europe faced a series of strong persistent heatwaves during the summer (Fink et al. 2004). The June-August period was 5°C warmer compared to the period of 1961-1990 and was the warmest summer since at least 1864 (Schär et al. 2004). The land temperature in Europe is predicted to increase between 2.5°C to 4.0° C by the end of 21st century compared to the temperature of 2005 (EEA 2012, Schröter et al. 2005a). Moreover, the highest increase of temperature is projected to occur over eastern and northern Europe in winter and over southern Europe in summer (Schröter et al., 2005a, Zebisch et al., 2005). From the 1950s, annual precipitation increased across the northern parts of but declined in southern Europe (EAA 2012). Annual precipitation and its distribution over the seasons are among the most important factors affecting ecosystems. Most scenarios predict that precipitation will continue to increase in the northern part during winter and decrease in the southern part during summer (EEA 2012, Schröter et al. 2005a).

In Germany, long-term weather recordings reveal that climate change is occurring (Schröter et al. 2005b). The annual temperature increased by ca. 0.85°C in the 20th century (Zebisch et al. 2005). By the end of this century, the annual temperature in Germany is forecasted to increase between 1.6°C and 3.8°C (Schröter et al. 2005b) (Fig.1.2). In the last 30 years, a definite increase of precipitation was recorded in winter and a decrease was observed in summer in Germany (Zebisch et al. 2005). All climate scenarios predicted that summer precipitation will decrease between 16.6 to 33.3% up to the year 2080 (Fig.1.3). These scenarios forecasted increases in winter precipitation in the southern part and decreases of summer precipitation in the southwest and central parts of Germany (Enke et al., 2005, Jacob et al., 2008, Schröter et al., 2005b).

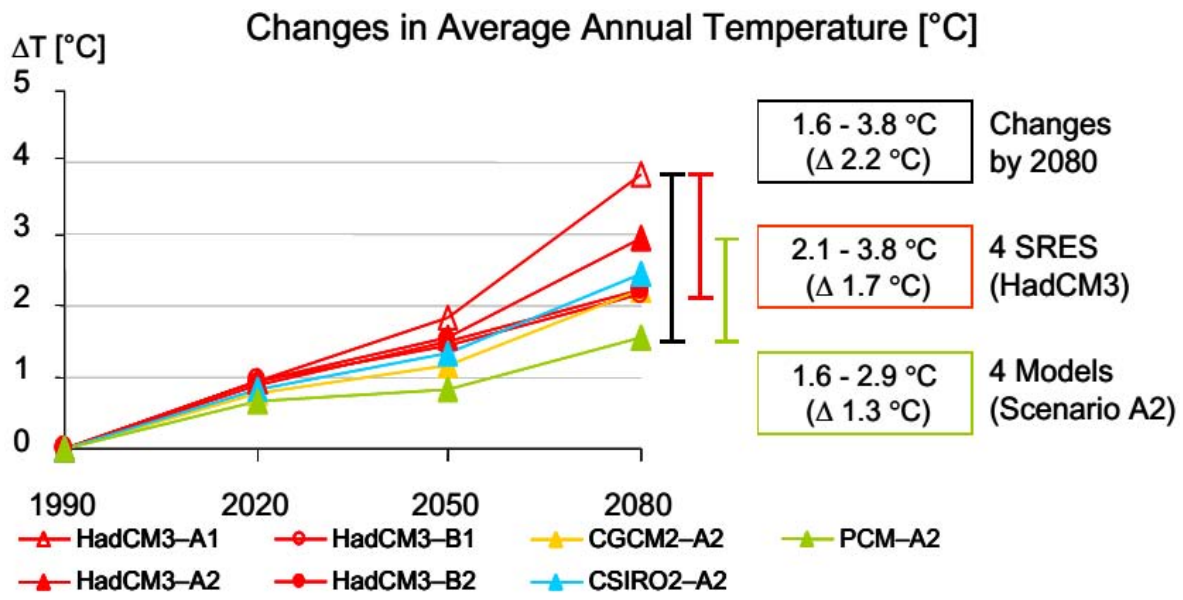


Figure 1.2: Scenarios of long-term annual average temperature change compared to 1990 in Germany up to 2080 (Schröter et al. 2005b).

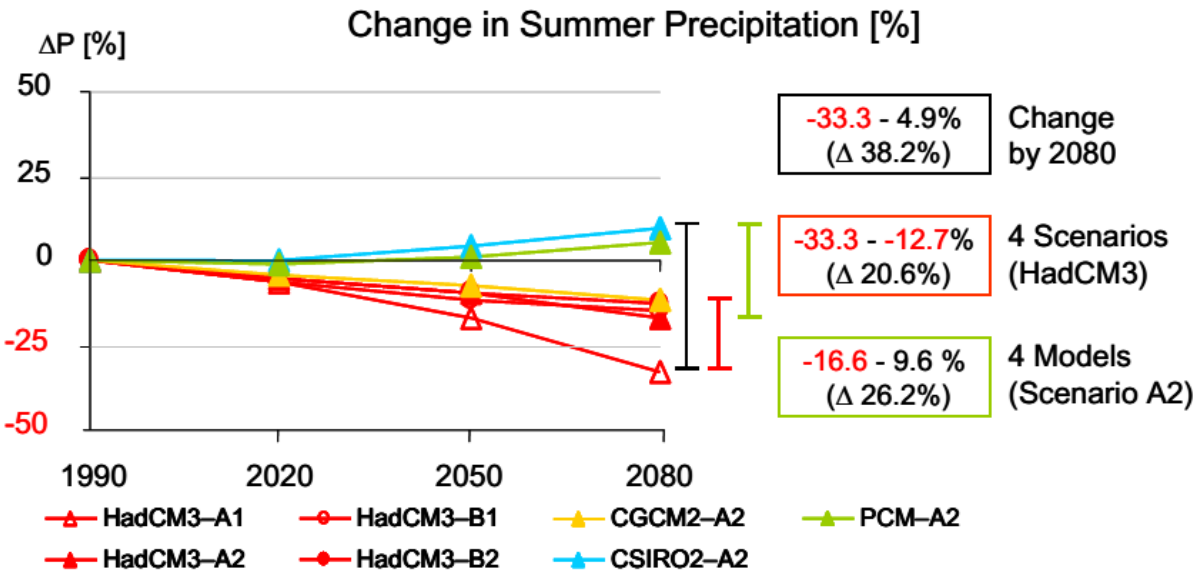


Figure 1.3: Change in summer precipitation compared to 1990 of seven scenarios in Germany up to 2080 (Schröter et al. 2005b)

1.2. European beech forests in Germany

European beech (*Fagus sylvatica* L.) is one of the ecologically and economically most important deciduous tree species of the vegetation in Germany (Wühlisch and Muhs 2010). The total forest area of Germany is 11,075 million ha (publicly 33.3%, corporate bodies 19.5% and private and to be privatized 47.2%). 1,565 million ha (approx.14.8%) are covered by beech.

Most of the beech forest are natural (60%) or managed close to nature (22.5%) (Federal Ministry 2002). Most beech forests (80%) occur in the southwest and central parts of Germany mainly in Rhineland-Palatinate, Saarland, Hessen, part of Bavaria and the southern parts of Lower Saxony and North Rhine-Westphalia. The data of the second Inventory of the German National Forests (Federal Ministry 2002) indicated that about 81,754 ha in 15 years or 5,450 ha/year of mainly conifer forest were placed by broadleaved forest tree species. Thus, beech forests will be more widespread in Germany in the coming decades.

1.3. Plant responses to drought stress

Drought was defined as "*a period of abnormally dry weather sufficiently prolonged for the lack of precipitation to cause a serious hydrological imbalance and carries connotations of a moisture deficiency with respect to water use requirements*" (McMahon and Arenas 1982). Among environmental factors, water balance is considered as the most important factor for plants. Moreover, drought is estimated to be the most stressful factor, which reduces plant productivity alone more than any other climatic stress (Lambers et al. 2008). Drought not only influences trees' growth but also cause changes at anatomical, physiological and biochemical levels (Micco and Aronne, 2012).

Adaptation to drought of anatomical properties can be achieved by balancing between the need to maintain high conductivity when water supply is satisfactory, and to avoid embolism when drought occurs (Sperry 2003). The main ecological trends in wood anatomy indicate that, moving from mesic to xeric conditions, woods tend to lower their conductive efficiency, but are more resistant to cavitation (Micco and Aronne 2012). Wood properties that indicate adaptation to drought are generally a reduction of vessel lumina and increases of vessel frequencies as well as changes in internal structure of vessels such as intervessel pitting and pit membrane pores (Wheeler et al. 2005, Sperry et al. 2006). Narrow vessels only permit low water transport but they are safer because they maintain hydraulic conductivity and are less prone to embolism (Sperry et al. 2006, Carlquist 2013). Thickness and density of vessel helical sculpturing are strongly correlated with resistance to cavitation, and play an important role in preventing spreading of cavitation and in increasing of mechanical strength (Lens et al. 2011).

Plants have developed different strategies to deal with drought for example by increasing the root: shoot ratio in order to explore larger soil volumes and to acquire more water from deeper soil layers or by minimizing the water loss by stomatal closure (Verslues et al.

2006, Zollinger et al. 2006). Another mechanism involves a continuation of plant metabolic activities at a low tissue water potential, for example, by osmotic adjustment, changes in cell wall elasticity, etc. (Anjum et al. 2012).

The plant hormone abscisic acid (ABA) plays a very important role in plants in response to drought stress. A key pathway for drought acclimation involves ABA signaling to recruit drought defense responses and which result in stomatal closure, thereby, regulating plant water consumption (Shinozaki and Yamaguchi-Shinozaki 2007, Popko et al. 2010, Raghavendra et al. 2010). A further common feature of drought stress is an increased production of reactive oxygen species (Cruz de Carvalho 2008). Thus, activation of protective enzymes, especially of antioxidative defenses is important to combat oxidative degradation of vulnerable structures such cell membranes (Polle et al. 2006, Fischer and Polle 2010).

1.4. Responses of European beech (*Fagus sylvatica* L.) to drought stress

In natural forests, beech dominates from moderately dry to moist environment (Ellenberg, 1996). However in dry locations beech is replaced by other broadleaves species like *Quercus petrea* or Q. *pubescens* (Wühlisch and Muhs, 2010). Water ability is the main limiting factor of the natural distribution of beech to the south of Germany (Ellenberg 1996). It has been predicted increased frequencies and duration of summer droughts in central European areas may lead to negative effects on the water balance, growth and competitive capacity of beech, especially on limestone-derived and sandy soils with low water retention capacity (Gessler et al. 2007).

Under severe drought stress (several weeks), young beech trees show decreased growth and reduced nitrogen uptake from the soil (soil water potential < - 0.4 Mpa) (Fotelli et al. 2001, 2002). Biomass accumulation of beech seedlings was significantly affected by irrigation regimes during a 52-day exposed drought (Fotelli et al. 2001). In addition, drought also significantly reduced transpiration rates (Fotelli et al. 2001) and led to embolism when the predawn water potentials of beech seedlings under controlled condition went below – 1.9 MP (Hacke and Sauter 1995).

Drought also lowered stomatal conductance and gross primary productivity of adult beech trees. The water deficit and extreme summer heat of 2003 were the main reasons reducing 75% mean stomatal conductance compared with the 2002 values and 30 % in gross productivity

of beech stand growing in forests in eastern France (Ciais et al. 2005). Keitel (2003) and Keitel et al. (2006) measured foliar carbon isotope composition along a climate gradient. Their studies indicated that ^{13}C depletion was reduced with decreasing summer precipitation exhibiting an exponential relationship when rainfall amounts were lower than 500 mm (Fotelli et al. 2003, Keitel et al. 2003, Keitel et al. 2006). Moreover, when average stomatal conductance (G_s) decreased below 25-30mmol m^{-2} s^{-1}, ^{13}C discrimination during CO_2 fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was reduced, and that increased $\delta^{13}C$ in the organic matter (Fotelli et al., 2003, Geßler et al., 2001). A summer drought further decreased the mean of fine root diameter and changed carbon allocation in the fine root of mature beech trees (Meier and Leuschner 2008).

Because of the expected drought sensitivity of European beech, it is likely that the physiological performance, growth and competitive ability of the species will be negatively affected by climate change with drastic consequences for current forests. It is, therefore, highly desirable to understand anatomical, physiological and molecular responses of European beech to low precipitation climate and drought stress.

1.5. Scope of the present study

The main aims of this research were to elucidate anatomical, physiological and molecular responses of European beech (*Fagus sylvatica*, L.) to drought. For this purpose, the following hypotheses were tested:

- Beech trees from drier habitats possess some changes in the xylem anatomy that enables them to cope with low precipitation (Chapter 2).
- Dry climate negatively affects carbon and nitrogen content of beech wood (Chapter 2)
- Beech progenies from dry sites exhibit constitutively higher expression levels of ABA-and stress-related genes and are therefore less drought responsive than progenies from dry sites (Chapter 3).

Beech originating from a low-precipitation climate show a stronger drought avoidance and beech from mesic habitats adopt a stronger drought tolerance strategy than those originating from xeric habitats when exposed to decreasing soil water availability (Chapter 4).

1.6. References

Anjum SA, Farooq M, Xie X, Liu X, Ijaz MF (2012) Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. Scientia Horticulturae 140:66–73.

Brohan P, Kennedy JJ, Harris I, Tett SFB, Jones PD (2006) Uncertainty estimates in regional and global observed temperature changes: A new data set from 1850. J Geophys Res 111:D12106.

Carlquist S (2013) Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer Science & Business Media.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533.

Cruz de Carvalho MH (2008) Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signaling & Behavior 3:156–165.

EEA (European Environment Agency) (2012) Climate change, impact and vulnerability in Europe 2012, an indicator-based report. European Environment Agency, Copenhagen

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5th edn. Ulmer, Stuttgart, Germany.

Enke W, Deutschländer T, Schneider F, Küchler W (2005) Results of five regional climate studies applying a weather pattern based downscaling method to ECHAM4 climate simulation. Meteorologische Zeitschrift 14:247–257.

Federal Ministry of Food, Agriculture and Consumer Protection (2002) The Second National Forest Inventory -NFI, Covering the National Forest Inventory Survey of 2001-2002 and 1986-1988.

Fink AH, Brücher T, Krüger A, Leckebusch GC, Pinto JG, Ulbrich U (2004) The 2003 European summer heatwaves and drought–synoptic diagnosis and impacts. Weather 59:209–216.

Fischer U, Polle A (2010) Populus Responses to Abiotic Stress. In: Jansson S, Bhalerao R, Groover A (Eds) Genetics and Genomics of Populus. Springer New York, pp 225–246.

Fotelli MN, Geßler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus*

fruticosus: responses of growth, water status and δ13C composition. New Phytologist 151:427–435.

Fotelli MN, Rennenberg H, Gessler A (2002) Effects of Drought on the Competitive Interference of an Early Successional Species (*Rubus fruticosus*) on *Fagus sylvatica* L. Seedlings: 15N Uptake and Partitioning, Responses of Amino Acids and other N Compounds. Plant Biology 4:311–320.

Fotelli MN, Rennenberg H, Holst T, Mayer H, Geßler A (2003) Carbon isotope composition of various tissues of beech (*Fagus sylvatica*) regeneration is indicative of recent environmental conditions within the forest understorey. New Phytologist 159:229–244.

Gessler A, Keitel C, Kreuzwieser J, Matyssek R, Seiler W, Rennenberg H (2007) Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21:1–11.

Geßler A, Schrempp S, Matzarakis A, Mayer H, Rennenberg H, Adams MA (2001) Radiation modifies the effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). New Phytologist 150:653–664.

Hacke U, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica f. purpurea* and *Populus balsamifera*. J Exp Bot 46:1177–1183.

IPCC (2001) Climate change 2001: The scientific basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jacob D, Göttel H, Kotlarski S, Lorenz P, Sieck K (2008) Klimaauswirkungen und Anpassung in Deutschland–Phase 1: Erstellung regionaler Klimaszenarien für Deutschland. Abschlussbericht zum UFOPLAN-Vorhaben 204 41 138. Umweltbundesamt (Eds), Climate Change 11.

Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Geßler A (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). Plant, Cell & Environment 26:1157–1168.

Keitel C, Matzarakis A, Rennenberg H, Gessler A (2006) Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. Plant, Cell & Environment 29:1492–1507.

Lambers H, Iii FSC, Pons TL (2008) Plant Water Relations. In: Plant Physiological Ecology. Springer New York, pp 163–223.

Lens F, Sperry JS, Christman MA, Choat B, Rabaey D, Jansen S (2011) Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. New Phytologist 190:709–723.

Mayer H, Holst T, Brugger U, Kirchgassner A (2005) Trends of the forest significant climate variables air temperature and precipitation in south-west Germany from 1950 to 2000. Allegemeine Forst und Jagdzeitung: 45–46.

McMahon, T. A., and A. Diaz Arenas, eds. Methods of computation of low streamflow. Paris: UNESCO, 1982.

Meier IC, Leuschner C (2008) Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. Global Change Biology 14:2081–2095.

Micco VD, Aronne G (2012) Morpho-Anatomical Traits for Plant Adaptation to Drought. In: Aroca R (Eds) Plant Responses to Drought Stress. Springer Berlin Heidelberg, pp 37–61.

Polle A, Altman A, Jiang X (2006) Towards Genetic Engineering for Drought Tolerance in Trees. In: Fladung DM, Ewald DD (Eds) Tree Transgenesis. Springer Berlin Heidelberg, pp 275–297.

Popko J, Hänsch R, Mendel R-R, Polle A, Teichmann T (2010) The role of abscisic acid and auxin in the response of poplar to abiotic stress. Plant Biology 12:242–258.

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends in Plant Science 15:395–401.

Schär C, Vidale PL, Lüthi D, Frei C, Häberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. Nature 427:332–336.

Schröter D, Cramer W, Leemans R, Prentice IC, Araújo MB, Arnell NW, Bondeau A, Bugmann H, Carter TR, Gracia CA, Vega-Leinert AC de la, Erhard M, Ewert F, Glendining M, House JI, Kankaanpää S, Klein RJT, Lavorel S, Lindner M, Metzger MJ, Meyer J, Mitchell TD, Reginster I, Rounsevell M, Sabaté S, Sitch S, Smith B, Smith J, Smith P, Sykes MT, Thonicke K, Thuiller W, Tuck G, Zaehle S, Zierl B (2005a) Ecosystem Service Supply and Vulnerability to Global Change in Europe. Science 310:1333–1337.

Schröter D, Zebisch M, Grothmann T (2005b) Climate change in Germany-vulnerability and adaptation of climate-sensitive sectors. Klimastatusbericht des DWD 2005:44–56.

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221–227.

Sperry JS (2003) Evolution of Water Transport and Xylem Structure. International Journal of Plant Sciences 164:S115–S127.

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93: 1490-1500.

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal 45:523–539.

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in woody *Rosaceae* and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. Plant, Cell & Environment 28:800–812.

Wühlisch G. V., Muhs H.J. (2010) Current state of European beech forests in Germany.

Zebisch M, Grothmann T, Schröter D, Hasse C, Fritsch U, Cramer W (2005) Climate Change in Germany. Vulnerability and Adaptation Strategies of Climate-Sensitive Sectors. Potsdam Institute for Climate Impact Research.

Zollinger N, Kjelgren R, Cerny-Koenig T, Kopp K, Koenig R (2006) Drought responses of six ornamental herbaceous perennials. Scientia Horticulturae 109:267–274.

Chapter 2: Anatomical responses of mature beech trees along a gradient of precipitation

2.1. Introduction

All climate scenarios forecasted that summer precipitation will decrease strongly in the 21st century compared to the last century (Jacob et al. 2008, Schröter et al., 2005). As the consequence of climate change, a reduction of precipitation is expected to decrease the water supply (Zebisch, et al. 2005). The impacts of reduced water availability are drought stress, weakened growth and drought damage (Zebisch et al. 2005). Because of the long lifespan of forest trees, forest ecosystems are more vulnerable to predicted drier and warmer climate than other agricultural ecosystems (Gessler et al. 2007). Thus, increasing numbers of extreme weather events like drought will have negative impacts on forest ecosystems and key forest species. Moreover, it is difficult for foresters to choose the suitable tree species and to manage forest stands in the context of climate change. Therefore, it is necessary to study the ability to adapt to water limitation of key forest species.

European beech (*Fagus sylvatica* L.) is the dominant and economically most important broad-leaf deciduous tree species of the natural potential vegetation in Germany (Wühlisch and Muhs 2010). In Germany, as the centre of distribution, 14.8% of the forest area is covered by European beech (*Fagus sylvatica* L.) (Hofmann et al. 2000). The beech forest proportion is currently increasing in Germany because of a forest conversion program in which coniferous forests are being converted to pure and mixed deciduous forests (Geßler et al. 2007). Water shortage is the main limiting factor of the natural area distribution (Ellenberg 1996) and the competitive ability and natural regeneration of beech (Gessler et al. 2007). The predicted climate change is expected to lead to more negative effects on beech forests in the future. Moreover, the ability of European beech (*Fagus sylvatica* L.) to adapt to the changing environmental conditions is not yet well-known and therefore studies are needed to address this issue.

Increasing frequency of drought events does not only affect the C-gains and C-losses of ecosystems, but also might impact tree growth by affecting the biochemical, physiological and anatomical responses (Schwartz 1999, Morison and Morecroft 2008). Wood anatomical traits have been increasingly studied with regard to the relationships between wood anatomy and

environmental factors (Sperry 2003). Drought can directly affect wood increment, through effects on cambial cells and their derivatives or, indirectly, through an effect on photosynthesis and the translocation of assimilates (Arend and Fromm 2007). In most studies, drought resulted in smaller vessel lumina but increased vessel density compared to well-watered plants (Sperry 2003, Sperry et al. 2006, Arend and Fromm 2007, Carlquist 2013, Beniwal et al. 2010). These changes of vessel properties resulted in a similar total cross-sectional vessel lumen area compared to non-stressed trees. The sum of vessel lumina remains unchanged and helped stressed plants to maintain water uptake because the xylem:vessel area ratio did not change (Sperry 2003, Arend and Fromm 2007). Other common traits of wood from dry habitat plants are the presence of helical thickening in vessels and thick wall cells (Carlquist 1989, Sperry 2003). These modifications help plants to prevent spreading of cavitation and increase their mechanical strength (Lens et al. 2011). Anatomical changes may allow plants adapt to dry conditions (Micco and Aronne 2008). Anatomical properties have the advantage that the tree´s development in response to environmental changes is archived in wood and may be evaluated retrospectively (Hacke and Sperry 2001, Carlquist 2013).

Some recent studies indicate that beech may be more vulnerable to the predicted warmer future climate than co-occurring forest species such as *Quercus, Tilia, Carpinus, Fraxinus* or *Pinus* species (Kölling et al., 2007, Köcher et al. 2009). The current climate-related drought events led to extensive growth restriction and mortality in some beech forest areas (Rennenberg et al. 2006, Fang and Lechowicz 2006, Gessler et al. 2007, Granier et al. 2007, Zang et al. 2014). For example, the 2003 drought event resulted in strong reduction of net gross primary productivity of beech forests (Ciais et al. 2005) and in the growth of beech (Czajkowski 2006). Similar results were observed by other researchers (Granier et al. 2007, Nielsen and Jørgensen 2003, Jump et al., 2006, Scharnweber et al. 2011, Eilmann et al. 2014). Van der Werf et al. (2007) found that, during drought stress, wood formation of beech ceased and recovered after drought treatment. Vessel lumen area of beech trees was strongly positively correlated with the monthly amount of precipitation during the growing season (Sass and Eckstein 1995). By analyzing vessel properties of beech branches of mature beech trees along a precipitation gradient (855-594 mm yr^{-1}), Schuldt et al. (2015) found that vessel diameter decreased 7% and embolism resistance increased 10% with climatic aridity. However, changes of other cells in beech xylem such as fibre and ray parenchyma under water stress were not yet well investigated.

Old beech trees which existed for long times at different sites might have acclimation to cope with wide range of ecosystems differing in water availability. Among these traits, the plasticity of wood anatomical properties may exist and might enable beech trees to deal with different water conditions. The present study focused on analyzing anatomical properties of different cell types in the xylem such as vessels, fibres and ray parenchyma of old beech trees. Mature beech trees from three locations differing in long-term annual precipitation were chosen for the analyses. In each location, two neighboring forest stands (loamy soil and one sandy soil) were chosen in order to include the influence of location and soil water storage capacity. It was expected that beech trees originating from dry conditions exhibited changes of anatomical properties of the xylem to adapt to dry conditions. We tested the hypotheses: (1) mature beech trees from drier habitats possess anatomical changes in the xylem to cope with low precipitation climate, (2) and dry climate negatively affects the carbon and nitrogen content of beech wood.

2.2. Materials and Methods

2.2.1. Study locations

Beech (*Fagus sylvatica* L.) trees were collected in 3 areas differing in long-term (1971-2000) mean annual precipitation (Deutscher Wetterdienst-DWD): 766 mm Unterlüss (high precipitation), 665 mm Göhrde (intermediate precipitation), and 544 mm Calvörde (low precipitation) in the North German Plain (Lower Saxony and Saxony-Anhalt, Germany). In order to evaluate the impact of soil water storage capacity on the water availability of trees, two neighboring plots were selected with different soil texture (sandy vs. loamy). Thereby, beech trees on six plots were studies. The forest structures, topography, climatic and edaphic characteristics of six plots are shown in Table 2.1.

Table 2.1. Survey of topographic, climatic, stand structural and edaphic characteristics of six European beech (Fagus sylvatica L.) forest stands along a precipitation gradient in Northern Germany. Climatic data were provided by National Climate Monitoring of Germany's National Meteorological Service (Deutscher Wetterdienst-DWD). Other data provided by Hilmar Müller-Haubold (Plant Ecology and Ecosystems Research Department, Georg-August-University Göttingen).

[1] – Mean values 1971-2000. Annual values/values referring to vegetation period April – September.
[2] – Soil chemical properties refer to the top mineral soil 0 – 30 cm soil depth, cation exchange capacity, DM = Dry mass.
[3] – Soil physical properties – water storage capacity as the sum, particle size distribution as the mean value of 0 – 120 cm soil depth.
[4] – Diameter at breast height and timber volume refer to all beech trees > 7 cm stem diameter, tree height refers to all beech trees constituting the upper stand canopy.
[5] – Stem density (N ha^{-1}) and cumulative basal area (G) include all trees > 7 cm stem diameter per plot, irrespective of tree species.
[6] – Mean values during the sampling period (2009-2012). Annual values/values referring to vegetation period April – September

Parameter	Sites					
	Unterüss		Göhrde		Calvörde	
	Clay soil	Sandy soil	Clay soil	Sandy soil	Clay soil	Sandy soil
Latitude	52°50' N	52°50' N	53°07' N	53°08' N	52°24' N	52°23' N
Longitude	10°19' E	10°19' E	10°49 E	10°52' E	11°16' E	11°17' E
Elevation (m a.s.l.)	120	117	85	85	72	75
Mean temperature (°C) [1]	8.5/13.6	8.5/13.6	8.7/13.8	8.7/13.9	9.1/14.5	9.2/14.5
Mean precipitation (mm) [1]	766/374	766/374	675/349	665/347	543/294	544/294
pH value (H_2O/KCl) mineral soil [2]	4.42/4.05	4.31/4.05	4.25/3.88	4.33/4.08	4.17/3.76	4.25/3.95
C (mg g^{-1} DM) [2]	10.21	11.17	9.52	13.31	5.67	5.67
N (mg g^{-1} DM) [2]	0.40	0.46	0.42	0.52	0.36	0.43
C/N ratio (g g^{-1}) [2]	25.8	24.1	22.9	25.5	15.9	13.2
Cation exchange capacity (μmol_c g^{-1}) [2]	18.4	24.2	20.2	26.5	18.6	14.7
Base saturation (%) [2]	14.8	8.3	6.7	2.8	7.4	5.0
Soil texture particle size distribution [3] ∑ Vol.% < 63 µm (silt+clay)	21.0	14.9	17.7	4.6	53.5	9.6
Water storage capacity (mm/120 cm) [3]	95	79	78	80	140	81
Stand age (year)	115	115	142	133	131	97
Mean diameter at breast height (cm) [4]	26.1	18.6	51.0	30.7	36.6	23.4
Stem density (N ha^{-1}) [5]	411	611	122	289	300	711
Stand basal area *G* (m^2 ha^{-1}) [5]	28.5	24.3	26.6	24.4	33.3	33.2
Proportion of beech of *G* (%)	100	81	100	94	97	100
Mean temperature (°C) during samplings period [6]	8.8/14.6	8.8/14.6	8.9/14.8	9.0/14.9	9.2/15.4	9.3/15.5
Mean precipitation (mm) during samplings period [6]	786/372	786/372	707/361	692/359	611/332	615/335

2.2.2. Sampling

Woody samples for this study were harvested from April 2009 to October 2012. Sampling was conducted on April 22nd, June 5th, August 22nd and October 6th during 2009 – 2012. In total, 15 harvests took place during the field work. In each plot, five randomly chosen mature beech trees were used for harvesting. From each beech tree, samples for anatomical analysis, consisting of wood cores with 2.0 cm sample diameter and 1.5 cm depth, were harvested with a chisel and a hammer at the height of 2.0 m above ground and transferred immediately into 50 ml tubes (Falcon tube 50 ml, 115 x 20 mm, Sarstedt, Nümbrecht, Germany) containing FAE solution (37% formaldehyde,100% glacial acetic acid,70% ethyl alcohol in a ratio of 5%,5%,90% (v/v)). The FAE solution was already prepared in the laboratory before sampling. The woody samples for carbon and nitrogen measurements were frozen in dry ice at -78°C, and were transferred to the laboratory where they were stored at -80°C.

2.2.3. Wood anatomical analyses

Woody samples that had been stored in FAE solution were washed three times with double distilled water for 5 minutes to remove the FAE solution. 20 µm-thick woody slices were cut using a sledge microtome (Reichert-Jung, Heidelberg, Germany). The cutting was done with a steel blade (16 cm) with c-grinding. The suitable angle of intersection was 10°. The optimal angle had to be tested for each tissue. For storing the cross-sections, freshly boiled distilled water was always used, and cross-sections were gently moved from the sledge microtome to microscope slides and stored at room temperature in double distilled water. Well-cut sections were chosen and stained with Mäule-stain (Mäule 1901). For this purpose, sections were incubated for 3 min in 2% (w/v) potassium permanganate ($KMnO_4$) solution, and then washed three times with double distilled water. Then, cross-sections were incubated about 2 min in 5% (v/v) hydrochloric acid (HCl) for the formation of chlorlignin. Double distilled water was used to gently wash cross-sections again. The cross-sections were incubated in 10% (v/v) ammonia (NH_3) solution. They were, then, mounted on glass slides with a drop of 50% (v/v) glycerin for microscopic viewing. By placing the slides on a 50°C warm plate (SD 12, MEDAX; Nagel GmBh, Kiel, Germany) cross-sections were flattened. Well-stained sections were viewed under a light microscope (Axioskop, Zeiss, Oberkochen, Germany) at 2.5-fold and 40-fold magnifications. Photographs were taken with an integrated digital camera (Axiocam, Zeiss, Oberkochen, Germany). Microphotographs of wood were analyzed using the software ImageJ (Abramoff et al. 2004) for the following parameters: thickness of annual growth ring (wood

increment), vessel lumen (VLA) and fibre lumen area (FLA), ray parenchyma area (RA), thickness of the double fibre wall (the wall between two adjacent fibre cells, TDFW), thickness of the vessel wall (VCW) as well as the frequency of vessel (VF) and frequency of fibre (FF) per unit area of 1.0 mm^2 as indicated in Fig. 2.1A. The percentage of cell wall area (PCWA) was determined as described by (Luo et al. 2004):

PCWA (%) = [total cross-section area – (vessel lumen area + fibre lumen area + ray parenchyma area)] × 100/total cross-section area.

Measurements of vessel and fibre anatomical properties and percentages of cell wall areas were also carried out in three different regions of a year ring (early wood, transition wood and latewood). The early wood region was characterized by large vessel lumen area and was defined as from 0-20% area of the ring width, the transition wood region in the region from 55-75% and the late wood region was defined as the wood area from 80 – 100% area of the whole year ring (Figure 2.1B).

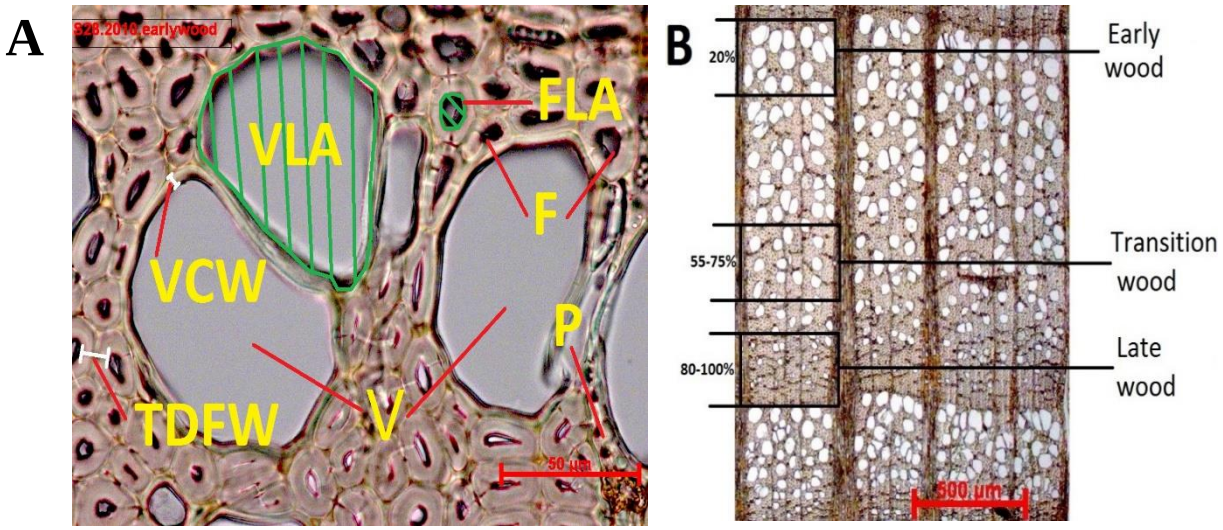


Figure 2.1. Typical microscopic pictures of beech wood at 40-fold magnification (A) and at 2.5-fold magnification (B). Vessel (V), fibre (F) and parenchyma ray (P), thickness of vessel cell wall (VCW) are shown in the figure and their anatomical properties were measured: thickness of vessel cell wall (VCW), vessel lumen area (VLA), fibre lumen area (FLA) and thickness of the double fibre wall (TDFW). Different regions in an annual ring of beech wood (Early wood, transition wood and late wood) are indicated. Magnifications are indicated by scale bars.

2.2.4. Carbon and nitrogen measurements

Annual rings from each beech tree were separated from frozen woody samples by using a scalpel under a dissecting microscope (Stemi SV11, Zeiss, Oberkochen, Germany). Four year

rings (2009-2012) derived from beech trees stocking in clay soil and sandy soil were prepared for this way. Five trees in each site were chosen as biological replicates. Thirty beech trees were used in total. The woody samples were dried for 48 hours in a drying oven at 60ºC. Dry samples were ground to fine powder using a ball mill (Type MM2, Retsch, Hann, Germany). Milled dry woody samples were weighted using a super-micro balance (S4, Sartorius, Göttingen, Germany) into tin capsules (4x6 mm, IVA Analysentechnik, Meerbusch, Germany). One sample of wood consisted of 0.7 to 0.9 mg dry mass. Carbon and nitrogen content were determined using an analyzer (EA 1108 Elemental Analyzer, Carlo Erba Instruments, Rodano, Milan, Italy). Acetanilide standard (C_6H_5NH ($COCH_3$)) was used as the standard.

To determine ^{13}C within annual rings, the annual ring of 2010 from beech trees on in sandy soil were split by using a scalpel under a dissecting microscope (Stemi SV11, Zeiss, Oberkochen, Germany). Five beech trees were chosen as biological replicates. Early wood, transition wood and late wood were separated from frozen woody samples (Figure 2.1B). All samples were dried for 48 hours in a drying oven at 60ºC. Dry samples were ground to fine powder using a ball mill (Type MM2, Retsch, Hann, Germany). Milled dry woody samples were weighed using a super-micro balance (S4, Sartorius, Göttingen, Germany) into tin capsules (4x6 mm, IVA Analysentechnik, Meerbusch, Germany). 0.2 to 0.5 mg of dry mass per sample were necessary for the analysis of ^{13}C. Samples were combusted in an elemental analyzer (EA 1108, Fisons, Rodano, Italy), CO_2 was separated by chromatography and directly injected into a continuous-flow isotope ratio mass spectrometer (IRMS Delta ^{plus} Thermo Finigan Mat, Bremen, Germany). The analyses were conducted in the KOSI laboratory (Centre for Isotope Stable Research and Analysis, University of Göttingen). Acetanilide standard (C_6H_5NH ($COCH_3$)) was run every six samples. $\delta^{13}C$ values (‰) were determined by the following formula:

$$\delta^{13}C_{sample}\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R is the ratio of $^{13}C/^{12}C$. Pee Dee Belemnite was referred to as the standard.

2.2.5. Data analysis

Statistical data analysis was carried out with the software R 3.1.2 (the R Project for Statistical Computing www.r-project.org). Normal distribution was tested with the Shapiro – Wilk's test and homogeneity of variances was tested with Levene's test. Where necessary, data

were transformed to fulfill the requirements of normality and homogeneity of variances. Multi-factor ANOVA was performed to determine the variation of the main variables precipitation, soil type, and the interactions between them. Values of $P \leq 0.05$ were considered to indicate significant effects. When the ANOVA revealed significant differences among the means with the $P < 0.05$, a post-hoc test (Tukey HSD) was performed. To test for relationships between wood increment or anatomical properties of beech trees with environmental factors (precipitation and temperature), regression analysis was carried out. The best models for linear or exponential correlation between wood increment and precipitation and temperature were chosen according to the coefficient of correlation. For investigating relationships between wood anatomical properties and precipitation were investigated by Pearson correlation analyses in Statgraphics (Centurion XVI, St. Louis, Mo, USA)). Graphs were generated using Origin Pro Lab 8.5 (OriginLab Corporation Northampton, USA).

2.3. Results

2.3.1. Growth along a precipitation gradient

The mean annual increment of beech trees declined significantly with decreasing precipitation (Figure 2.2A). Beech trees in Unterlüss showed the widest ring width and followed by beech trees in Göhrde, and the smallest was observed in Calvörde (Figure 2.2B). Analysis of annual increment of beech wood showed no significant differences between beech trees stocking in sandy or loamy soil ($F = 1.556$, $P = 0.228$), whereas significant changes were found among the locations ($F = 16.004$, $P < 0.001$). General linear models analysis indicated that average monthly temperature did not affect annual increment of beech wood ($F = 1.19$, $P = 0.288$). The average yearly wood increment of beech trees in this studied was strongly exponentially correlated to the annual precipitation amount of the studied sites ($R = 0.915$, $P < 0.001$, Fig.2.3).

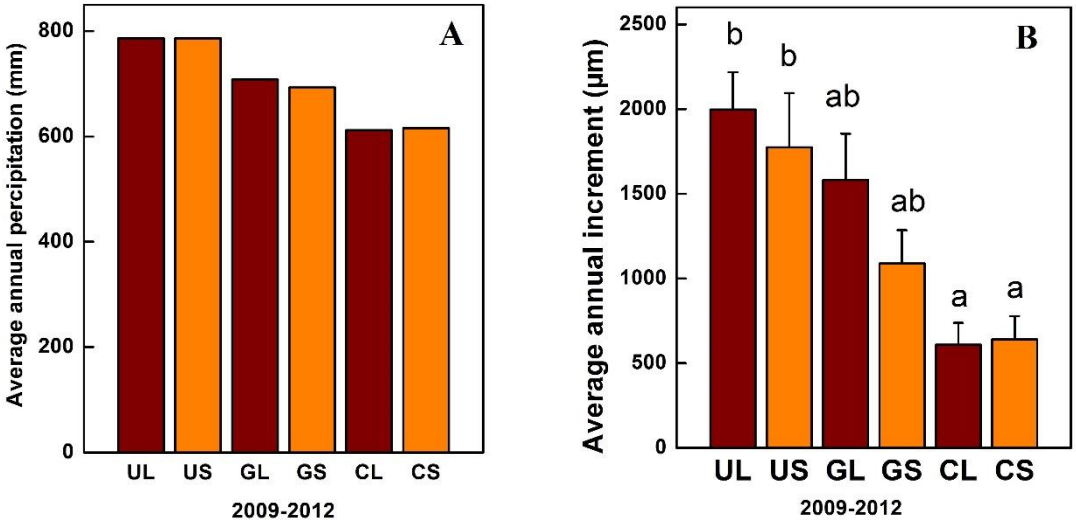


Figure 2.2. Average annual precipitation were observed from 2009-2012 in six locations (A) and average annual increment of beech (Fagus sylvatica L.) trees in in six sites (B) (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)). Increment data indicate means ± SE, n = 5. Different letters indicate significant differences at $P \leq 0.05$.

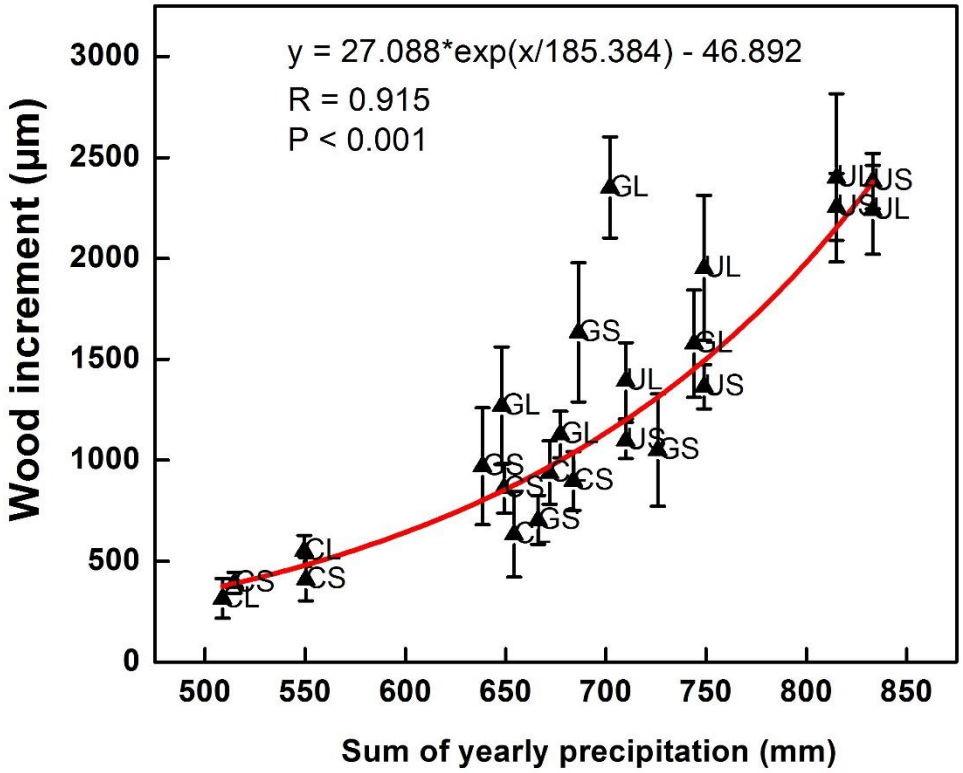


Figure 2.3. Relationship between the mean annual wood increment of adult beech trees (Fagus sylvatica L.) and the sum of yearly precipitation in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)) during the period from 2009-2012. Symbols indicate means ± SE, n = 5.

2.3.2. Anatomical characteristics of beech trees along a precipitation gradient

In order to compare the differences between anatomical properties of the year ring 2010 and 2011, T-test statistical analysis was carried out. There was no significant difference between the year ring 2010 and 2011 (Table 2.2). Therefore, we combined anatomical data of 2010 and 2011 for further analyses.

Table 2.2: Comparison of anatomical traits from the year 2010 and 2011. Traits were compared by T-test. P values were shown in the table.

Site	*P*-values						
	VF	VLA	VCW	FF	FLA	TDFW	PCWA
UL	0.1191	0.1130	0.7446	0.4074	0.7184	0.5871	0.4557
US	0.7647	0.1032	0.3991	0.1532	0.8018	0.7240	0.4946
GL	0.4161	0.2737	0.3434	0.8878	0.2940	0.7352	0.9858
GS	0.0923	0.7043	0.9784	0.7014	0.4920	0.9325	0.7318
CL	0.3591	0.0784	0.9540	0.9120	0.1700	0.9853	0.3595
CS	0.2172	0.0731	0.2742	0.5481	0.3923	0.9980	0.1852

The effect of precipitation and soil types (sandy and loamy soil texture) on the anatomical features of wood in beech trees were analyzed in cross-sectional samples in whole year rings (2010 and 2011). A significant difference was found among the different sites for the vessel frequencies in wood formed in 2010 and 2011 ($F = 23.162$, $P < 0.001$, Fig. 2.4A). The forest with the highest precipitation (UL/US) had the lowest vessel frequency and followed by the intermediate precipitation (GL/GS) and the driest one (CL/CS) had the highest vessel frequency (Fig. 2.4A). In all three locations, soil types did not lead to significant changes in vessel frequency ($F = 0.058$, $P = 0.811$, Fig. 2.4A). At sites with low precipitation, vessel lumen areas were significantly smaller than those in wetter site ($F = 25.746$, $P < 0.001$, Fig. 2.4B). Soil type did not change vessel lumen area of beech trees ($F = 0.082$, $P = 0.777$). Cross-sectional lumen area of vessel element increased significantly from the driest site to the wettest site: Calvörde ≤ Göhrde ≤ Unterlüss (Figure 2.4B). No significant differences in the thickness of vessel cell walls were observed among the forest sites ($F = 0.836$, $P = 0.447$), neither between the soil types ($F = 0.248$, $P = 0.624$) (Figure 2.4C).

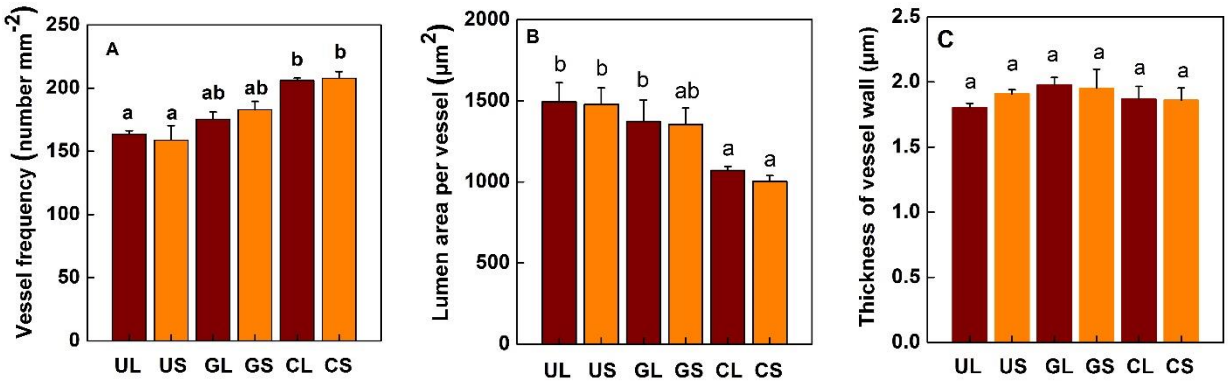


Figure 2.4: Vessel properties of beech trees (*Fagus sylvatica* L.) in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL) and Calvörde sand (CS)): vessel frequency (A), vessel lumen area (B) and the thickness of vessel cell wall (C). Data indicate means ± SE, n = 5. Different letters indicate significant differences at $P \leq 0.05$.

In order to find out whether precipitation and soil types affected fibre properties, fibre frequency, fibre lumen area and fibre cell wall thickness were also determined. However, neither precipitation nor soil type resulted in any remarkable changes of fibre properties ($P > 0.05$, Figure 2.5A, B and C).

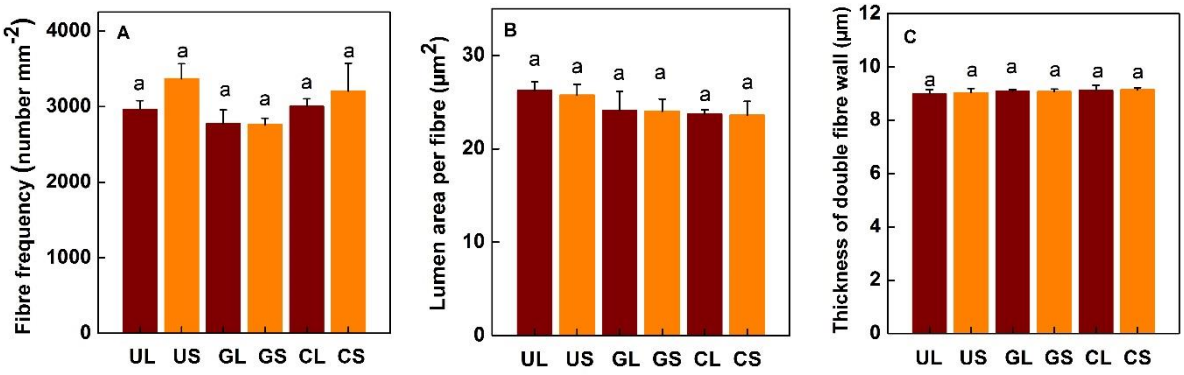


Figure 2.5: Fibre properties of beech trees (*Fagus sylvatica* L.) in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)): fibre frequency (A), fibre lumen area (B) and the thickness of double fibre cell wall (C). Data indicate means ± SE, n = 5. Different letters indicate significant differences at $P \leq 0.05$.

The fractions of cell wall area per cross-sectional area of beech trees from different sites were not significantly different among forest sites ($F = 0.081$, $P = 0.9283$) and between soil types ($F = 0.070$, $P = 0.794$) (Figure 2.6).

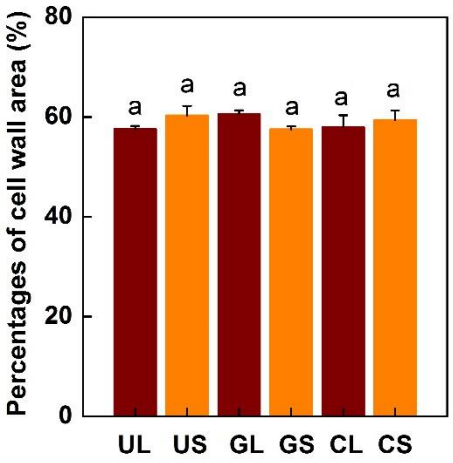


Figure 2.6: Percentage of cell wall areas of beech trees (*Fagus sylvatica* L.) in 6 sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)). Data indicate means ± SE, n = 5. Different letters indicate significant differences at $P \leq 0.05$.

The relationships between anatomical properties of beech trees and annual precipitation and average monthly temperature were analyzed. Vessel frequency (VF) and the thickness of double fibre cell wall (TDFW) were strongly negative correlated with annual precipitation, whereas vessel lumen area (VLA) and fibre lumen area (FLA) were significantly negative correlated with annual precipitation (Table 2.3 and Fig.2.7 A, B, C, D). No relationships were found between the thickness of vessel wall (VCW) or fibre frequency (FF) or fractions of cell wall area (PCWA) and yearly precipitation (Table 2.3). Vessel lumen area and fibre lumen were negatively correlated with average monthly temperature, but other anatomical properties showed no significant relationship with this parameter (Table 2.3 and Fig.2.8 A, B).

Table 2.3: Summary of the linear regression models relating the annual precipitation, temperature to anatomical properties of beech trees adult beech trees (*Fagus sylvatica* L.) living in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)). Asterisks in columns indicate significant differences with $P \leq 0.05$.

Variables	Annual precipitation			Average monthly temperature		
	R	**P**		**R**	**P**	
VF	-0.991	< **0.001**	***	0.445	0.147	
VLA	0.992	< **0.001**	***	-0.680	**0.042**	*
VCW	-0.564	0.060		0.544	0.067	
FF	-0.247	0.439		0.349	0.266	
FLA	0.958	< **0.001**	***	-0.820	**0.009**	**
TDFW	-0.810	**0.039**	*	0.243	0.446	
PCWA	-0.242	0.449		0.319	0.313	

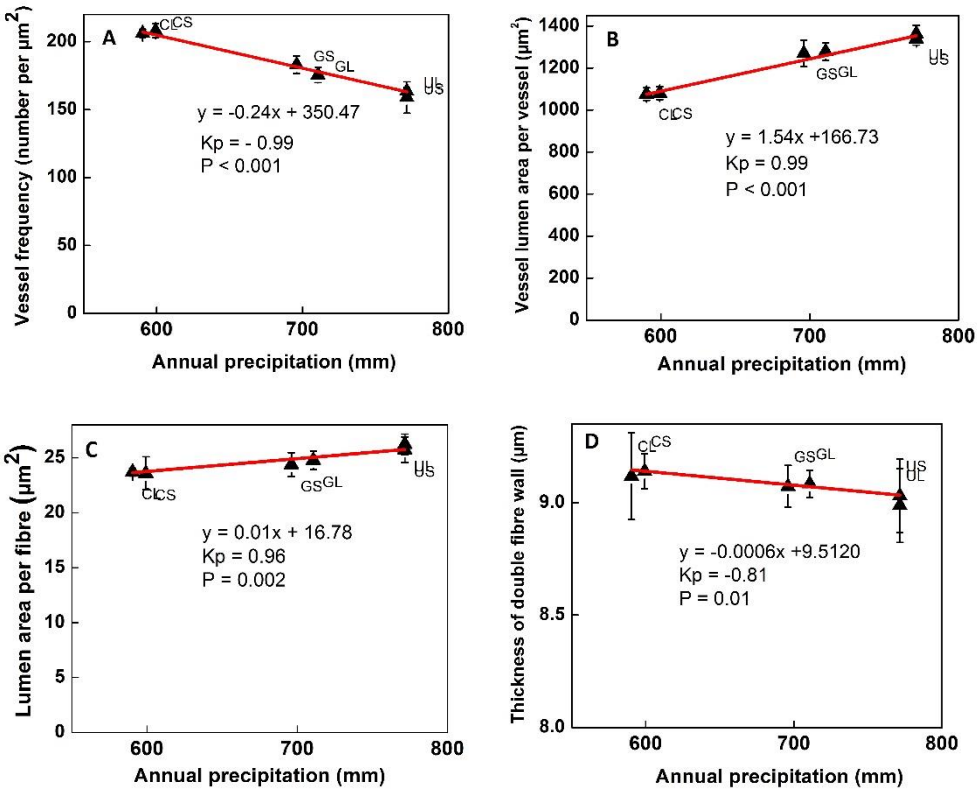


Figure 2.7: Relationships in adult beech trees (*Fagus sylvatica* L.) between annual precipitation and (A) vessel frequency, (B) vessel lumen area, (C) fibre lumen area, (D) the thickness of double fibre cell wall. The Pearson correlation coefficients (K_P) and P-value for H_0: $K_P = 0$ are given.

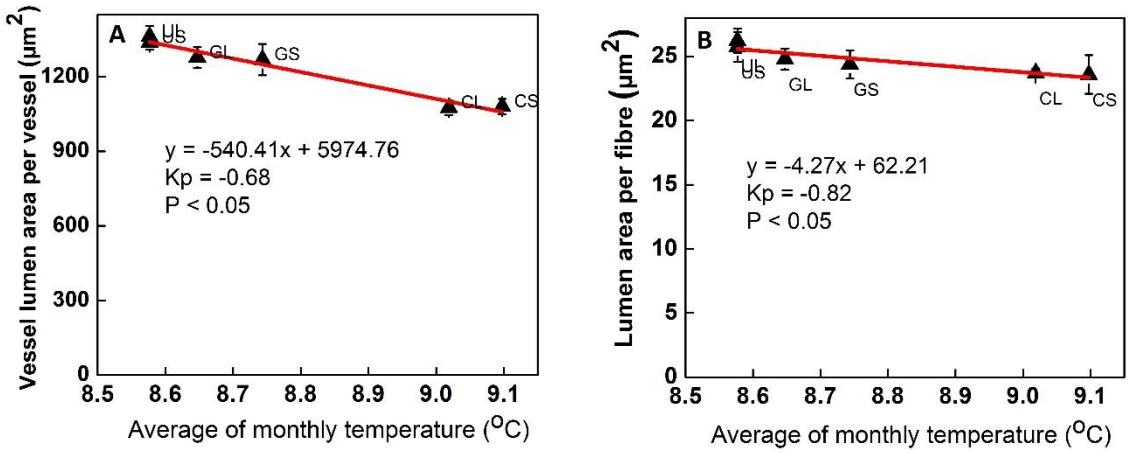


Figure 2.8: Relationships in adult beech trees (*Fagus sylvatica* L.) between average monthly temperature and (A) vessel lumen area, (B) fibre lumen area. The Pearson correlation coefficients (K_P) and *P*-value for H_0: $K_P = 0$ are given. Data indicate means ± SE, n = 5.

2.3.3. Variation of anatomical features within the year rings

In order to compare anatomical features of different regions within the annual rings (2010 and 2011), anatomical features of early wood, transition wood and late wood regions were analyzed.

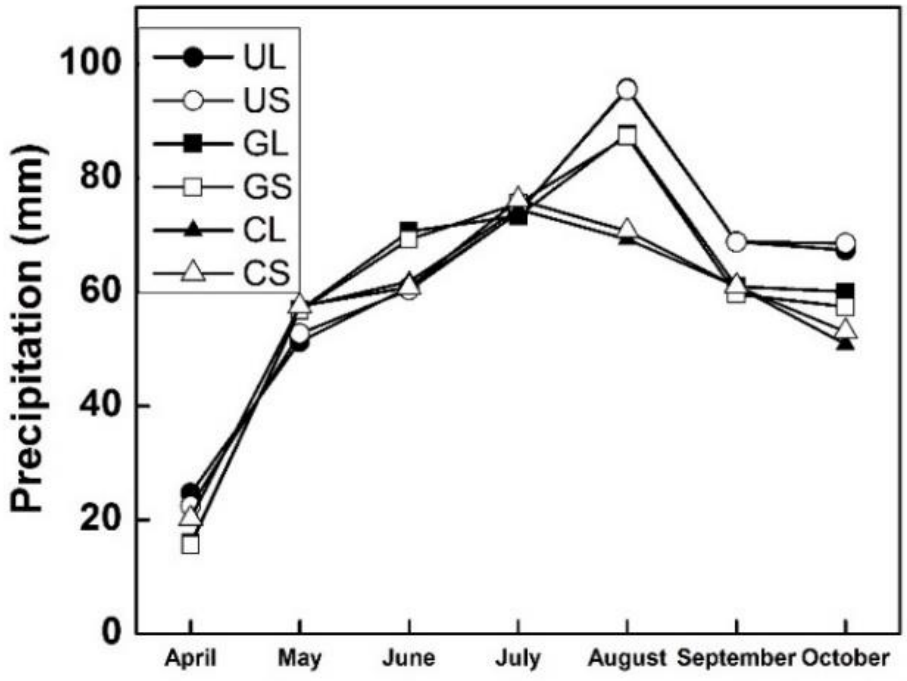


Figure 2.9: Precipitation patterns during the growing season (from April – October) of 2010 and 2011 in 6 sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL), Calvörde sand (CS)).

In early wood, none of the analyzed anatomical features of vessels and fibres showed any significant difference among the six sites ($P > 0.05$, Figure 2.10, Figure 2.11). A slight change of vessel lumen area and vessel frequency were observed but were not significant among beech trees stocking in the six sites.

In transition wood, the average vessel lumen area (VLA) increased significantly ($F = 23.336$, $P < 0.001$) and the vessel frequency (VF) increased strongly ($F = 15.886$, $P < 0.001$) from dry sites to moist sites (Figure 2.10A, B). In these woody regions, no changes were observed for the thickness of vessel cell wall (VCW) and fibre properties under different sites and soil types ($P > 0.05$, Figure 2.10C, Figure 2.11).

In late wood formed in 2010 and 2011, vessel frequency increased significantly from the moist to the dry site ($F = 7.685$, $P < 0.001$, Figure 2.10A) whereas vessel lumen areas

showed strong decreases (F = 19.151, P < 0.001, Figure 2.10B). The thickness of vessel cell walls exhibited no significant differences in the beech wood of the six sites (F = 0.484, P = 0.784, Figure 2.10C). However, beech trees which stocked in low precipitation climate (Calvörde), possessed significantly narrower fibre lumen (Figure 2.11B) and thicker thickness of double fibre cell wall compared to the beech trees growing in forests with higher precipitation (Figure 2.11C). Fibre frequency stayed constant in the beech wood of beech trees stocking in six sites (Figure 2.11A).

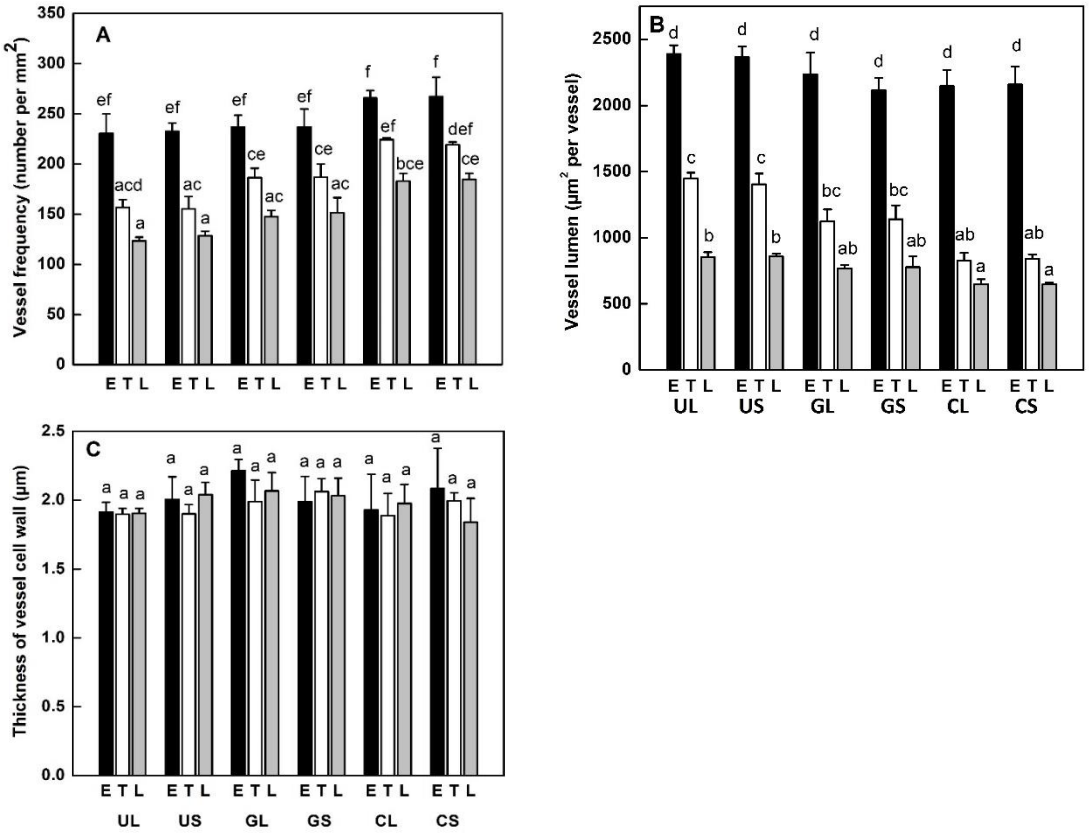


Figure 2.10. Variation of vessel properties of beech (*Fagus sylvatica* L.) trees in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL) and Calvörde sand (CS)) within the annual rings 2010 and 2011: vessel frequency (A), vessel lumen area (B) and the thickness of vessel cell wall (C). Early wood (E, black bars), transition wood (T, white bars) and late wood (L, grey bars). Data indicate means ± SE, n = 5. Different letters indicate significant differences at $P \leq 0.05$.

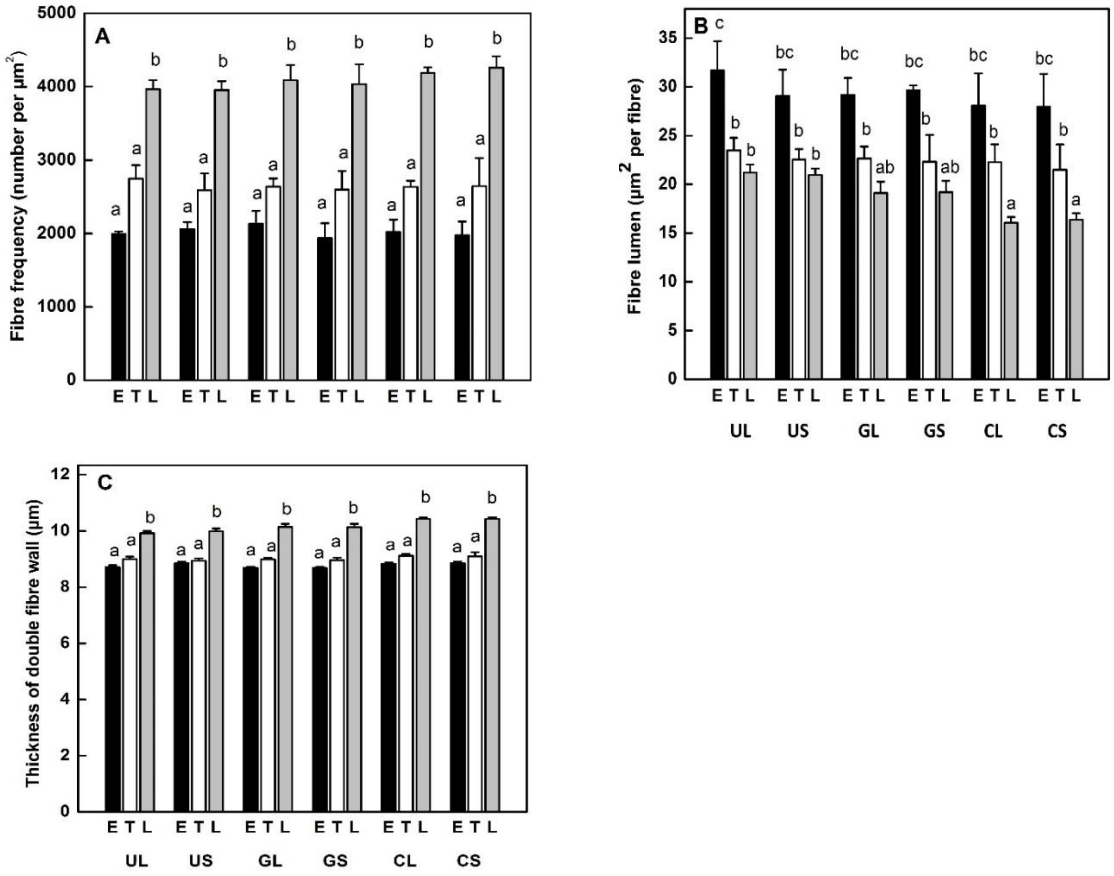


Figure 2.11. Variation of fibre properties of beech (*Fagus sylvatica* L.) trees in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL) and Calvörde sand (CS)) within the annual rings 2010 and 2011: fibre frequency (A), fibre lumen area (B) and the thickness of double fibre cell wall (C). Early wood (E, black bars), transition wood (T, white bars) and late wood (L, grey bars). Data indicate means ± SE, n = 5. Different letters show significant differences at $P \leq 0.05$.

The percentages of cell wall area did not reveal significant differences among six sites in wood regions formed at different times during the growing season (early wood, transition wood and late wood) ($P > 0.05$, Figure 2.12).

Seasonal changes also affected some anatomical features but not all. Vessel frequency, vessel lumen area, fibre lumen area decreased towards the end of the growing season (Figure 2.10A, B; Figure 2.11B, respectively), whereas fibre frequency and thickness of double fibre walls and percentage of cell wall area (Figure 2.11A, C; Figure 2.12, respectively) increased from early wood to late wood in all beech trees from the six sites. The thickness of vessel cell wall remained constant during the growing season (Figure 2.10C).

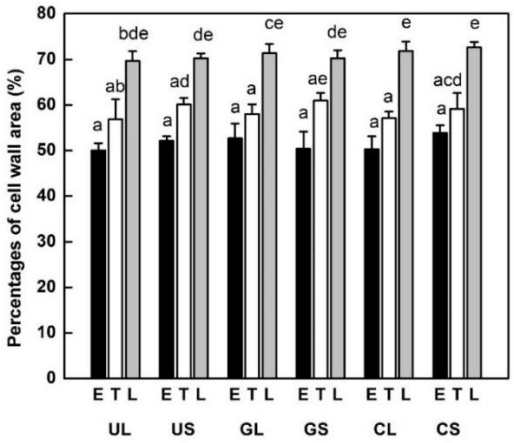


Figure 2.12. Fractions cell wall area per cross-section area of beech (*Fagus sylvatica* L.) trees in six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam (CL) and Calvörde sand (CS)) within the annual rings 2010 and 2011. Early wood (E, black bars), transition wood (T, white bars) and late wood (L, grey bars). Data indicate means ± SE, n = 5. Different letters show significant differences at $P \leq 0.05$

2.3.4. Nitrogen and carbon in wood

Nitrogen concentration in the annual rings (2009-2012) showed no significant changes among beech tree along the precipitation gradient ($P = 0.192$, $F = 1.513$) or stocking in different soil types ($F = 1.164$, $P = 0.283$) (Figure 2.13A). The carbon concentration revealed no effect of precipitation ($F = 2.226$, $P = 0.113$) or soil types ($F = 0.313$, $P = 0.577$, Figure 2.13B). The carbon-to-nitrogen ratios (C: N), were neither affected by precipitation ($F = 1.189$, $P = 0.308$) nor soil type ($F = 1.151$, $P = 0.226$) (Figure 2.13C).

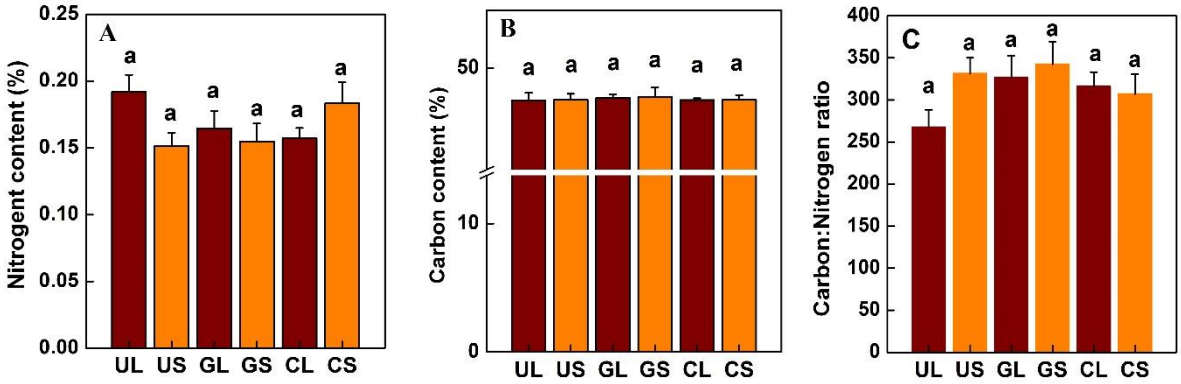


Figure 2.13: Nitrogen concentration (A), carbon concentration (B) and Carbon – to – Nitrogen ratio (C) of woody samples of beech (*Fagus sylvatica* L.) trees living in the six sites (Unterlüss loam (UL), Unterlüss sand (US), Göhrde loam (GL), Göhrde sand (GS), Calvörde loam(CL), Calvörde sand (CS)). Data indicate means ± SE, n = 20. Different letters indicate significant differences at $P \leq 0.05$.

To find out whether precipitation affected wood formation, the $\delta^{13}C$ signature was determined in early, transition and late wood regions of the 2010 annual ring of beech trees stocking in sandy soil locations.

In the early wood and transition wood, the $\delta^{13}C$ ‰ signatures of beech wood samples from three locations were not significantly different among beech trees ($P > 0.05$, Figure 2.14A, B). But in the late wood, a significant increase was found in Calvörde (sandy soil) compared to Unterlüss (sandy soil) ($F = 3.287$, $P = 0.043$, Fig. 2.14C).

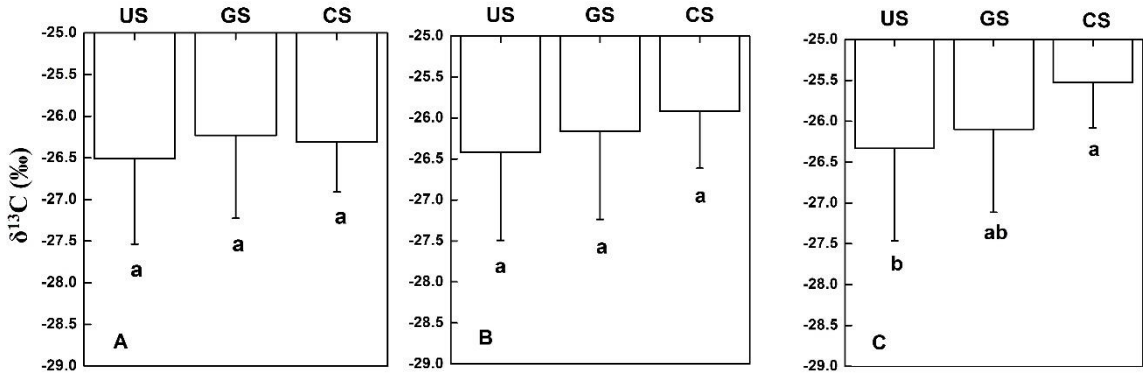


Figure 2.14: Carbon isotope composition in the early wood region (A), in transition region (B) and late wood region (C) of beech (*Fagus sylvatica* L.) trees stocking in the three sandy locations (CS = Calvörde sand, GS = Göhrde sand, US = Unterlüss Sand). Data indicate means ± SE, n = 5. Different letters show significant differences at $P \leq 0.05$. Year ring 2010 was investigated.

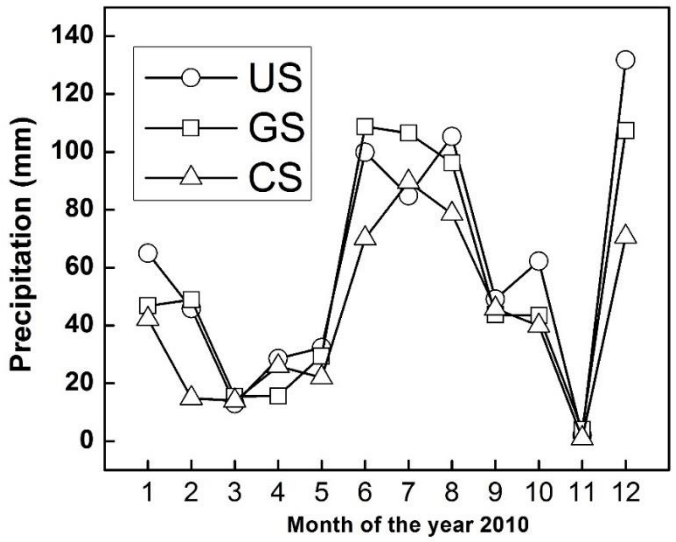


Figure 2.15: Precipitation patterns during the growing season of 2010 in 3 sites Unterlüss sand (US), Göhrde sand (GS), Calvörde sand (CS)).

2.4. Discussion

One of the major questions in this study was whether precipitation or soil type affected secondary xylem development and xylem properties. Mature beech trees from six sites along a precipitation gradient showed significant differences in annual increment, and these changes were strongly related to sites but not soil types. Moreover, a strong negative correlation between annual precipitation and wood increment was found. It is clear that the reduction of wood increment (ring-width) will lead to a decrease gross primary productivity of beech trees at low precipitation locations. Some other studies found remarkable reduction in growth of beech trees because of low soil water supply (Bolte et al. 2007, Van Hees 1997, Fotelli et al. 2001, van der Werf et al. 2007, Piovesan et al. 2008), drought and heat waves (Ciais et al. 2005). Therefore, a decline of precipitation in Germany may reduce the productivity of European beech (*Fagus sylvatica* L.) in the future and make beech less competitive than other broadleaves species like oaks.

Anatomical analysis of beech trees revealed that vessel lumen area and vessel frequency were significantly affected by the conditions of the sites. Under limited water condition, beech trees in the driest site (Calvörde) had the smallest vessels, but displayed a significant increase of vessel frequency. These changes of vessel properties of beech trees might, probably, be an adaptation to cope with low precipitation because narrow vessels prevent cavitation (Tyree et al. 1994, Hargrave et al. 1994, Sperry 2011). However, beech trees probably needed to balance their efficiency in water transport through secondary xylem because they have to maintain their water tissue status. Vessel density is another important anatomical parameter for water conduit in the xylem. Increased vessel frequency in beech trees in drier locations indicated a compensation for small vessel lumina since the total cross-sectional vessel lumen area was unchanged (Bacelar et al. 2007). The present study also found the similar results. Some other studies also showed the same results in other species (Lovisolo and Schubert 1998, Sperry et al. 2006, Arend and Fromm 2007, Bacelar et al. 2007). Schuldt et al. (2015) found that in branches of beech vessel diameter increased and beech vessel density decreased with increasing precipitation.

In transition wood and late wood, the increased vessel frequencies and decreased vessel lumina were found compared to changes of the whole ring-width of beech trees. This suggests that in the middle and the late period of the growing season, beech trees, on the dry site, could

change their vessel features to acclimate to low precipitation (Fig. 2.9). Interestingly, patterns of precipitation during growing season of all six sites indicated that the precipitation as higher in early summer and was similar among the sites. However, from July to October monthly precipitation data in driest sites (CL/CS) were clearly lower than those in wettest sites (UL/US) (Fig. 2.9). Therefore, observed changes of anatomical properties suggest that beech trees may have an adaptive drought strategy to maintain a stable water status in habitats often exposed to low precipitation.

Regarding fibre characteristics of early and transition wood, site and soil types did not affect these values. However, thicker fibre wall and narrower fibre lumina were found in beech trees living in the dry site (Calvörde). The same features of wood were described for xeric environments (Sperry 2003, Micco and Aronne 2012). Cell wall thickening increases the mechanical strength of wood and is correlated with resistance to cavitation (Carlquist 1989, Kohonen and Helland 2009, Lens et al. 2011). Therefore, thicker fibre walls and narrower fibre lumina found in the late wood region of beech trees suggest that not only vessels but also fibres could be changed because of low precipitation.

The percentage of cell wall area per cross-sectional area is another important parameter representing the mechanical strength of wood since it is strongly correlated with wood density. Notably, despite some anatomical changes, the percentages of cell wall remained constant in the wood of all beech trees. Schuldt et al. (2015) also found that beech trees along a precipitation did not change their wood densities although these trees showed decreased vessels lumnia and increased vessel frequencies. Therefore, the mechanical strength of beech wood might be not affected by conditions of six beech forests.

$\delta^{13}C$ from dry sites is inversely correlated to the precipitation amount of summer months (Saurer et al. 1995) and soil conditions (Saurer and Siegenthaler 1989). Leavitt and Long (1988) found that $\delta^{13}C$ showed a significant correlation with the drought index (Palmer Hydrological Drought Indices) of trees chronologies. Gessler et al. (2001) found that $\delta^{13}C$ signatures of sap reflected short-term fluctuations in water availability. Here, carbon isotope analysis of beech trees on the dry site indicated that carbon discrimination in the late period of the growing season was stronger than early in the season. Water shortage can induce stomatal closure and thus increase $\delta^{13}C$ of the incorporated carbon. This finding suggests that beech trees probably closed their stomata to prevent water loss and maintain their water tissue status. This change of $\delta^{13}C$

signatures and anatomical changes of late wood also confirmed that beech trees on the dry site may have a drought avoidance strategy to cope with low water availability in nature.

Here, beech trees living in different habitats which differ in precipitation showed significant changes in annual increment values. However, carbon and nitrogen content in their wood did not vary among different locations. Since beech trees in dry habitats probably had an avoidance mechanism, and this mechanism contributes remaining their water tissue status and probably remained the rate of assimilation in plants. It is explained that carbon and nitrogen content did not differ among locations. However, the total C content of beech trees on dry site was significantly lower than those in wetter sites because a reduction wood increment of beech trees was clearly shown in dry sites. Decreased wood growth could be the cost that beech trees have to deal with low precipitation.

In conclusion, the comparison of beech trees at wet and dry sites suggests that water availability caused anatomical changes. Beech trees seem to have a drought avoidance strategy to deal with dry conditions. However, other factors as genetic factors may also contribute to better adaptedness of beech to low precipitation.

2.5. References

Abramoff M.D., Magelhaes P.J., Ram S.J. (2004) Image processing with ImageJ. Biophotonics International 11:36–42.

Arend M., Fromm J. (2007) Seasonal change in the drought response of wood cell development in poplar. Tree Physiology 27:985–992.

Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environmental and Experimental Botany 60:183–192.

Beniwal RS, Langenfeld-Heyser R, Polle A (2010) Ectomycorrhiza and hydrogel protect hybrid poplar from water deficit and unravel plastic responses of xylem anatomy. Environmental and Experimental Botany 69:189–197.

Bolte A, Czajkowski T, Kompa T (2007) The north-eastern distribution range of European beech-a review. Forestry 80:413–429.

Carlquist S (1989) Adaptive wood anatomy of chaparral shrubs. The California chaparral: paradigms re-examined Los Angeles Country Museum of Natural History Contributions, Los Angeles: 25–35.

Carlquist S (2013) Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer Science & Business Media.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533.

Czajkowski T, Bolte A (2006) Unterschiedliche reaktion deutscher und polnischer Herkünfte der Buche (Fagus sylvatica L.) auf Trockenheit. Allg Forst Jagdztg 177:30–40.

Eilmann B, Sterck F, Wegner L, Vries SMG de, Arx G von, Mohren GMJ, Ouden J den, Sass-Klaassen U (2014) Wood structural differences between northern and southern beech provenances growing at a moderate site. Tree Physiol 34:882–893.

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5th edn. Ulmer, Stuttgart, Germany.

Fang J, Lechowicz MJ (2006) Climatic limits for the present distribution of beech (*Fagus* L.) species in the world. Journal of Biogeography 33:1804–1819.

Fotelli MN, Geßler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and δ13C composition. New Phytologist 151:427–435.

Gessler A, Keitel C, Kreuzwieser J, Matyssek R, Seiler W, Rennenberg H (2007) Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21:1–11.

Geßler A, Schrempp S, Matzarakis A, Mayer H, Rennenberg H, Adams MA (2001) Radiation modifies the effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). New Phytologist 150:653–664.

Granier A, Reichstein M, Bréda N, Janssens IA, Falge E, Ciais P, Grünwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Köstner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala T, Wang Q (2007) Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. Agricultural and Forest Meteorology 143:123–145.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics 4:97–115.

Hargrave KR, Kolb KJ, Ewers FW, Davis SD (1994) Conduit Diameter and Drought-Induced Embolism in *Salvia mellifera* Greene (*Labiatae*). New Phytologist 126:695–705.

Hofmann G, 1960-, Anders S, Matthes B (2000) Potentiell-natürliche und derzeitige Waldbild in den ostdeutschen Wäldern.

Jacob D, Göttel H, Kotlarski S, Lorenz P, Sieck K (2008) Klimaauswirkungen und Anpassung in Deutschland–Phase 1: Erstellung regionaler Klimaszenarien für Deutschland. Abschlussbericht zum UFOPLAN-Vorhaben 204 41 138. Umweltbundesamt (ed), Climate Change 11.

Jump AS, Hunt JM, PeñUelas J (2006) Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. Global Change Biology 12:2163–2174.

Köcher P, Gebauer T, Horna V, Leuschner C (2009) Leaf water status and stem xylem flux in relation to soil drought in five temperate broad-leaved tree species with contrasting water use strategies. Annals of Forest Science 66:101–101.

Kölling, C., Walentowski, H., & Borchert, H. (2005) Die Buche in Mitteleuropa. AFZ-Der Wald, 13: 696-701.

Kohonen MM, Helland Å (2009) On the Function of Wall Sculpturing in Xylem Conduits. Journal of Bionic Engineering 6:324–329.

Leavitt SW, Long A (1988) Stable carbon isotope chronologies from trees in the southwestern United States. Global Biogeochemical Cycles 2:189–198.

Lens F, Sperry JS, Christman MA, Choat B, Rabaey D, Jansen S (2011) Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. New Phytologist 190:709–723.

Lovisolo C, Schubert A (1998) Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. Journal of Experimental Botany 49:693–700.

Luo Z-B, Langenfeld-Heyser R, Calfapietra C, Polle A (2004) Influence of free air CO2 enrichment (EUROFACE) and nitrogen fertilisation on the anatomy of juvenile wood of three poplar species after coppicing. Trees 19:109–118.

Mäule C (1901) Das Verhalten verholzter Membranen gegen Kaliumpermanganat, eine Holzreaktion neuer Art. A. Zimmer's Verlag (Ernst Mohrmann).

Micco VD, Aronne G (2008) Twig morphology and anatomy of Mediterranean trees and shrubs related to drought tolerance. Bot Helv 118:139–148.

Micco VD, Aronne G (2012) Morpho-Anatomical Traits for Plant Adaptation to Drought. In: Aroca R (Eds) Plant Responses to Drought Stress. Springer Berlin Heidelberg, pp 37–61.

Morison JIL, Morecroft MD (2008) Plant Growth and Climate Change. Boston, MA, USA: Blackwell Publishing.

Nielsen CN, Jørgensen FV (2003) Phenology and diameter increment in seedlings of European beech (*Fagus sylvatica* L.) as affected by different soil water contents: variation between and within provenances. Forest Ecology and Management 174:233–249.

Piovesan G, Biondi F, Filippo AD, Alessandrini A, Maugeri M (2008) Drought-driven growth reduction in old beech (*Fagus sylvatica* L.) forests of the central Apennines, Italy. Global Change Biology 14:1265–1281.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological Responses of Forest Trees to Heat and Drought. Plant Biology 8:556–571.

Sass U, Eckstein D (1995) The variability of vessel size in beech (*Fagus sylvatica* L.) and its ecophysiological interpretation. Trees 9:247–252.

Saurer M, Siegenthaler U (1989) 13C/12C isotope ratios in trees are sensitive to relative humidity. Dendrochronologia 7:9–13.

Saurer M, Siegenthaler U, Schweingruber F (1995) The climate-carbon isotope relationship in tree rings and the significance of site conditions. Tellus B 47:320–330.

Scharnweber T, Manthey M, Criegee C, Bauwe A, Schröder C, Wilmking M (2011) Drought matters – Declining precipitation influences growth of *Fagus sylvatica* L. and *Quercus robur* L. in north-eastern Germany. Forest Ecology and Management 262: 947–961.

Schröter D, Zebisch M, Grothmann T (2005) Climate change in Germany-vulnerability and adaptation of climate-sensitive sectors. Klimastatusbericht des DWD 2005: 44–56.

Schuldt B, Knutzen F, Delzon S, Jansen S, Müller-Haubold H, Burlett R, Clough Y, Leuschner C (2015) How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? New Phytol: n/a-n/a.

Schwartz MD (1999) Advancing to full bloom: planning phenological research for the 21st century. Int J Biometeorol 42: 113–118.

Sperry JS (2003) Evolution of Water Transport and Xylem Structure. International Journal of Plant Sciences 164: S115–S127.

Sperry JS (2011) Hydraulics of Vascular Water Transport. In: Wojtaszek P (Eds) Mechanical Integration of Plant Cells and Plants. Springer Berlin Heidelberg, pp 303–327.

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93: 1490 - 1500.

Tyree MT, Davis SD and Cochard H (1994) Biophysical Perspectives of Xylem Evolution: is there a Tradeoff of Hydraulic Efficiency for Vulnerability to Dysfunction? IAWA Journal 15:335–360.

Van Hees AFM (1997) Growth and morphology of pedunculate oak (*Quercus robur* L) and beech (*Fagus sylvatica* L) seedlings in relation to shading and drought. In: Annales des Sciences Forestières. EDP Sciences, pp 9–18.

Van der Werf GW, Sass-Klaassen UGW, Mohren GMJ (2007) The impact of the 2003 summer drought on the intra-annual growth pattern of beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) on a dry site in the Netherlands. Dendrochronologia 25:103–112.

Wühlisch GV, Muhs HJ (2010) Current state of European beech (*Fagus sylvatica* L.) Forests in Germany. In Evaluation of beech genetic resources for sustainable forestry (2006-2010). Johann Heinrich von Thünen-Institut Federal Research Institute for Rural Areas, Forestry and Fisheries, Germany.

Zang C, Hartl-Meier C, Dittmar C, Rothe A, Menzel A (2014) Patterns of drought tolerance in major European temperate forest trees: climatic drivers and levels of variability. Glob Change Biol 20:3767–3779.

Zebisch M, Grothmann T, Schröter D, Hasse C, Fritsch U, Cramer W (2005) Climate Change in Germany. Vulnerability and Adaptation Strategies of Climate-Sensitive Sectors. Potsdam Institute for Climate Impact Research.

2.6. Declaration

The following work in this chapter has been conducted by Ngoc Quynh Nguyen:

- Wood sample harvest
- Anatomy analysis
- Carbon and Nitrogen measurement
- Carbon isotopic measurements (sample preparation and data analysis)
- Statistical analysis

Chapter 3: Intraspecific variations in expression of stress-related genes in beech progenies are stronger than drought-induced responses

Caroline Carsjens[1], Ngoc Quynh Nguyen[1], Jonas Guzy[1], Florian Knutzen[2], Ina Christin Meier[2], Markus Müller[3], Reiner Finkeldey[3], Christoph Leuschner[2], Andrea Polle[1,4]

Published in Tree Physiology (2014) 34: 1348-1361.

[1]Forstbotanik und Baumphysiologie, Büsgen-Institute, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[2]Plant Ecology, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany

[3]Forstgenetik und Forstpflanzenzüchtung, Büsgen-Institute, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[4]Corresponding author

3.1. Introduction

European beech (*Fagus sylvatica*, L.) is a dominant forest tree species of high economic and ecological value (Ellenberg and Strutt 2009). Within its distribution range across Central Europe, the species grow preferentially on well-drained, moist soils; but at the margins of its occurrence beech stands also exist under moderately dry conditions (Paule et al. 1994, Bolte et al. 2007). It has been forecast that climate change will lead to extended periods of drought stress in this century, especially during summer (Lindner et al. 2010). Extended periods of drought stress have profound, negative effects on tree vitality and forest productivity (Ciais *et al.*, 2005, Williams et al. 2013, Hanewinkel et al. 2013). Current beech forests are, therefore, most likely endangered by the consequences of global change (Rennenberg et al. 2006; Geßler et al. 2007). It is, thus, important to understand the drought adaptation potential of this species.

The physiological drought responsiveness of beech has been studied in provenances from sites differing in annual precipitation. Progenies from different locations exhibit significant variations in growth, photosynthetic activity, leaf traits and leaf area, nutrient element concentrations, and accumulation of osmoprotectants under stress indicating

physiological plasticity (García-Plazaola and Becerril 2000, Peuke et al. 2002, Czaikowski and Bolte 2006, Rose et al. 2009, Peuke and Rennenberg 2011, Robson et al. 2012) and the potential availability of local, drought-adapted beech provenances (Bolte et al. 2007, Pluess and Weber 2012, Weber et al. 2013). The molecular and cellular mechanisms that may lead to differences in drought performance of beech are currently unknown.

A key pathway for drought acclimation involves abscisic acid (ABA) signaling to recruit drought defense responses and which results in stomatal closure, thereby, regulating plant water consumption (Shinozaki and Yamaguchi-Shinozaki 2007, Popko et al. 2010, Raghavendra et al. 2010). A further common feature of drought stress is an increased production of reactive oxygen species. Therefore, activation of protective enzymes, especially of antioxidative defenses, is important to combat oxidative degradation of vulnerable structures such as cell membranes (Polle et al. 2006, Fischer and Polle 2010).

To address the plasticity and adaptation of beech in response to drought, we selected key genes involved in ABA signaling [nine-cis-epoxy-dioxygenase (*NCED*), protein phosphatase 2C (*PP2C*), early responsive to dehydration (*ERD*)] and stress protection [ascorbate peroxidase (*APX*), superoxide dismutase (*Cu/ZnSOD*), aldehyde dehydrogenase (*ALDH*), glutamine amido transferase (*GAT*)]. Briefly, *NCED* is a crucial enzyme for ABA biosynthesis because it catalyzes the first committed step cleaving *cis*-xanthophyll to xanthoxin, which is then converted to ABA (Nambara and Marion-Poll 2005). Transgenic approaches in *Arabidopsis* have shown the involvement of *NCED* in drought tolerance (Iuchi et al. 2001, Frey et al. 2012). *PP2C* is induced by high ABA levels and is an essential component in ABA signal transduction (Lorenzo et al. 2001, Saavedra et al. 2010). *ERD* proteins are transcription factors that act as regulators of ABA signaling. Because *ERD* overexpression renders plants less ABA-responsive and more drought susceptible, it exerts negative control on the down-stream events (Kariola et al. 2006, Aalto et al. 2012). The beech homologs of the genes *ERD15* and *NCED1* have been studied in response to ozone (Jehnes et al. 2007) and *PP2C* from beech was cloned and overexpressed in *Arabidopsis* supporting its role in ABA signal transduction (Reyes et al. 2006).

The antioxidative enzymes *SOD* and *APX* detoxify superoxide radicals and hydrogen peroxide, respectively. Their role in mediating drought tolerance is known for a long time (Gupta et al. 1993, Mittler and Zilinskas 1994, Badawi et al. 2004). In beech, *APX* and *SOD*

undergo strong seasonal regulation (Polle and Morawe 1995a, Polle and Morawe 1995b) and the responsiveness of *SOD* to oxidative stress declines with increasing leaf age resulting in increased membrane leakage under acute oxidative stress (Polle et al. 2001). Here, homologs to cytosolic *Cu/ZnSOD1* and *APX1* were analyzed.

Unattended reactive oxygen species result in lipid peroxidation and increase the formation of aldehydes (Bartels and Sunkar 2005). Aldehydes are removed by *ALDH*. Overexpression of *ALDH* increases dehydration tolerance in *Arabidopsis* (Sunkar et al. 2003). *ALDH2B7* from *Arabidopsis*, to which *ALDH* from *Fagus sylvatica* shows the highest homology, is localized in the mitochondrion and can oxidize acetaldehyde and glycolaldehyde (Skibbe et al. 2002, Kirch et al. 2004).

Furthermore, we selected *GAT*, also known as asparagine synthase, which synthesizes asparagine from aspartate and glutamine (Heuvel et al. 2002). The expression of *GAT* is regulated by various stress factors and was identified as a constitutive drought marker in rice (Herrera-Rodríguez et al. 2007, Degenkolbe et al. 2013). In beech, *GAT* was induced after infection with *Phytophthora citricola* (Schlink 2009a). We expected that GAT. Which is involved in nitrogen metabolism, would also respond to drought because limited water availability suppresses beech nitrogen supply (Rennenberg et al. 2009).

In the present study we selected five stands along a precipitation gradient from moist to dry conditions and determined their genetic structures. The natural regeneration of these stands was used in a common garden experiment to investigate the expression of genes related to ABA signaling and stress. The responses of well-irrigated and drought stressed saplings were compared throughout summer at an early, mid- and late season time point. We hypothesized that progenies from dry sites would have constitutively increased expression levels of ABA- and stress-related genes and would be less drought responsive than progenies from moist sites. To link gene expression with plant performance we determined progeny- and drought-related effects on leaf area and membrane integrity in the absence and presence of acute oxidative stress.

3.2. Material and Methods

3.2.1. Field sites and plant material

Five old-growth mono-specific beech (*Fagus sylvatica* L.) forests (Sellhorn, Unterlüss, Göhrde, Klötze, Calvörde) were selected along a 130-km-long NW-SE precipitation gradient from the Lüneburg Heath to the Altmark in the North German Plain (Lower Saxony, Saxony-Anhalt, Germany, Table 1). The long-term mean annual precipitation decreased from 816 mm a^{-1} at the moist to 544 mm a^{-1} at the driest site, and the mean annual temperature increased along this gradient from 8.5 to 9.1 °C (Table 3.1). The forest stands exhibited similar structures with closed canopies, similar tree age of 100 to 130 years and were stocking on similar soil substrates (Pleistocene fluvioglacial sands from the penultimate Ice Age (Saalian), up to 30 % sand fraction, moderate to intense podzolic Umbrisols).

Table 3.1 - Location, mean annual and summer precipitation, and mean annual and summer temperature of five beech forests. Mean annual climate data refer to long-term averages from 1971 to 2000. Summer is defined as the time period from May to September. Climate data were derived from weather station data provided by National Climate Monitoring of Deutscher Wetterdienst and were corrected for altitude

	Origin Latitude (N)	Longtitude (E)	Elevation (m)	Precipitation (mm)		Temperature (°C)	
				Annual	Summer	Annual	Summer
Calvörde	52°22'	11°17'	105	544	260	9.1	15.6
Klötze	53°09'	11°15'	85	614	284	8.9	15.4
Göhrde	53°09'	10°52'	85	665	300	8.7	15.1
Unterlüß	52°50'	10°19'	117	766	324	8.5	14.8
Sellhorn	53°10	10°09'	130	816	352	8.5	14.6

Fresh leaves from at least 99 adult trees were sampled in summer 2009 (Göhrde, Calvörde and Unterlüss) and in summer 2012 (Sellhorn and Klötze) for genetic analysis. In summer 2011, 160 about three-year-old beech saplings (height of about 0.2 m) from the natural regeneration were collected in each stand and transferred to the Experimental Botanical Garden of the University of Göttingen (51°33' N, 9°57' E, 177 m a.s.l.) for a common garden experiment. For this purpose, the forest soil was carefully washed off and the bare-rooted trees were planted individually in 5-L containers filled with coarse sand (Oppermann, Hedemünden,

Germany) to a depth of 0.25 m. The unfertilized, fluviatile sand had a $pH_{(KCl)}$ of 6.2, a base saturation of 99.5 % and contained 0.01 µmol N g^{-1} and 0.6 µmol P g^{-1}. All saplings were kept well-watered and maintained outdoors during the growing season (mean air temperature: 17.7/11.9 °C day/night). In fall 2011, they were transferred to a climate chamber and kept at air temperatures of 4-7 °C and 70-80 % air humidity during the cold season. In April 2012, the plants were moved outdoors into the common garden and kept well-watered until the experimental treatments started.

3.2.2. Experimental treatments and harvests

A three-factorial randomized block design was set-up with the factors progeny (5), soil moisture (2) and time of the season (3). A total number of 150 trees (n = 5 per treatment) was placed under a mobile Perspex roof equipped with a rain sensor (Eltako, Fellbach, Germany), with the roof automatically moving over the plants when it rained. This allowed the control of the soil water content while providing outdoor conditions for the saplings. A shading net (Wunderlich, Osterode, Germany) reduced photosynthetically active radiation by approximately 70 %. Each plant was treated once before the beginning of the experiment with 150 ml of a biocide solution composed of 0.025 % dimethoate (Perfekthion 40EC, BASF, Ludwigshafen, Germany), 0.04 % fenazaquin (Magister 10EC, Margarita Internacional, Funchal, Portugal) and 0.15 % tebuconazole (Folicur, Bayer AG, Monheim, Germany), and was fertilized thrice during the growing season with 200 ml of a 0.2 % NPK 5-20-5 fertilizer solution (Wuxal P Profi; AGLUKON, Düsseldorf, Germany).

During the experimental phase from May to September the microclimatic conditions were monitored (HOBO U10 data loggers, Onset, Cape Cod, MA, USA, iButton Thermocron Temperature, Maxim, San Jose, CA, USA) showing mean air temperatures of 17.5/12.0 °C (day/night) and root temperatures of 16.9/11.6 °C at a depth of 0.1 m.

The drought treatments started on May 25, 2012, after the termination of leaf expansion and were continued until September 2012. Two soil moisture regimes were established according to the target precipitation amount (during this period) at plant origin: (i) a moist treatment with 260 mm, which resulted in average soil moisture of 10 % SWC v/v, and (ii) a dry treatment with 135 mm, which resulted in average soil moisture of 2 % SWC v/v. The SWC was monitored regularly to a soil depth of 0.16 m with a mobile TDR probe (time domain

reflectometry probe; TRIME-FM2, IMKO GmbH, Ettlingen, Germany) in 8 pots per treatment. Water lost by evapotranspiration was added every second day.

Whole trees of each progeny and soil moisture regime (n = 5) were sampled four (June 25, 2012), nine (July 30, 2012) and sixteen weeks (September 17, 2012) after the start of the drought stress. Leaves were removed from the stem and weighed. One leaf per tree (3^{rd} or 4^{th} fully expanded leaf from the top) was immediately frozen in liquid nitrogen for molecular analyses. Leaf disks were cut from fresh leaves and used for the determination of membrane leakage. The remaining leaves were used for leaf area analyses with WinFOLIA 2005b (Régent Instruments Inc., Quebec, QC, Canada). All fractions were dried (72 h, 70 °C) and weighed. Total leaf area per tree was calculated as specific leaf area (SLA; leaf area per leaf mass) x total leaf mass of the tree (cm^2).

3.2.3. Relative electrolyte leakage (REL)

Twenty leaf disks (6 mm in diameter) were cut and incubated at room temperature in 25 ml distilled water (ddH_2O) or 1 mM paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, Sigma-Aldrich, Saint Louis, MO, USA) as described by Polle et al. (2001). The conductivity (C) of the floating solution was measured at time zero (t_o), and after 4 and 24 h (LF 315/Set, WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). To disrupt the tissue and to release all electrolytes into the solution, the samples were autoclaved (121ºC, 20 min, HST 6x6x6 Zirbus Technology GmbH, Bad Grund, Germany) and cooled to room temperature before measurement of maximum electrolyte leakage (C_{max}). REL was calculated as: REL (%) = $(C_t - C_{to}) * 100/(C_{max} - C_{to})$.

3.2.4. RNA extraction and analysis of gene expression

Leaves of the beech progenies were powdered in liquid nitrogen and used for RNA extraction as described by Chang et al. (1993) with minor modifications: the extraction buffer contained 2% β-mercaptoethanol, but no spermidine. The isolated RNA was dissolved in 30 µl ddH_2O. The quality of 1 µl of the isolated RNA was controlled by gel electrophoresis and the concentration was measured in a BioPhotometer (Eppendorf, Hamburg, Germany). 1 µg RNA was used for the following steps: genomic DNA digestion with "TURBO DNA-free'' (Ambion, Austin, TX, USA) and subsequent cDNA synthesis with the "First Strand cDNA Synthesis Kit" (Thermo Scientific, Waltham, MA, USA), each according to the manufacturer's protocol. The

resulting cDNA was diluted 1:10 and used for real-time PCR in a LightCycler 480 (Roche, Mannheim, Germany). In each well 5 µl diluted cDNA, 10 µl SYBR Green I Master (Roche), 1 µl of each, forward and reverse primer (5 pmol) and 3 µl ddH_2O were mixed for the amplification reaction. Each biological sample was measured twice; five biological samples were measured per treatment and gene. The PCR program comprised the following steps: pre-incubation for 5 min at 95°C, 45 cycles of amplification for 10 sec at 95°C, 10 sec at 55°C, 20 sec at 72°C. The relative expression was calculated as: $E^{(Ct(actin)-Ct(target\ gene))}$ where E is the primer efficiency (Supplement Table S3.1). The primer efficiency was determined by a dilution series and ranged between 1.8 and 2 for the genes of this study. The primer sequences for the target genes were obtained from Olbrich et al. (2008) (Actin), Jehnes et al. (2007) (*NCED*) or designed using the programs Oligo Explorer and Oligo Analyzer (Gene Link, Hawthorne, NY, USA). Gene names, accession numbers and primer sequences have been compiled in Supplement Table S3.1.

3.2.5. DNA extraction and microsatellite analysis

Total DNA was extracted from leaves of the mature forest trees using the DNeasy™ 96 Plant Kit (Qiagen, Hilden, Germany). The amount and the quality of the DNA were analyzed by 1% agarose gel electrophoresis with 1 X TAE as running buffer (Sambrook et al. 1989). DNA was stained with Roti®-Safe GelStain (Roth, Karlsruhe, Germany), visualized by UV illumination and compared to a Lambda DNA size marker (Roche, Mannheim, Germany).

To analyze the genetic structures of the beech populations, nine highly polymorphic microsatellite markers were used. Four of them were originally developed for *Fagus crenata* (sfc_0018, sfc_0161, sfc_1063, sfc_1143; Asuka et al. 2004), whereas two of them were directly developed for *F. sylvatica* (FS 3-04, Pastorelli et al. 2003, mfs 11, Vornam et al. 2004). In addition, three EST microsatellite markers were applied originally developed and transferred from *Quercus robur* (GOT 066, FIR 065, FIR 004; Durand et al. 2010). We performed multiplexing of two to four primers, labeled with different fluorescent dyes (6-Carboxyfluorescein: sfc1063 sfc0161, FIR004, mfs11; 6-Hexafluorescein: sfc0018, sfc1143FIR065, FS 3-04), in set 1: all sfc loci, set 2: FS 3-04 and mfs 11, set 3: GOT 066, FIR 065, FIR 004.

PCR amplifications were conducted in a 15 µl volume containing 2 µl of genomic DNA (~10 ng), 10 x reaction buffer (0.8 M Tris-HCl pH 9.0, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20; Solis BioDyne, Tartu, Estonia), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 unit of *Taq* DNA polymerase (HOT FIREPol® DNA Polymerase, Solis BioDyne, Estonia), 0.3 µM of each forward and reverse primer. The PCR protocol consisted of an initial denaturation step of 95 °C for 15 min followed by 30 cycles of 94 °C for 1 min (denaturation), 47 °C (for the EST primer set 3) or 55 °C (for primer set 1 and 2) for 30 sec (annealing), 72 °C for 1 min (denaturation) and a final extension step of 72 °C for 20 min. Microsatellite fragments were separated on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). Data were collected and aligned with the help of the internal size standard GS 500 ROX™ using GeneScan 3.7® (Applied Biosystems). Fragments were scored with the software Genotyper 3.7® (Applied Biosystems).

3.2.6. Data analysis

Nei's genetic distance (Nei 1972) and the molecular diversity indices "number of alleles" (N_a), "observed heterozygosity" (H_o), "expected heterozygosity" (H_e) as well as the analysis of molecular variance (AMOVA) and pairwise F_{ST} (both based on 9,999 permutations) between the adult stands were calculated with the software GenAlEx, version 6.5 (Peakall and Smouse 2012). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram, based on Nei's distance, and the bootstrap values based on 1000 permutations were calculated with the software Populations, version 1.2.32 (Langella 1999). The dendrogram was visualized with the program TreeView, version 1.6.6 (Page 1996) using the phylogram tree style.

Statistical data analysis was conducted with Statgraphics (Centurion XVI, St. Louis, Mo, USA). Data for REL, leaf area and relative transcript abundance are shown as means (±SE). After testing for normality (skewness, kurtosis), multivariate analysis of variance (MANOVA) was conducted to determine the effects of the main factors progeny, drought and time and their interactions. Treatment effects were considered to be significant when the *P* values were ≤ 0.05. Post-hoc test was performed to discriminate between means using Fisher´s least significant differences procedure; homogenous group were considered to be significantly different, when *P* value was < 0.05. The *P* values are indicated in the results sections as post-hoc *P*. Correlation between the expression levels of different genes were tested by Spearman rank correlations.

Principle component analysis (PCA) was performed using the free PAST software package 2.17c (http://folk.uio.no/ohammer/past/ Hammer et al. 2001). Data were analyzed as correlation matrix based on Euclidean distances.

3.3. Results

3.3.1. Genetic structure of beech stands along a precipitation gradient

The genetic distances (Nei 1972) between the adult stands were relatively low and ranged from 0.015 between the stands Göhrde and Calvörde and 0.053 between Unterlüß and Göhrde (Table 3.2). The mean genetic distance between all stands was 0.037. In the dendrogram, only the stands Göhrde and Calvörde grouped close together whereas the remaining stands did not show a distinct clustering (Fig. 3.1). All clades were supported by significant bootstrap values. The genetic differentiation between the stands did not reflect the geographic distances or mean precipitation, but clearly showed significant differences between all populations (mean pairwise $F_{st} = 0.012$, $p < 0.01$). However, the differences of the molecular diversity indices between the different populations were low (Table 3.3). The number of alleles ranged from 7.556 for the population Klötze to 8.444 for the population Göhrde. The observed heterozygosity ranged from 0.594 for the population Unterlüß to 0.611 for the population Sellhorn. The mean expected heterozygosity was 0.610, whereas the lowest value was found for the population Klötze ($H_e = 0.582$) and the highest one for the population Göhrde ($H_e = 0.638$). The AMOVA revealed that 97 % of the molecular variance can be found within populations and 3% among them.

Table 3.2: Nei's genetic distances (Nei 1972) between the different adult stand

Calvörde	Klötze	Göhrde	Unterlüß	Sellhorn	
0.000					Sellhon
0.050	0.000				Unterlüß
0.027	0.053	0.000			Göhrde
0.035	0.041	0.048	0.000		Klötze
0.025	0.043	0.015	0.037	0.000	Calvörde

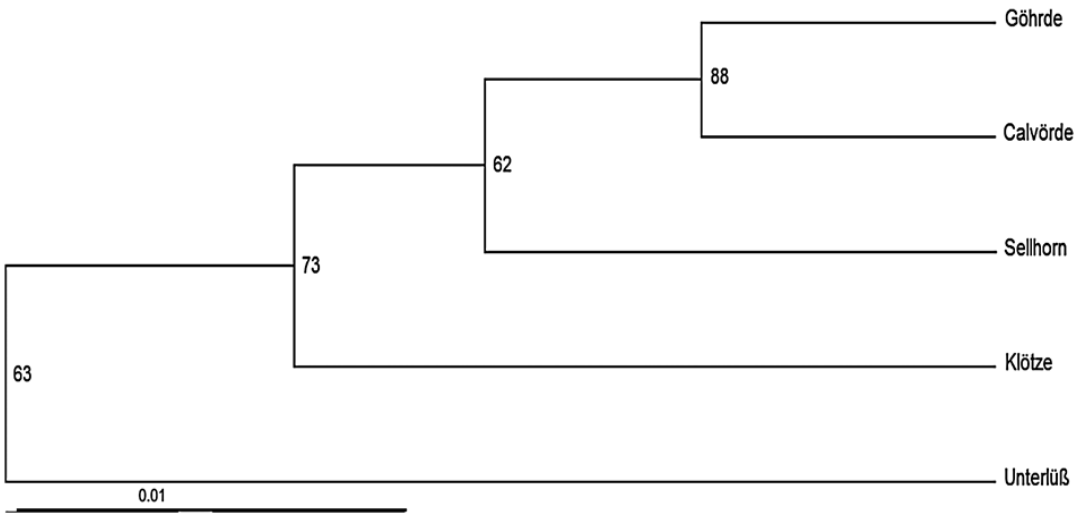


Figure 3.1: Dendrogram of European beech (*F. sylvatica*) populations from five locations (Sellhorn, Unterlüß, Göhrde, Klötze and Calvörde) along a precipitation gradient. The dendrogram was calculated with Nei's distance based on the analysis of nine microsatellite markers. Bootstrap values (1000 permutations) are indicated. Details of the sites are summarized in Table 1.

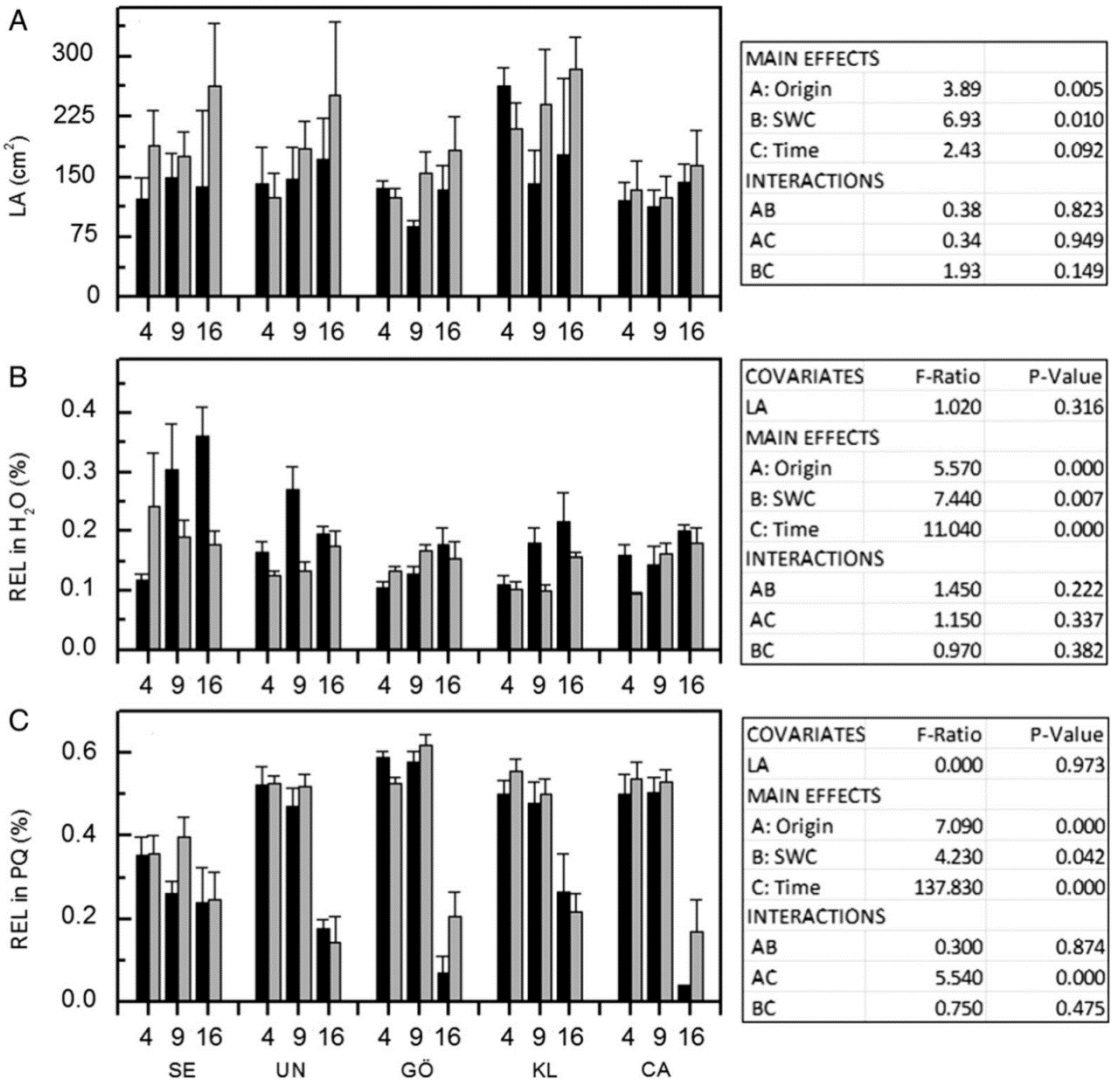
Table 3.3: Molecular diversity indices for the different populations. *N*, number of individuals; *N*a, number of alleles; *H*o, observed heterozygosity; *H*e, expected heterozygosity.

Population	N	Na	Ho	He
Sellhorn	99.9	7.778	0.611	0.615
Unterlüß	99.4	8.000	0.594	0.595
Göhrde	103.0	8.444	0.602	0.638
Klötze	99.7	7.556	0.598	0.582
Calvörde	104.0	8.111	0.599	0.619
Mean	101.2	7.978	0.601	0.610

3.3.2. Stress responses of beech progenies from a precipitation gradient

Leaf area is an indicator of plant growth and critically determines drought responses. The natural regeneration of the five genetically distinct beech forests showed significant differences in leaf area, generally with larger leaf areas present on progenies from wetter sites than on those from drier sites ($P = 0.005$, Fig. 3.2A). Drought stress resulted in decreased leaf area compared with well-watered trees ($P = 0.010$, Fig. 3.2A).

To test whether drought stress affected the cellular integrity, REL was determined after 24h incubation of leaf disks in water (Fig. 3.2B). Progenies from drier origins showed lower REL than those from moister origins ($P < 0.001$, Fig. 3.2B). REL increased towards the end of the growing season (post-hoc $P < 0.05$) and increased in response to drought stress ($P < 0.001$, Fig. 3.2B). Higher drought sensitivity of the progeny from Sellhorn (moist site) than of those of the drier sites is indicated by significant interactions between SWC x time and progeny x SWC x time (post-hoc $P < 0.05$, Fig. 3.2B).



MAIN EFFECTS		
A: Origin	3.89	0.005
B: SWC	6.93	0.010
C: Time	2.43	0.092
INTERACTIONS		
AB	0.38	0.823
AC	0.34	0.949
BC	1.93	0.149

COVARIATES	F-Ratio	P-Value
LA	1.020	0.316
MAIN EFFECTS		
A: Origin	5.570	0.000
B: SWC	7.440	0.007
C: Time	11.040	0.000
INTERACTIONS		
AB	1.450	0.222
AC	1.150	0.337
BC	0.970	0.382

COVARIATES	F-Ratio	P-Value
LA	0.000	0.973
MAIN EFFECTS		
A: Origin	7.090	0.000
B: SWC	4.230	0.042
C: Time	137.830	0.000
INTERACTIONS		
AB	0.300	0.874
AC	5.540	0.000
BC	0.750	0.475

Figure 3.2: Leaf area (LA) of young beech (*F. sylvatica*) trees (A), relative electrolyte leakage (REL %) of leaf disks after incubation in water (B) or paraquat (PQ) (C). The beech trees originated from five locations (SE, Sellhorn; UN, Unterlüß; GÖ, Göhrde; KL, Klötze; and CA, Calvörde). Data are arranged from moist to dry sites. The trees were maintained in a common garden experiment with sufficient water availability (gray bars) or drought (black bars) imposed by soil water contents SWC of 10 and 2% v/v, respectively, and harvested in the growing season after 4, 9 and 16 weeks of drought. Scale bars indicate means ± SE (n = 5).

The ability to cope with an acute stress situation was tested by challenging the leaf disks with paraquat, an herbicide known to induce massive oxidative stress (Polle et al. 2001). Early in the growing season, leaves from Sellhorn progenies, which originate from the most mesic site, withstood oxidative stress better than those from drier sites (post-hoc $P < 0.05$, Fig. 3.2C).

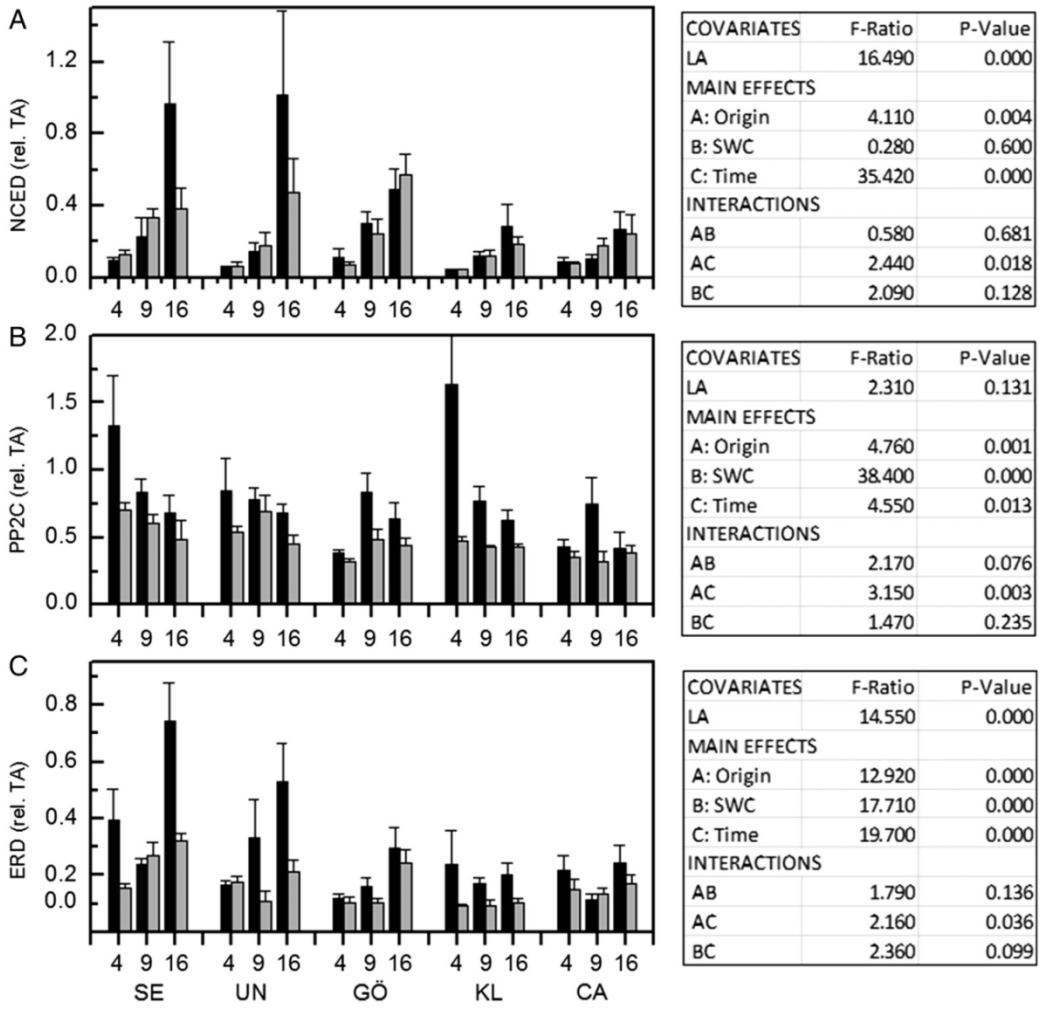
Towards the end of the growing season, REL was reduced indicating higher protection in all progenies (post-hoc $P < 0.05$, Fig. 3.2C). Leaves from drought-stressed trees exhibited generally lower REL than those from unstressed trees, but the magnitude of the ameliorating effect was very small ($P = 0.042$, Fig. 3.2C).

3.3.3. ABA-related gene expression in beech progenies from a precipitation gradient

The expression of *NCED*, which is required for ABA biosynthesis, increased from the beginning towards the end of the growing season (post-hoc $P < 0.05$) and was higher in progenies from wetter sites than in those from drier sites (post-hoc $P < 0.05$, Fig. 3.3A). Drought stress affected *NCED* only at the end of the growing season, with the strongest increases in progenies from Sellhorn and Unterlüss (moist sites, post-hoc $P < 0.05$, Fig. 3.3A).

In contrast to *NCED*, the expression of *PP2C*, a phosphatase kinase involved in ABA signal transduction, decreased towards the end of the growing season (post-hoc $P < 0.05$). Progeny-related differences in *PP2C* expression were found ($P = 0.001$), which were however, less pronounced than those found for *NCED*. Drought stress resulted in most cases in increases in *PP2C*, which were generally stronger in the early growing season than at the end of the growth phase (interaction progeny x drought, $P = 0.018$, Fig. 3.3B).

The expression of *ERD15*, an ABA-responsive transcription factor that attenuates ABA responses, was higher in progenies from moist than in those from drier sites (post-hoc $P < 0.05$), increased towards the end of the growing season ($P = 0.013$) and increased in response to drought stress ($P < 0.001$). The responses were stronger in progenies from Sellhorn and Unterlüss than in those from the other sites (interaction progeny x drought, Fig. 3.3C).



COVARIATES	F-Ratio	P-Value
LA	16.490	0.000
MAIN EFFECTS		
A: Origin	4.110	0.004
B: SWC	0.280	0.600
C: Time	35.420	0.000
INTERACTIONS		
AB	0.580	0.681
AC	2.440	0.018
BC	2.090	0.128

COVARIATES	F-Ratio	P-Value
LA	2.310	0.131
MAIN EFFECTS		
A: Origin	4.760	0.001
B: SWC	38.400	0.000
C: Time	4.550	0.013
INTERACTIONS		
AB	2.170	0.076
AC	3.150	0.003
BC	1.470	0.235

COVARIATES	F-Ratio	P-Value
LA	14.550	0.000
MAIN EFFECTS		
A: Origin	12.920	0.000
B: SWC	17.710	0.000
C: Time	19.700	0.000
INTERACTIONS		
AB	1.790	0.136
AC	2.160	0.036
BC	2.360	0.099

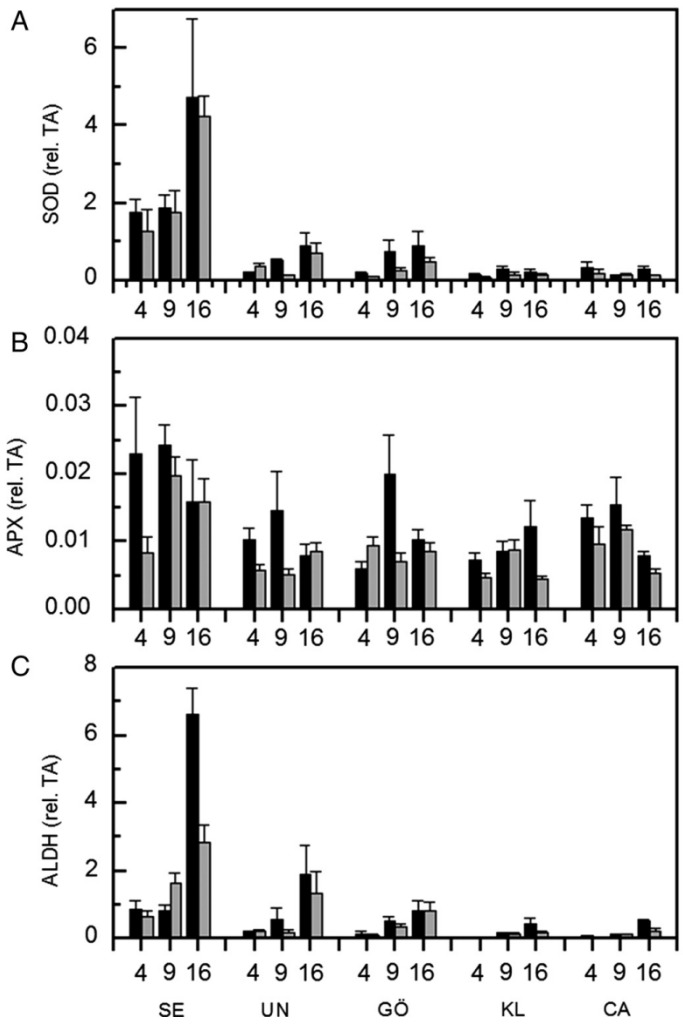
Figure 3.3: Relative transcript abundance (rel. TA) of NCED (A), PP2C (B) and ERD (C) in leaves from beech (*F. sylvatica*). The leaves were obtained from progenies originating from five sites (SE, Sellhorn; UN, Unterlüß; GÖ, Göhrde; KL, Klötze; CA, Calvörde). Data are arranged from moist to dry sites. The trees were maintained in a common garden with sufficient water availability (gray bars) or drought (black bars) imposed by SWC of 10 and 2%, respectively, and harvested in the growing season after 4, 9 and 16 weeks of drought. Scale bars indicate means ± SE (n = 4–5).

3.3.4. Stress-related gene expression in progenies from a precipitation gradient

The expression of *SOD*, *APX* and *ALDH*, genes encoding enzymes required for the scavenging of superoxide radicals, hydrogen peroxide and toxic aldehydes were higher in Sellhorn progenies than in those from the other sites (post-hoc $P < 0.05$ for each gene, Fig. 3.4). *SOD* and *ALDH* showed intermediate expression levels in progenies from Unterlüss and Göhrde and very low expression levels in progenies from the driest sites, Klötze and Calvörde

(Fig. 3.4A, C). The expression levels of *SOD* and *ALDH* increased at the end of the growing season in the Sellhorn progeny (post-hoc $P < 0.05$ for each gene, Fig. 3.4A, C).

The expression levels of *APX* were lower than those of *SOD* and *ALDH* (post-hoc $P < 0.001$ for both comparisons), and decreased towards the end of the growing season compared with mid-season values (post-hoc $P < 0.05$, Fig. 3.4B). Drought stress resulted in increased *APX* levels ($P = 0.001$), but the magnitude of this effect was relatively small (Fig. 3.4B).



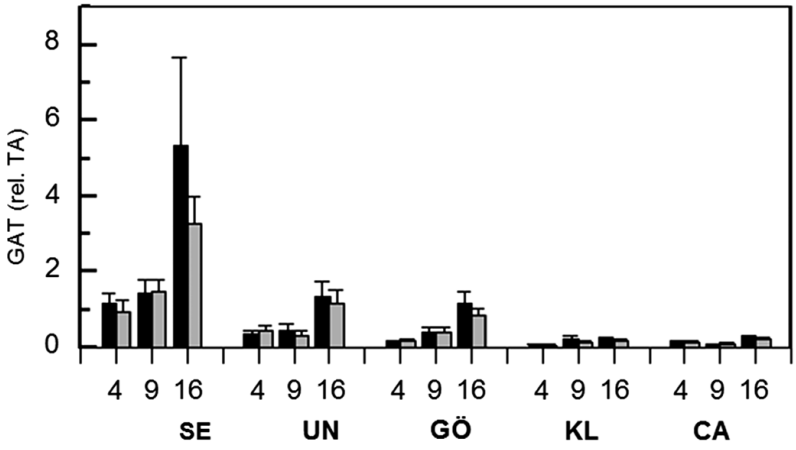
COVARIATES	F-Ratio	P-Value
LA	2.800	0.097
MAIN EFFECTS		
A: Origin	60.850	0.000
B: SWC	4.410	0.038
C: Time	15.850	0.000
INTERACTIONS		
AB	0.340	0.853
AC	8.140	0.000
BC	0.330	0.722

COVARIATES	F-Ratio	P-Value
LA	0.870	0.352
MAIN EFFECTS		
A: Origin	9.950	0.000
B: SWC	10.700	0.001
C: Time	4.340	0.015
INTERACTIONS		
AB	0.170	0.951
AC	0.650	0.738
BC	1.080	0.342

COVARIATES	F-Ratio	P-Value
LA	21.100	0.000
MAIN EFFECTS		
A: Origin	41.460	0.000
B: SWC	1.790	0.183
C: Time	56.800	0.000
INTERACTIONS		
AB	0.900	0.464
AC	12.490	0.000
BC	4.860	0.009

Figure 3.4. Relative transcript abundance (rel. TA) of superoxide dismutase (SOD, A), ascorbate peroxidase (APX, B) and aldehyde dehydrogenase (ALDH, C) in leaves from beech (*F. sylvatica*). The leaves were obtained from progenies originating from five sites (SE, Sellhorn; UN, Unterlüß; GÖ, Göhrde; KL, Klötze; CA, Calvörde). Data are arranged from moist to dry sites. The trees were maintained in a common garden with sufficient water availability (gray bars) or drought (black bars) imposed by SWC of 10 and 2%, respectively, and harvested in the growing season after 4, 9 and 16 weeks of drought. Scale bars indicate means ± SE (n = 4–5).

We also determined the expression of *GAT*, which is expected to respond to drought-induced changes in nutrient supply. *GAT* expression was highest in progenies from Sellhorn, intermediate in those from Unterlüss and Göhrde and lowest in those from Klötze and Calvörde ($P < 0.001$, Fig. 3.5). GAT expression levels increased towards the end of the growing season ($P < 0.001$, Fig. 3.5). In contrast to our expectation, they were not affected by drought ($P = 0.299$, Fig. 3.5).



COVARIATES	F-Ratio	P-Value
LA	2.520	0.115
MAIN EFFECTS		
A: Origin	36.840	0.000
B: SWC	1.090	0.299
C: Time	28.130	0.000
INTERACTIONS		
AB	0.660	0.619
AC	7.180	0.000
BC	1.110	0.332

Figure 3.5: Relative transcript abundance (rel. TA) of glutamine amidotransferase (GAT; syn. asparagine synthase) in leaves from beech (*F. sylvatica*). The leaves were obtained from progenies originating from five sites (SE, Sellhorn; UN, Unterlüß; GÖ, Göhrde; KL, Klötze; CA, Calvörde). Data are arranged from moist to dry sites. The trees were maintained in a common garden with sufficient water availability (gray bars) or drought (black bars) imposed by SWC of 10 and 2%, respectively, and harvested in the growing season after 4, 9 and 16 weeks of drought. Scale bars indicate means ± SE (n = 4–5).

Correlation analyses conducted for the transcript levels of the genes revealed significant relationships for most of the genes studied (Supplement Table S3.2). The exception were *PP2C* levels, which were only correlated with *ERD* and *APX*, and *APX* levels, which were not correlated with *NCED* and *ALDH* (Supplement Table S3.2).

3.3.5. Multivariate analysis of progeny- and drought-related performance of beech progenies

To determine the main factors responsible for progeny-related differences and stress responsiveness, PCAs were conducted for early, mid- and late time points during the growing season using all measuring variables (Fig. 3.6). PC1 was the main component representing 40.2

to 42.0 % of the variation and separating the five progenies according to the precipitation at the sites of their origin (Fig. 3.6A-C, Supplement Table S3.3). Although mean summer precipitation was included, *ALDH*, *GAT*, and *SOD* were the main positive loadings of PC1 with a high correlation of r ranging from 0.81 to 0.93 (Supplement Table S3.3). Negative loadings of the PC1 were acute stress responses (PQ4 with $r > 0.6$) at early and mid-season time points (Fig. 3.6 A, B), whereas no significant negative loading was determined for PC1 at the end of the growing season (Fig. 3.6 C).

PC2 and PC3 separated drought-stressed from unstressed treatments, but their contributions to the variation were less important than PC1 (13.1 to 17.6% for PC2; 10.7 to 13.0% for PC3, Supplement Table S3.3). For PC2 SWC was the most significant loading with $r = 0.83$ in the mid-season (Fig. 3.6B), whereas leaf area was the main loadings early and late in the season with $r = 0.61$ and 0.76, respectively (Fig. 3.6A, C). The main loadings of PCA3, which was similarly important as PC2, were SWC with -0.50 and 0.50 early and late in the growing season and leaf area ($r = 0.82$) in the mid-season (Supplement Table S3.3). SWC as the main loading was always opposed by *PP2C* as main loading for the opposite direction with 0.79 (early season), -0.70 (mid-season), and -0.57 (late season) on either the first or second PCA axis.

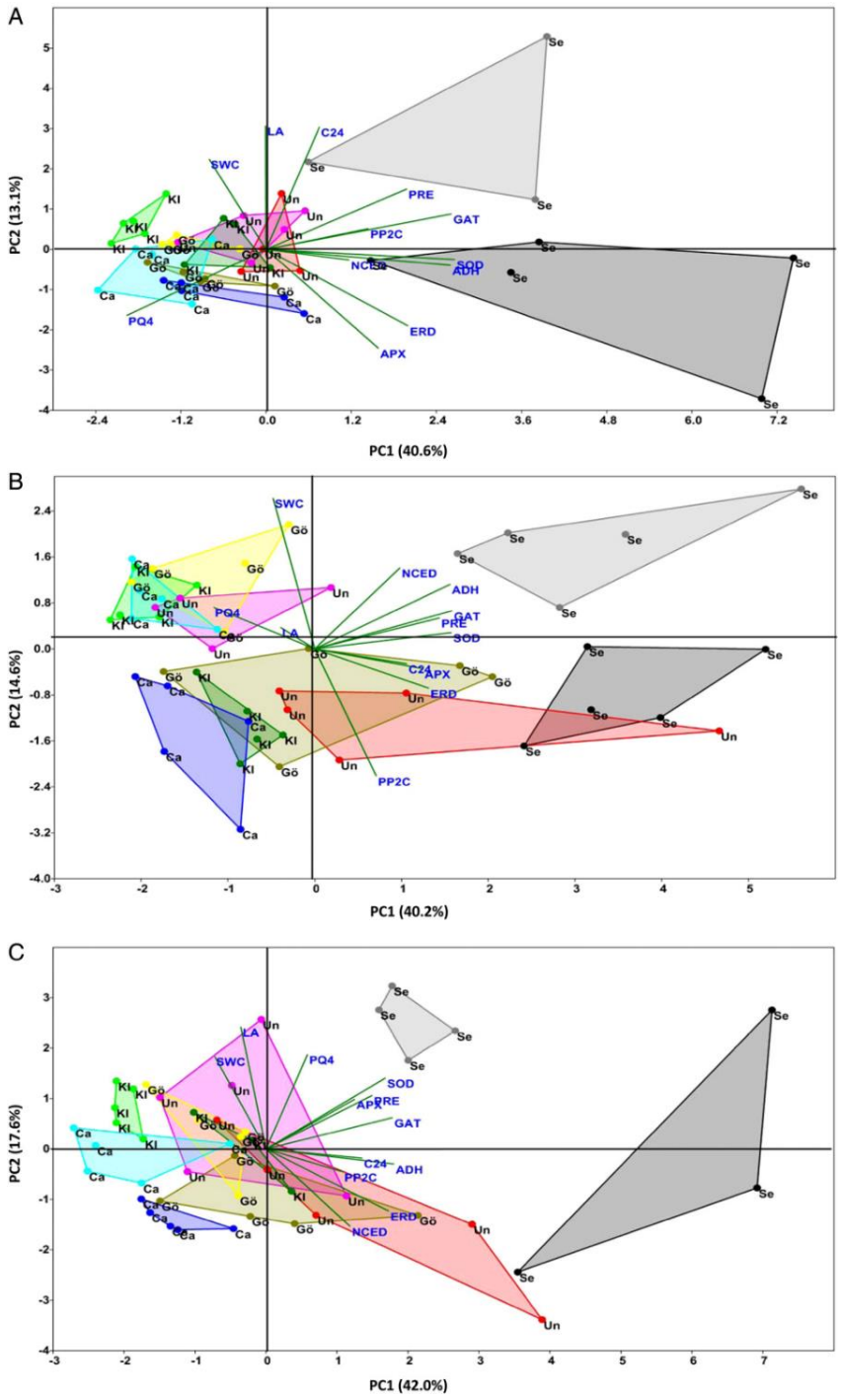


Figure 3.6: Principal component analysis of all measuring parameters in June (A), July (B) and September (C). Each progeny is indicated by the minimum hulls with light colors for drought-stressed and full colors for non-stress trees: gray, Sellhorn (Se); red, Unterlüß (Un); yellow, Göhrde (Gö); green, Klötze (Kl); blue, Calvörde (Ca). ADH, aldehyde dehydrogenase; APX, ascorbate peroxidase; C24, membrane leakage after water incubation; ERD, early dehydration responsive protein; GAT, glutamine amidotransferase; LA, leaf area; NCED, 9-*cis*-epoxy-dioxygenase; PP2C, protein phosphatase 2C; PQ4, membrane leakage after paraquat treatment; SWC, soil water content.

3.4. Discussion

3.4.1. Beech populations exhibit strong differentiation of stress-related gene transcription, but not of neutral genetic markers

Beech populations display high genetic variation in natural and managed stands and even in stands that are geographically close to each other (Buiteveld et al. 2007, Jump and Penuelas 2007). In concordance with other studies applying neutral genetic markers (Vornam et al. 2004, Chybicki et al. 2009, Gömöry et al. 2010), we found that the beech trees from the five sites analyzed here exhibited only very little variation between and high variation within the populations. The applied neutral markers were sufficient to distinguish the populations, but the genetic distances did not reflect the climatic gradient of the sites. A reason for this result was probably that neutral markers are under lower selection pressure than adaptive genes. However, some studies found evidence for on-going selection in beech. For example, Jump et al. (2006a) identified a gene locus whose frequency correlated with temperature in populations at the southern range edge of in Spain, where increasing temperatures enhanced evaporative demand and resulted in growth decline (Jump et al. 2006b). Jump et al. (2006a) suggested that this locus may indicate an adaptive *in situ* response to the changing environment.

Beech populations in Switzerland also exhibited higher differentiation than expected of some genetic markers in trees of a dry compared to those of a mesic site (Pluess and Weber 2012). This finding suggested ongoing selection, but no correlation between the genetic diversity indices and growth was observed (Pluess and Weber 2012).

In addition to neutral genetic markers, we used the transcript abundance and stress responsiveness of a number of structural genes to distinguish between genetic adaptation and flexible stress adjustment. PCA of transcript abundances of stress-related genes and physiological parameters ordered the populations according to mean precipitation and temperature. Numerous previous investigations using beech provenances or ecotypes from different (micro-)geographic areas have already identified physiological and phenotypical differences that suggested the presence of adaptive traits (Peuke et al. 2002, Meier et al. 2006, Rose et al. 2009, Czajkowski and Bolte 2006, Robson et al. 2012, Stajner et al. 2013). Our results show that GAT, ALDH, SOD and with slightly lower impact also ERD were the major

factors ordering the progenies according to precipitation gradient (SE > UN ≥ GÖ ≥ KL > CA). These findings suggest that these genes were either direct or indirect targets of selection.

In contrast to our initial hypothesis *SOD*, *ALDH*, and *GAT* were constitutively lower and not higher in progenies from dry than from mesic sites. Furthermore, the transcript levels increased towards fall more strongly in beeches from mesic than in those from dry sites. These observations suggest that beech origins from sites with higher precipitation may have an increased need to detoxify products of oxidative stress. In fact, leaves from unstressed Sellhorn beeches, the site with the highest annual precipitation, exhibited higher membrane leakage than those from the other sites pointing to the higher constitutive production of reactive oxygen species than in the other beech progenies. Currently, we can only speculate about the reasons for these results. It is possible that beeches from drier sites avoid production of toxic metabolites by yet unknown mechanisms, whereas those from sites with sufficient precipitation protect themselves by enhanced activation of scavenging mechanisms. As detoxification is energy and reductant consuming, we speculate that prevention of oxidant production may be more favorable that permanent scavenging in progenies from dry sites.

Other selected genes in our study also showed significant site-of-origin related differences in in transcript abundance. However, these genes contributed less than *SOD, GAT* and *ALDH* to the order of the progenies on PC1. Among these genes, the transcriptional regulation of *ERD* is noteworthy because *ERD* is a negative regulator of ABA sensitivity. Increased transcript levels of *ERD* in Sellhorn beeches, therefore, suggest that the sensitivity to ABA is lower in these progenies than in those from the other sites. ABA is known for its role in stomatal closure, but many other down-stream effects of ABA have been recognized such as its antagonizing effect on growth and up-regulation of proteins involved in osmotic adaptation, e.g. LEA proteins, dehydrins, etc. (Cutler et al. 2010). The present results may imply that selection acts on the balance between the capacity for detoxification of injurious metabolites and the presence of pre-formed anti-drought proteins. It is clear that more work is required in future to identify adaptive traits in beech and unravel their molecular basis.

3.4.2. Stress-related genes exhibit seasonal changes and differ in drought responsiveness

Antioxidative enzymes play important roles in plant development and adaptation to environmental stresses (Suzuki et al. 2012). It is therefore critical to balance the removal and

production of reactive species throughout the plant´s life (Suzuki et al. 2012). Antioxidative systems as well as transcript abundances in beech leaves vary with tissue age and physiological stage (Polle and Morawe 1995b, Luwe 1996, Olbrich et al. 2009). Furthermore, the stress susceptibility of beech leaves varies during the growing season and is generally higher in young than mature leaves (Polle et al. 2001). Here, acute stress imposed by paraquat also resulted in strong membrane injury at the earlier time points (June, July) than in September, with the exception of the progeny Sellhorn, whose leaves exhibited higher antioxidative capacities than those from the other sites. Notably, the leaves from drought exposed plants were either not or only marginally better protected from acute oxidative injury suggesting that stress did not trigger enhance responsiveness or generally higher activation of the antioxidative defenses. This notion is also supported by the relatively small increases in transcript levels of *SOD, APX* and *ALDH* in response to drought.

Many studies, mostly conducted with crops or herbaceous model plants, have shown that overexpression of enzymes such as SOD, APX and ALDH increased drought protection (Reddy et al. 2004, Kotchoni et al. 2006, Foyer and Noctor 2009, Kar 2010). A drawback of our analysis is that only a limited number of stress-related genes were analyzed and that the genes included here are members of larger families. Therefore, the current analysis provides only a glimpse into the drought regulation of antioxidative systems in beech. However, the finding that membrane leakage of drought stressed leaves was not much changed compared to that of non-drought stressed leaves supports our suggestion that plasticity or flexibility of antioxidative defenses may not play a major role in stress amelioration in beech.

In concordance with other studies (Cutler et al. 2010, Raghavendra et al. 2010), our results point to ABA signaling as a critical drought response. Among the analyzed genes, *PP2C* was the only one, whose transcript abundance was the main driver separating drought and non-drought stressed behavior in the PCA. *PP2C* loading was opposed to soil water content. However, the separation of drought and non-stressed treatments was less important (about 17% or 11% of the variation) than plant origin (40 % of the variation). *PP2C* is probably the best-studied gene in European beech (Lorenzo et al. 2001). It was isolated from beech nuts, induced by ABA in seeds during dormancy, but not in other vegetative tissues (Lorenzo et al. 2001). Our data show that its drought inducibility depends on the beech population studied and was generally stronger early than late in the growing season. Heterologous overexpression of beech *PP2C* in Arabidopsis rendered the plants more stress sensitive (Reyes et al. 2006). Furthermore,

beech PP2C interacts with PYL/RCAR in the presence of ABA and thereby enables the transcription of drought-responsive target genes (Saavedra et al. 2010). Here, decreased *PP2C* and increased *NCED* levels imply higher ABA biosynthesis, but lower sensitivity in fall than at earlier time points. Overall, this pattern was more pronounced in beech from moist than in those from drier sites underlining that intraspecific progeny-related differences exist to cope with drought.

There is now increasing awareness that our knowledge on stress responses and regulation, gained by in-depth analysis of model plants such as *Arabidopsis thaliana,* is often not applicable to other plants species (Martin 2013). Our attempts to increase understanding of non-model plants have to be re-enforced because climate change with decreasing water availability and increasing temperatures is expected to limit the current range of many plant species, especially of long-lived forest trees. The present study addresses the adaptability of beech, an economically important, widespread species in European temperate forests, to drought. Analyses of the transcriptional regulation of genes for drought signaling and defense throughout the growing season uncovered intraspecific differences in constitutive expression and drought responsiveness. The progeny-related differences were stronger than the stress responses suggesting that selection for drought adaptation may already take place in local beech populations. An important future task will be to elucidate the molecular reasons for the observed differentiation.

3.5. References

Aalto MK, Helenius E, Kariola T, Pennanen V, Heino P, Hõrak H, Puzõrjova I, Kollist H, Palva ET (2012) ERD15-An attenuator of plant ABA responses and stomatal aperture. Plant Sci 182: 19–28.

Asuka Y, Tani N, Tsumura Y, Tomaru N (2004) Development and characterization of microsatellite markers for *Fagus crenata* Blume. Mol Ecol Notes 4:101-103.

Badawi GH, Kawano N, Yamauchi Y, Shimada E, Sasaki R, Kubo A, Tanaka K (2004) Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol Plant 121:231–238.

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Critical Reviews in Plant Sciences 24:23–58.

Bolte A, Czajkowski T, Kompa T (2007) The north-eastern distribution range of European beech-a review. Forestry 80:413-429.

Buiteveld J, Vendramin GG, Leonardi S, Kamer K, Geburek T (2007) Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. For Ecol Manag 247:98-106.

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116.

Chybicki IJ, Trojankiewicz M, Oleksa A, Dzialuk A, Burcyk J (2009) Isolation-by-distance within naturally established populations of European beech (*Fagus sylvatica*). Can J Bot 87:791-798.

Ciais Ph, Reichstein, M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer Chr, Carrara A, Chevallier F, De Noblet N, Friend A D, Friedlingstein P, Grunwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529-533.

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Ann Rev Plant Biol 61:651-679.

Czajkowski T, Bolte A (2006) Unterschiedliche Reaktion deutscher und polnischer Herkünfte der Buche (*Fagus sylvatica* L.) auf Trockenheit. Allg Forst Jagdztg 177:30-40.

Degenkolbe T, Do PT, Kopka J, Zuther E, Hincha DK, Köhl K (2013) Identification of Drought tolerance markers in a diverse population of rice cultivars by expression and metabolite profiling. PLoS one 8(5): e63637.

Durand J, Bodenes C, Chancerel E, Frigerio J-M, Vendramin G, Sebastiani F, Buonamici A, Gailing O, Koelewijn H-P, Villani F, Mattioni C, Cherubini M, Goicoechea PG, Herran A, Ikaran Z, Cabane C, Ueno Saneyoshi, Alberto F, Dumoulin P-Y, Guichoux E, de Daruvar A, Kremer A, Plomion C (2010) A fast and cost-effective approach to develop and map EST-SSR markers: oak as a case study. BMC Genomics 11: 570.

Ellenberg H, Strutt GK. (2009). Vegetation Ecology of Central Europe. Cambridge University Press: Cambridge, p. 756.

Fischer U, Polle A. (2010) *Populus* responses to abiotic stress. In: Genetics and genomics of *Populus*. (Eds). S. Jansson, R. Bhalerao, Groover A), Springer-Verlag, Berlin, pp. 225-247

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxidants & Redox Signaling 11:861-905.

Frey A, Effroy D, Lefebvre V, Seo M, Perreau F, Berger A, Sechet J, To A, North HM, Marion-Poll A (2012) Epoxycarotenoid cleavage by NCED5 fine-tunes ABA accumulation and affects seed dormancy and drought tolerance with other NCED family members. Plant J 70:501–512.

García-Plazaola JI, Becerril JM (2000) Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. Trees 14:485–490.

Gessler A, Keitel C, Kreuzwieser J, Matyssek R, Seiler W, Rennenberg H (2007) Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21:1-11.

Gömöry D, Longauer R, Paule L, Krajmerova, D Schmidtová (2010) Across-species patterns of genetic variation in forest trees of Central Europe. Biodivers Conserv 19:2025-2038.

Gupta AS, Heinen JL, Holaday AS, Burke JJ, Allen RD (1993) Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Natl Acad Sci USA 90:1629–1633.

Hammer O, Harper DAT, Ryan PD (2001) PAST: Palaeontological Statistics software package for education and data analysis. Palaeontol Electron 4: 9-17.

Hanewinkel M, Cullmann D, Schelhaas MJ, Nabuurs GJ, Zimmermann NE (2013) Climate change may cause severe loss in the economic value of European forest land. Nature Climate Change 3:204-207.

Herrera-Rodríguez MB, Pérez-Vicente R, Maldonado JM (2007) Expression of asparagine synthetase genes in sunflower (*Helianthus annuus*) under various environmental stresses. Plant Physiol Biochem 45:33-38.

Heuvel RHH van den, Ferrari D, Bossi RT, Ravasio S, Curti B, Vanoni MA, Florencio FJ, Mattevi A (2002) Structural studies on the synchronization of catalytic centers in glutamate synthase. J Biol Chem 277:24579–24583.

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J 27:325–333.

Jehnes S, Betz G, Bahnweg G, Haberer K, Sandermann H, Rennenberg H (2007) Tree internal signalling and defence reactions under ozone exposure in sun and shade leaves of European Beech (*Fagus sylvatica* L.) trees. Plant Biol 9:253–264.

Jump AS, Hunt JM, Martinez-Izquierdo JA, Penuelas J (2006a) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Mol Ecol 15:3469–3480.

Jump AS, Hunt JM, Penuelas J (2006b) Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. Global Change Biol 12:2163-2174.

Jump AS, Penuelas J (2007) Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the wind-pollinated tree, *Fagus sylvatica*. Mol Ecol 16:925-936.

Kar RK (2011) Plant responses to water stress: Role of reactive oxygen species. Plant Signaling & Behavior 6:1741–1745.

Kariola T, Brader G, Helenius E, Li J, Heino P, Palva ET (2006) EARLY RESPONSIVE TO DEHYDRATION 15, a negative regulator of abscisic acid responses in Arabidopsis. Plant Physiol 142:1559–1573.

Kirch H-H, Bartels D, Wei Y, Schnable PS, Wood AJ (2004) The ALDH gene superfamily of *Arabidopsis*. Trends Plant Sci 9:371–377.

Kotchoni SO, Kuhns C, Ditzer A, Kirch HH, Bartels D (2006) Over-expression of different aldehyde dehydrogenase genes in *Arabidopsis thaliana* confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant Cell Environ 29:1033-1048.

Langella O (1999) Populations, version 1.2.32. http://www.bioinformatics.org/project/?group_id=84. Accessed June 2012

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manag 259:698-709.

Lorenzo O, Rodríguez D, Nicolas G, Rodríguez PL, Nicolás C (2001) A new protein phosphatase 2C (FsPP2C1) induced by abscisic acid is specifically expressed in dormant beechnut seeds. Plant Physiol 125:1949–1956.

Luwe M (1996) Antioxidants in the apoplast and symplast of beech (*Fagus sylvatica*) leaves: seasonal variations and responses to changing ozone concentrations in air. Plant Cell Environ 19:321-328.

Martin C (2013). The plant science decadal vision. Plant Cell 25:4773–4774.

Meier IC, Leuschner C (2008) Leaf size and leaf area index in *Fagus sylvatica* forests: Competing effects of precipitation, temperature, and nitrogen availability. Ecosystems 11:655-669.

Mittler R, Zilinskas BA (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J 5:397–405.

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56:165–185.

Nei M (1972) Genetic distance between populations. Am Nat 106: 283-292.

Olbrich M, Gerstner E, Welzl G, Fleischmann F, Osswald W, Bahnweg G, Ernst D (2008) Quantification of mRNAs and housekeeping gene selection for quantitative real-time RT-PCR normalization in European beech (*Fagus sylvatica* L.) during abiotic and biotic stress. Z f Naturforsch C 63: 574-582

Olbrich M, Gerstner E, Welzl G, Winkler JB, Ernst D (2009) Transcript responses in leaves of ozone-treated beech saplings seasons at an outdoor free air model fumigation site over two growing seasons. Plant Soil 323:61–74.

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357-358.

Pastorelli R, Schmulders MJM, Van´t Westende WPC, Vosman B, Giannini R, Vettori C, Vendramin GG (2003) Characterization of microsatellite markers in *Fagus sylvatica* L. and *Fagus orientalis* Lipsky. Mol Ecol Notes 3:76-78.

Paule L, Krizo M, Pagan J (1994) Genetics and improvement of common beech (*Fagus sylvatica* L.). Ann Forest Sci 11:1–22.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Peuke AD, Rennenberg H (2011) Impacts of drought on mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings. Tree Physiol 31:196–207.

Peuke AD, Schraml C, Hartung W, Rennenberg H (2002) Identification of drought-sensitive beech ecotypes by physiological parameters. New Phytol 154:373–387.

Pluess A, Weber P (2012) Drought-adaptation potential in *Fagus sylvatica*: linking moisture availability with genetic diversity and dendrochronology. PLoS one 7(3): e33636. doi:10.1371/journal.pone.0033636

Polle A, Altman A, Jiang XN (2006) Towards Genetic Engineering for Drought Tolerance in Trees. In: Tree Transgenesis: recent developments (Fladung M, Ewald D, eds). Springer-Verlag, pp 275-297.

Polle A, Morawe B (1995a) Properties of ascorbate-related enzyme activities in foliar extracts from beech (*Fagus sylvatica*, L.). Phyton 35:117-129.

Polle A, Morawe B (1995b) Seasonal changes of antioxidative systems in foliar buds and leaves of field grown beech trees (*Fagus sylvatica*, L.) in a stressful climate. Bot Acta 108:314-320.

Polle A, Schwanz P, Rudolf C (2001) Developmental and seasonal changes of stress responsiveness in beech leaves (*Fagus sylvatica, L.*). Plant Cell and Environ 24:821-829.

Popko J, Hänsch R, Mendel RR, Polle A, Teichmann T (2010) The role of abscisic acid and auxin in the response of poplar to abiotic stress. Plant Biol 12:242–258.

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signaling. Trends Plant Sci 15: 395-401.

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189-1202.

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol 11:4–23.

Rennenberg H, Loreto L, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological responses of forest trees to heat and drought. Plant Biol 8: 556-571.

Reyes D, Rodriguez D, Gonzalez-Garcia MP, Lorenzo O, Nicolas G, Garcia-Martinez JL, Nicolas C (2006) Overexpression of a protein phosphatase 2C from beech seeds in *Arabidopsis* shows phenotypes related to abscisic acid responses and gibberellin biosynthesis. Plant Physiol 141:1414-1424.

Robson TM, Sanchez-Gomez D, Cano FJ, Aranda I (2012) Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. Tree Genetics Genomes 8:1111-1121.

Rose L, Leuschner C, Kockemann B, Buschmann H (2009) Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? Eur J Forest Res 128:335–343

Saavedra X, Modrego A, Rodriguez D, Gonzalez-Garcia MP, Sanz L, Nicolas G, Lorenzo O (2010) The nuclear interactor PYL8/RCAR3 of *Fagus sylvatica* FsPP2C1 is a positive regulator of abscisic acid signaling in seeds and stress. Plant Physiol 152:133–150.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Habor N.Y. Cold Spring Habor Laboratory

Schlink K (2009) Identification and characterization of differentially expressed genes from *Fagus sylvatica* roots after infection with *Phytophthora citricola*. Plant Cell Reports 28:873–882.

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58: 221-227.

Skibbe DS, Liu F, Wen T-J, Yandeau MD, Cui X, Cao J, Simmons CR, Schnable PS (2002) Characterization of the aldehyde dehydrogenase gene families of *Zea mays* and *Arabidopsis*. Plant Mol Biol 48:751–764.

Stajner D, Orlovic S, Popovic BM, Kebert M, Stojnic S, Klasnja B (2013) Chemical parameters of oxidative stress adaptability in beech. Journal of Chemistry, Article ID: 592695, dx.doi.org/10.1155/2013/592695

Sunkar R, Bartels D, Kirch H-H (2003) Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. Plant J 35:452–464.

Suzuki N, Koussevitzky S, Mittler R, Miller G (2012) ROS and redox signaling in the response of plants to abiotic stress. Plant Cell Environ 35:259-270.

Vornam B, Decarli N, Gailing O (2004) Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. Conserv Genet 5:561-570.

Weber P, Bugmann H, Pluess AR, Walthert L, Rigling A (2013) Drought response and changing mean sensitivity of European beech close to the dry distribution limit. Trees 27:171-181

Williams AP, Allen CD, Macalady AK, Griffin D, Woodhouse CA, Meko DM, Swetnam TW, Rauscher SA, Seager R, Grissino-Mayer HD, Dean JS, Cook ER, Gangodagamage C, Cai M, McDowell NG (2013) Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change 3:292-297.

3.6. Declaration

The following work in this chapter has been conducted by Ngoc Quynh Nguyen

- Sample harvest
- Relative Electrolyte Leakage measurement
- Leaf specific area measurement
- Molecular analysis
- Statistical analysis

The manuscript was written by Ngoc Quynh Nguyen, Caroline Carsjens and Andrea Polle

All authors commented on the final version.

Table S3.1: Target genes and primers used for the analysis of beech (*Fagus sylvatica*) leaves.

Gene name	Accession no.	Forward primer	Reverse primer	Tm	Primer efficiency
Actin	AM063027	AGAGATTCCGTTGCCCAGAA	TGGATTCCAGCAGCTTCCA	57°C	1.93
PP2C	AJ277743	GGAGGTGCAAGAGTGGAGAG	AGTCTGGACGTCGCATCTG	59°C	1.88
GAT	Fs_Pc_009_C08	AAGGCTCAACAGCATTCCAC	TCAGCTATTGTGAGTCCCACTG	60°C	1.91
ALDH	FR774766	ACGAGGTGATACGAAGAGCAAAT	CGTGTCAAAGTGTTAGCAGTGTC	59°C	1.90
APX1	FR774767	ATGCCTGAGGATTTGAGGAACA	AAGAGGGCGGAAGACGG	58°C	2.00
ERD	FR775803	CCTCGTCAAGTCCTCACCT	GGATCGTCAATATCGGGAAAGT	59°C	1.83
CuZnSOD	AJ586519	TTATCGGAAGGGCTGTTGTTG	GGCCACCAGCATTTCCAGT	59°C	1.92
NCED	DQ787262	GCAACCTATGTCTCCCGCTATG	GAATAATCCAAACAGCCCCTTGA	59°C	1.95

Table S3.2: Spearman Rank Correlations of the genes studied in 5 progenies. Lower part: Correlation coefficients (black), upper panel: p-values (red: significant), blue = insignificant).

	PP2C	ERD	ALDH	SOD	GAT	APX	NCED
PP2C		0.033	0.171	0.051	0.060	0.049	0.804
ERD	0.396		0.000	0.000	0.000	0.010	0.007
ALDH	0.254	0.712		0.000	0.000	0.058	0.000
SOD	0.362	0.781	0.841		0.000	0.002	0.002
GAT	0.350	0.693	0.952	0.877		0.040	0.000
APX	0.366	0.479	0.352	0.565	0.381		0.282
NCED	-0.046	0.505	0.796	0.571	0.675	0.200	

Table S3.3: Principal component analyis all measuring parameters after 4 weeks, 9 weeks and 16 weeks of drought treatment.

4 weeks

PC	Eigenvalue	% variance	cumulative variance	loadings	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12
1	4.878	40.65	**40.7**	PRE	0.696	0.298	-0.112	-0.064	0.209	-0.450	0.327	0.048	0.220	0.068	-0.043	-0.013
2	1.573	13.11	53.8	SWC	-0.282	0.447	-0.502	0.060	0.536	0.353	-0.112	0.157	0.118	-0.048	0.019	-0.014
3	1.562	13.02	66.8	LA	-0.002	0.611	0.536	0.446	-0.026	0.250	0.227	-0.018	-0.072	0.134	-0.029	0.039
4	0.957	7.97	74.8	PP2C	0.507	0.103	0.793	0.082	0.115	-0.048	-0.105	0.075	0.111	-0.211	0.088	-0.031
5	0.844	7.03	81.8	ERD	0.702	-0.379	0.324	-0.057	0.137	0.121	-0.373	0.159	0.115	0.203	-0.061	0.007
6	0.651	5.42	87.2	ADH	0.912	-0.079	-0.254	0.103	0.102	-0.042	0.037	0.027	-0.133	0.114	0.202	0.021
7	0.561	4.68	91.9	SOD	0.934	-0.050	-0.111	0.143	0.091	0.081	0.056	0.001	-0.194	-0.045	-0.083	-0.170
8	0.469	3.91	95.8	GAT	0.917	0.175	-0.135	-0.030	0.095	-0.080	-0.112	0.080	-0.164	-0.128	-0.086	0.155
9	0.227	1.90	97.7	APX	0.556	-0.490	0.009	-0.220	-0.069	0.442	0.430	0.054	0.101	-0.055	-0.004	0.040
10	0.148	1.24	98.9	NCED	0.412	-0.052	-0.417	0.612	-0.476	0.021	-0.109	0.084	0.177	-0.051	-0.004	0.004
11	0.070	0.59	99.5	PQ4	-0.688	-0.328	0.082	0.200	0.083	-0.162	0.153	0.553	-0.102	-0.006	-0.005	0.001
12	0.059	0.49	100.0	C24	0.264	0.606	-0.033	-0.496	-0.464	0.089	-0.059	0.296	-0.014	0.025	0.022	-0.037

Table S3.3: Continued

9 weeks

PC	Eigenvalue	% variance	cumulative variance	loadings	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12
1	4.822	40.19	40.2	PRE	0.764	0.172	0.310	-0.090	-0.163	0.256	-0.117	0.300	0.177	-0.198	-0.118	-0.043
2	1.750	14.58	54.8	SWC	-0.255	0.838	0.210	0.204	-0.082	0.067	0.054	-0.187	0.284	0.073	0.107	0.024
3	1.476	12.30	67.1	LA	-0.209	0.120	0.817	-0.086	0.311	0.153	0.324	0.121	-0.136	0.095	-0.018	0.010
4	0.890	7.42	74.5	PP2C	0.377	-0.706	0.098	0.215	0.042	0.492	-0.117	-0.098	0.102	0.121	0.080	0.077
5	0.742	6.18	80.7	ERD	0.698	-0.218	-0.058	0.372	-0.232	-0.004	0.475	-0.161	-0.007	-0.082	-0.054	-0.096
6	0.646	5.39	86.1	ADH	0.828	0.359	-0.108	0.274	-0.069	-0.059	0.022	0.081	-0.157	-0.015	-0.042	0.237
7	0.515	4.29	90.3	SOD	0.836	0.091	0.235	-0.139	0.204	-0.023	-0.109	-0.196	-0.113	-0.216	0.256	-0.028
8	0.386	3.22	93.6	GAT	0.840	0.212	0.087	0.103	-0.255	-0.053	-0.169	0.135	-0.134	0.271	0.092	-0.129
9	0.264	2.20	95.8	APX	0.662	-0.109	-0.334	0.090	0.498	-0.228	0.145	0.233	0.210	0.063	0.083	-0.011
10	0.217	1.80	97.6	NCED	0.521	0.451	-0.436	-0.160	0.341	0.331	-0.030	-0.191	-0.074	0.059	-0.183	-0.060
11	0.192	1.60	99.2	PQ4	-0.618	0.231	-0.518	0.016	-0.095	0.379	0.187	0.233	-0.105	-0.066	0.197	-0.017
12	0.101	0.84	100.0	C24	0.563	-0.082	-0.109	-0.714	-0.268	-0.006	0.237	-0.053	0.085	0.096	0.047	0.074

Table S3.3: Continued

16 weeks

PC	Eigenvalue	% variance	cumulative variance	loadings	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12
1	5.048	42.06	42.1	PRE	0.726	0.335	0.306	0.087	-0.353	-0.083	0.025	-0.141	-0.319	0.033	-0.019	-0.061
2	2.117	17.65	59.7	SWC	-0.351	0.578	0.500	0.313	0.221	0.020	-0.255	0.272	-0.052	0.017	0.055	-0.013
3	1.296	10.80	70.5	LA	-0.172	0.761	-0.080	-0.063	-0.350	0.312	0.365	0.121	0.095	0.040	0.017	0.034
4	0.886	7.39	77.9	PP2C	0.525	-0.142	-0.574	0.390	-0.357	-0.103	-0.180	0.202	-0.018	-0.102	-0.007	0.029
5	0.718	5.99	83.9	ERD	0.838	-0.386	0.056	0.196	-0.085	0.104	-0.041	-0.027	0.128	0.189	0.187	-0.023
6	0.617	5.14	89.0	ADH	0.875	-0.094	0.348	-0.069	0.108	0.102	0.112	-0.022	-0.047	-0.183	0.111	0.132
7	0.524	4.37	93.4	SOD	0.816	0.443	0.022	-0.165	0.013	0.047	-0.137	-0.053	0.214	-0.143	0.007	-0.144
8	0.269	2.24	95.6	GAT	0.865	0.196	0.064	-0.077	0.031	0.233	-0.303	-0.042	0.065	0.103	-0.174	0.098
9	0.238	1.98	97.6	APX	0.607	0.311	-0.092	-0.457	0.043	-0.529	0.042	0.161	0.014	0.081	0.030	0.036
10	0.121	1.01	98.6	NCED	0.576	-0.483	0.437	0.243	0.026	-0.104	0.339	0.165	0.099	0.001	-0.144	-0.042
11	0.103	0.86	99.5	PQ4	0.283	0.589	-0.284	0.515	0.329	-0.207	0.183	-0.196	0.052	0.018	-0.009	0.032
12	0.062	0.52	100.0	C24	0.658	-0.057	-0.456	-0.121	0.405	0.302	0.133	0.148	-0.207	0.026	-0.002	-0.061

Chapter 4: Drought avoidance and drought tolerance: evidence for intraspecific variation in juvenile beech (*Fagus sylvatica* L.)

Ngoc Quynh Nguyen[1, 2], Andrea Polle[1], Rodica Pena[1*]

(Submitted to Journal: Frontiers in Plant Sciences; the manuscript is being reviewed)

[1]Forest Botany and Tree Physiology, Büsgen-Institute, Georg-August Universität Göttingen, Germany

[2] Hung Vuong University, Nong Trang, Viet Tri, Phu Tho, Viet Nam

[*]Corresponding author

4.1. Introduction

The current climate change-related weather extremes have a severe, negative impact on forest ecosystems (Ciais et al., 2005, Bréda et al., 2006, Lindner et al., 2010). The most recent report of the International Panel on Climate Change (IPCC, 2014) states that with "very high confidence" ecosystems are highly vulnerable to these conditions. Particularly, the scenarios emphasize the increased frequency, intensity, and duration of drought events (IPCC, 2014). European beech (*Fagus sylvatica* L.) is the most abundant and dominant tree of natural vegetation in Central Europe (Ellenberg, 1996). Its distribution is mainly determined by water availability (Ellenberg, 1988, Stojanović et al., 2013). As the direct consequence of current drought events beech forests suffer already from extensive growth restriction and tree mortality in some areas (Fang and Lechowicz, 2006, Rennenberg et al., 2006, Geßler et al., 2006, Granier et al., 2007, Zang et al., 2014). Therefore, there are concerns about the adaptedness and adaptability of beech to survive in habitats that have been forecasted to experience extensive summer drought in the future.

Plant species exhibit two main strategies to cope with drought: avoidance or tolerance (Levitt, 1972, Jones, 1993). Drought avoidance implies that the plant maintains a high water status under stress, for example by acquiring more water from the soil by increasing root growth or by minimizing the water loss by the stomatal closure (Verslues et al., 2006). Drought tolerance mechanisms involve a continuation of plant metabolic activities at a low tissue water

potential, for example, by osmotic adjustment, changes in cell wall elasticity, the activation of antioxidant defense systems, etc. (Polle and Fischer 2010, Anjum et al., 2012). The basic mechanism of either strategy involves isohydric or anisohydric stomatal regulation (Tardieu and Simonneau, 1998, McDowell et al., 2008, Skelton et al., 2015). Isohydric plants close stomata before any changes occur in plant water status, whereas anisohydric species show a slow stomatal reaction in response to a decrease in the water potential. Species that grow in habitats where only mild drought or drought events of limited duration usually occur employ a drought avoidance strategy (Verslues et al., 2006). Divergent drought resistance strategies were observed in closely related species (Ambrose et al., 2015) reflecting adaptation to the contrasting habitat conditions that each species experienced.

European beech forests cover an area with wide climatic variation from mesic to semi-dry conditions, and, therefore, intraspecific variation in drought adaptedness is expected (Pluess and Weber, 2012, Weber et al., 2012). Studies with beech provenances from different geographic origins showed that plant water status and photosynthesis were less affected by drought in provenances from drought-prone habitats than in those from mesic environments (Tognetti et al., 1995, Peuke et al., 2002, Robson et al., 2012, Sánchez-Gómez et al., 2013). Recently, Aranda et al. (2015) found that vulnerability to xylem cavitation is higher in drought-sensitive mesic beech populations than in drought-adapted populations. However, the capability of drought-adapted beech progenies to acclimate flexibly to varying water availability is questionable because cell wall elastic adjustment and the activation of molecular defenses was impeded in progenies from low- compared with those from high-precipitation habitats (Carsjens et al., 2014, Knutzen et al., 2015). Carsjens et al. (2014) speculated that beech progenies from dry habitats may have evolved different strategies to cope with low water availability than those from mesic environments.

Here, we investigated whether there is intraspecific variation in the drought resistance mechanisms employed by beech progenies from different habitats. We tested the hypothesis that beech trees originating from a low-precipitation climate show a stronger drought avoidance and beech from a relative mesic habitat adopt a stronger drought tolerance strategy than those originating from the reciprocal habitat when exposed to decreasing soil water availability. Specifically, we anticipated a prompt isohydric stomatal reaction in progenies from the low-precipitation climate and anisohydric stomatal regulation in the plants from mesic conditions since beech is known to exhibit high stomatal sensitivity to water shortage (Cano et al., 2013, Aranda et al., 2015a). Furthermore, we expected differences in drought-induced damage to

photosystem II (PSII) among the low- and higher-precipitation climate provenances because drought-avoiding trees suffer more from limited carbon assimilation than drought-tolerant tree species (Picon et al., 1996, Schwanz et al., 1996). To address these hypotheses, three beech provenances from habitats differing in precipitation, 544 mm $year^{-1}$ (low-precipitation, designed as LP), 665 mm $year^{-1}$ (intermediate-precipitation, IP), and 766 mm $year^{-1}$ (higher-precipitation, HP) were subjected to progressive drought, which lasted until the leaf predawn water potential dropped below the cavitation-inducing threshold of Ψ_{cav}= -1.9 MPa (Hacke and Sauter, 1995). Soil and plant water status, the maximum quantum yield of PSII, and stomatal conductance of control and drought-treated seedlings were regularly measured. Furthermore, transcript levels of *OST1* (open stomata 1), a protein kinase that links the guard cell reaction to the abscisic acid (ABA) signaling network (Mustilli et al., 2002, Sirichandra et al., 2010) were determined. We expected that transcriptional up-regulation of *OST1* would characterize the beech progenies with drought avoidance compared to those drought tolerance mechanisms at the molecular level.

4.2. Materials and Methods

4.2.1. Plant material

Beech (*Fagus sylvatica* L.) nuts were collected in three locations differing in long-term annual precipitation: 766 mm Unterlüss (HP), 665 mm Göhrde (IP), and 544 mm Calvörde (LP) in Lower Saxony and Saxony-Anhalt, Germany (Table 4.1). The provenances were selected to represent the gradient in long-term yearly precipitation that determines natural distribution regions of European beech (EUFORGEN, http://www.euforgen.org/distribution-maps). The three forests exhibited similar stand structure with closed canopies. The tree age was about 100 to 130 years. The soil substrates, Pleistocene fluvioglacial sands from the penultimate Ice Age (Saalian), up to 30 % sand fraction, moderate to intense podzolic Umbrisols, was similar for all the three locations (for further details see Carsjens et al., 2014). All stands originated from natural regeneration (Müller-Haubold et al. 2013). In autumn 2009, beech nuts were collected after a scheme including at least 100 mother trees per plot with about 100 nuts harvested from each tree.

Table 4.1. Location, mean annual temperature, and mean annual sum of precipitation in three beech forest stands. Mean annual climate data refer to long-term the averages from 1971 to 2000. Climate data were provided by National Climate Monitoring of Germany's National Meteorological Service (Deutscher Wetterdienst-DWD). Low precipitation (LP), intermediate precipitation (IP), high precipitation (HP).

Origin	Latitude (N)	Longitude (E)	Elevation (m)	Annual precipitation (mm)	Annual average temperature (°C)
Calvörde (LP)	52°22'	11°17'	105	544	9.1
Göhrde (IP)	53°09'	10°52'	85	665	8.7
Unterlüss (HP)	52°50'	10°19'	117	766	8.5

Beech nuts were germinated on moist filter paper at 4°C in darkness in April 2010. After five-six weeks, when the radicles of beech nuts had developed a length of 1-2 cm, the seedlings were transferred in 30-cell growing trays (Herkuplast Kubern GmbH, Ering, Germany). The substrate consisted of a mixture of sand (WOLFF & MÜLLER Baustoffe GmbH, Röderland OT Haida, Germany) and perlite (HAWITA GRUPPE GmbH, Vechta, Germany) with a ratio of 3:1 (v/v). The plants were fertilized once with a fertilizer (Wuxal Top N 12-4-6, AGLUKON, Spezialdünger GmbH& Co. Kg, Düsseldorf, Germany). After two months, on 6th and 7th July 2010, the seedlings were transferred to 2L pots containing a mixture of soil (Type N, HAWITA GRUPPE GmbH, Vechta, Germany), sand of 1.5 mm and sand of 0.8 mm particle size (WOLFF & MÜLLER Baustoffe GmbH, Röderland OT Haida, Germany) in a ratio of 4:3:1 (v/v). All beech plants were kept well-watered and maintained outdoors (Forest Botany, Georg August University, Göttingen, Germany). To control the soil water content, exposure to rain was prevented by installing a transparent roof. The light was adjusted to the requirements of beech by a shading net (mesh width of 5 mm, Mayer, Rellingen, Germany).

4.2.2. Drought treatment and harvests

From August 5th, 2010, half of the 4-month-old beech seedlings from each provenance were subjected to drought treatment by withholding water. The control plants were irrigated daily with 31.3 ml tap water that represented the mean daily precipitation in the high-precipitation site. The soil moisture was daily monitored in eight pots per treatment using a moisture meter (HH2 Moisture Meter, Delta-T Devices, Cambridge, UK).

Plants were harvested after 0, 33, 45, 47, and 60 days of withholding water. The harvests took place at the same day time from 8 to 11 a.m. At each harvest, a number of eight plants

were completely harvested. Roots were briefly washed with tap water to remove adhering soil particles. Leaves, stems, and fine and coarse roots were separated and weighed. Aliquots of all fractions were taken, immediately weighed, dried until constant mass in an oven at 60ºC for 48h and weighed again to determine the fresh and dry biomass. Leaves of the harvested plants were immediately shock-frozen in liquid nitrogen and stored at -80°C.

4.2.3. Physiological Measurements

Eight beech plants in each treatment were randomly selected for physiological measurements throughout the experiment. Leaf predawn water potential (Ψ) was regularly measured using a Scholander pressure chamber (Soil-moisture Equipment Corp., Santa Barbara, CA) (Scholander et al., 1965). A leaf of each plant was cut using a razor, then immediately was placed inside the chamber, with its petiole projecting to the exterior through the sealing port. Then, the pressure in the chamber was gradually increased until a drop of liquid appeared at the cut end of the petiole. The value revealed by the gauge of the chamber was immediately recorded. The leaves used for Ψ were weighted, and immediately plastic wrapped and further subjected to estimation of relative water content (RWC).

Leaf RWC was calculated as:

RWC (%) = [(w-dw) / (tw-dw)] x 100

where: w = sample fresh weight; tw = sample turgid weight; dw = sample dry weight

The leaves used for measuring the turgid weight were previously hydrated to full turgidity for 24h at room temperature under a constant light. Dry weight was registered after drying the leaves in the oven at 60ºC for 48h.

Chlorophyll fluorescence parameters of beech plants were regularly determined with a portable Chlorophyll Fluorometer (Mini-PAM, Walz, Effeltrich, Germany) connected to a leaf clip holder (Model 2030-B, Walz, Effeltrich, Germany). Fully expanded leaves of control and stressed plants were used for measurements of the maximum fluorescence yield (F_m) and the initial fluorescence (F_0) of PSII on the dark-adapted leaves just before dawn. From these measurements, the maximum quantum yield of PSII was calculated as described by Maxwell and Johnson (Maxwell & Johnson, 2000):

$$\Phi_{PSI} = F_v/F_m = (F_m - F_o)/F_m$$

Stomatal conductance (G_s) was measured in control and drought-stressed plants in the time interval between 1 pm and 2 pm using a transient state diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). A single leaf of a plant was randomly chosen to carry out stomatal conductance measurements.

We assessed the percentage of tree mortality by visual estimation: a tree was considered dead when all leaves were scorched, curled or cracked indicated a complete desiccation.

4.2.4. Total RNA isolation and gene expression analysis

4.2.4.1. RNA extraction from frozen beech leaves

Frozen eaves of beech seedlings were stored at -80ºC. Frozen beech leaves were ground with a mortar and a pestle in liquid nitrogen. A modified RNA extraction method was used to isolate total RNA (Chang et al., 1993) as follows:

Day 1: 800 µl CTAB extraction buffer (1 liter CTAB buffer contains 2% CTAB (hexadecyltrimethylammonium bromide), 2% PVP (polyvinylpyrrolidinone K30), 100 mM Tris-HCl (pH = 8.0), 25 mM EDTA (Ethylenediaminetetraacetic acid), 2.0 M NaCl, pH = 8.0) pre-warmed to 65ºC was added to 150 mg of the ground frozen leaves powder in a 2.0 ml plastic tube. Afterward, 16 µl mercaptoethanol was added into each tube and then mixed for 15 min at 65ºC (Thermo-mixer Comfort, Eppendorf, Hamburg, Germany). Each tube was taken out and cooled to room temperature with regular shaking for 5 min. Then, 800 µl chloroform: isomylalcohol (24:1) was added to the CTAB extraction mixture. Samples were mixed at 14000 rpm for 5 min at room temperature (5417 R, Eppendorf, Hamburg, Germany). Then, the upper phase was transferred to a new 2.0 ml tube. 800 µl chloroform: isomylalcohol (24:1) was added to each new tube. 200 µl of the volumes 10 M Lithiumchlorid (-20ºC) was added to the upper phase, mixed and kept on ice at 4ºC overnight.

Day 2: Samples were centrifuged at 14000 rpm for 20 min at 4ºC. The upper phases were discarded and the pellets were dissolved with 400 µl SSTE buffer (100 ml SSTE buffer contains 0.5% SDS (Sodium dodecyl sulfate), 10 mM Tris – HCl, 1 mM EDTA (Ethylenediaminetetraacetic acid), 1.0 M NaCl, pH = 8.0) for 10 min at 42ºC by using a Thermo-Mixer Comfort (Eppendorf, Hamburg, Germany). Samples were shortly centrifuged one more (5417 R, Eppendorf, Hamburg, Germany). Adding 400 µl chloroform: isomylalcohol (24:1) to the mixture and mixed it for 5 min at room temperature by centrifuging at 14000 rpm (5417 R, Eppendorf, Hamburg, Germany) and upper phases were transferred to new tubes.

RNA was precipitated by using 800 µl pre-cooled 96% ethanol for 60 min at -80ºC (or 2h at -20ºC) and spun down at 14000 rpm for 20 min at 4ºC. Then, 500 µl 70% ethanol was added to each tube and centrifuged at 14000 rpm for 10 min at room temperature to wash pellets and to remove residual salts. RNA was dried using a concentrator (Concentrator 5301, Eppendorf, Hamburg, Germany) for 4 min and was dissolved by adding 30 µl RNase-free water and mixing at 850 rpm for 10 min at 42ºC (Thermo - mixer Comfort, Eppendorf, Hamburg, Germany). Finally, RNA was store at -80ºC for further steps.

4.2.4.2. Evaluation of RNA concentration and purity

Total RNA yield and purity were estimated by using a Nanodrop™ 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA integrity was determined by gel-electrophoresis. Intact RNA was on a denaturing gel should have at least two bands 28S and 18S rRNA. The 28S rRNA band should be absent more than the 18S rRNA band. Degraded RNA is shown as a smear and lacks the sharp rRNA bands, or does not satisfy the 2:1 ratio of high quality RNA.

Highly degraded RNA was excluded from further analysis. RNA gel electrophoresis was carried out with the protocol of the manufacturer (Thermo Scientific, Waltham, MA, USA) as follows: 1.2 g agarose was used and dissolved in 70 ml double distilled H_2O and 10 ml 10x running buffer (1 liter running buffer contains 0.2 M MOPS (3-(*N*-morpholino) propane sulfonic acid), 50 mM Sodium acetate, 10 mM EDTA (Ethylenediaminetetraacetic acid)). Then a microwave oven was used to heat the mixture for 2 min to dissolve this mixture. After that, 10 ml of 37% (w/v) formaldehyde solution was added to the mixture under a fume hood and shaken. The mixture was poured in a prepared gel electrophoresis tray and equipped with a comb. The gel polymerized at room temperature for 15 min. The gel was placed in a tank containing 1x running buffer after removing the comb. 1.0 µl RNA extract and 1.5 µl double distilled H_2O were mixed with 2.5 µl 2 x loading buffer (1 ml loading buffer contains 660 µl formamide (deionized), 80 µl formaldehyde 37% (w/v), 140 µl nuclease-free water, 10 µl 10% bromophenol blue, 10 µl ethidium bromide) for RNA in a 1.5 ml tube. The mixture was heated for 10 min at 70ºC, then kept on ice at least 5 min and was shortly centrifuged. After loading RNA to the gel, the electrophoresis was run for 5 min at 100 V, then 40 min at 120 V under the fume hood. After that, gels were moved out and scanned using 300 nm excitation as indicated (Fluorescence-Multiimager, Bio-Rad, Munich, Germany).

4.2.4.3. DNase treatment

In order to obtain pure RNA, DNase I treatment (Turbo DNA - Free kit, Ambion, Austin, TX, USA) was applied to clean from DNA contamination as follows: 1 µg RNA, which was extracted from previous steps, was added to 2 µl of 10x Turbo DNase buffer (Turbo DNA - Free kit, Ambion, Austin, TX, USA), 1 µl of Turbo DNase (2U) (Turbo DNA - Free kit, Ambion, Austin, TX, USA) and mixed. The mixture was incubated at 37°C for 20 min and spun down for 1 min at 14000 rpm. 2 µl of resuspended DNase Inactivation Reagent (Turbo DNA - Free kit, Ambion, Austin, TX, USA) was added to the mixture and mixed. Then, the tubes were incubated for at least 2 min at room temperature and mixed 3 times during incubation. Finally, the mixture was centrifuged at 10000 rpm for 2 min and was transferred supernatant to a new tube. The RNA was stored at -80°C for further use.

4.2.4.5. Synthesis of first strand complementary DNA (cDNA)

RNA was treated with DNase I treatment (Turbo DNA - Free kit, Ambion, Austin, TX, USA) to remove DNA contamination. Subsequently, the pure RNA was used to perform cDNA synthesis using First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) as follow: 1 µg of DNA-free total RNA (was treated above) added to 1 µl Oligo (dT) (1.5 µg/µl) primers and nucleic acid free water was added to reach a volume of 11 µl. The mixture was incubated at 65°C for 5 min and cooled on ice. 4 µl of 5x Reaction buffers (Thermo Scientific, Waltham, MA, USA) was added to the mixture (1 µl RiboLock™ Ribonuclease Inhibitor (20U/µl) (Thermo Scientific, Waltham, MA, USA) and 2 µl dNTP mix (10 mM)), well mixed. The mixture was incubated at 37°C for 5 min and 2 µl Reverse Transcriptase M-MuLV RT (200 U/µL) (Thermo Scientific, Waltham, MA, USA) was added. The final mixture was incubated at 37°C for 1 hour and 70°C for 5 min in a PCR machine (GeneAmp PCR System 9700, A&B Applied Biosystems, Thermo Scientific, Waltham, MA, USA). 10 times of Nuclease – Free Water was added to the cDNA for dilution and stored at -80°C in the fridge.

4.2.4.6 Quantitative real time PCR (qRT-PCR)

Five micro liters of 1:10 diluted cDNA were used for quantitative real-time PCR (qRT-PCR) in a LightCycler®480 (Roche, Mannheim, Germany). The master mix for amplification reaction was prepared with 10 µl of SYBR Green I Master (Roche), 1 µl of a pair of forward and reverse primers and 3 µl of ddH_2O. The PCR program was same as described by Carsjens et al. (2014). Each sample was measured twice; three samples were measured per treatment.

The primer sequences for the housekeeping gene (Actin: 5'-AGAGATTCCGTTGCCCAGAA-3' and 3'-TGGATTCCAGCAGCTTCCA-5') was obtained from (Olbrich et al., 2008). The cDNA sequences of Fagus OST1 (FR612317) were obtained from NCBI, http://www.ncbi.nlm.nih.gov/genbank. Specific primers (5'-GGAGTGGCAAGGCTTATGAG-3' and 3'-TGGGATGCCTCAATGACCTG-5') for qRT-PCR were designed for Fagus OST1 with the programs Oligo Explorer (Gene Link, Hawthorne, NY, USA). The designed primers were tested by using Oligo Analyzer (Both Gene Link, Hawthorne, NY, USA) for checking melting temperature (T_m), primer dimers, and primers loops. Primers were obtained from Microsynth (Microsynth Austria GmbH, Vienna, Austria). A 5-fold dilution series was applied to determine the primer real-time PCR efficiencies. These values were 2.0 for actin and 1.89 for OST1. OST1 transcript level was normalized to actin as the housekeeping gene according to the following equation: $RE = E^{(Ct\,(Actin) - Ct(Target)}$, where *E* is the primer efficiency, Ct is the cycle threshold, RE is the relative expression (Pfaffl, 2004).

4.2.5. Data analyses

Statistical data analysis was carried out with the software R 3.1.2 (the R Project for Statistical Computing www.r-project.org). Normal distribution was tested with the Shapiro – Wilk's test and homogeneity of variances were tested with Levene's test. Where necessary, data were transformed to fulfill to assumptions of normality and homogeneity of variance. After testing for normality (skewness, kurtosis), multi-factor ANOVA analysis was performed to determine the significance of the main variables provenance, time and treatment, and interactions between them. Data obtained from the same individual plant measured at distinct time points were subjected to repeated measures ANOVA, where the individual plant were considered a random factor and Provenance (P), Treatment (D), and Time (T) were fixed factors. When the ANOVA revealed significant differences among the means with the $P < 0.05$, a posthoc test (Tukey HSD) was performed. A t-test was performed to test the differences of ecophysiological data between control and drought-stressed plants at each harvest. Graphs were generated using Origin Pro Lab 8.5 (OriginLab Corporation Northampton, USA).

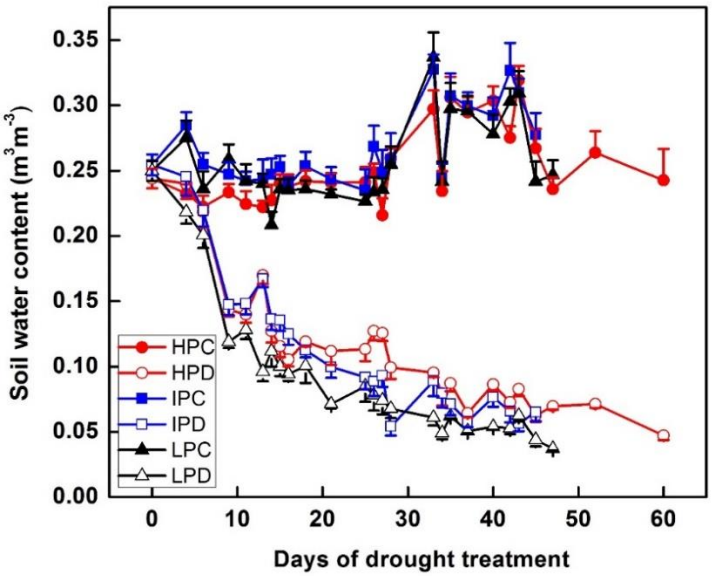
4.3. Results

4.3.1. Juvenile beech dry matter remains unaffected by drought

A significant decline in soil water content (SWC) was observed after a 10-day period of withholding water (Fig. 4.1). In the control treatment, SWC was maintained constant at 0.259

± 0.030 m^3m^{-3} throughout the experiment, with no significant differences among the provenances (P = 0.526, F = 0.644). In the drought treatment, SWC progressively decreased relative to controls (P < 0.001, F = 51.913) by about 67% after four weeks and 80% after six weeks (Fig. 4.1). The provenance had a significant effect on SWC in drought treatment (P < 0.001, F = 102.106) with the lowest SWC values found in LP (Fig. 4.1).

No significant differences in plant biomass were found among the provenances either in well-watered or among drought-treated plants (P = 0.376, F = 0.983). The drought treatment did not lead to reduction in biomass (Table 4.2) or affected its allocation between the shoot and root compartments (root-to-shoot ratio of HP, IP, and LP: 1.62 ± 0.4, 1.63 ± 0.38, 1.56 ± 0.45, P = 0.996, F = 0.001). However, the IP provenances died earlier than HP and LP provenances (Table 4.2).



Variables	*F*-Ratio	*P*-Value
Provenance	19.219	< **0.001**
Time	48.047	< **0.001**
Treatment	7786.092	< **0.001**
Provenance* Time	0.886	0.686
Time * Treatment	51.913	< **0.001**

Figure 4.1. Soil water content (SWC) at ten cm-depth in pots of well-watered controls (C) and drought-treated (D) young beech (*Fagus sylvatica* L.) trees. Beech nuts were collected in locations differing in mean annual precipitation: 766 mm Unterlüss (HP), 665 mm Göhrde (IP), and 544 mm Calvörde (LP) and exposed to drought. Symbols indicate means ± SE (n=8). *F*- and *P*-values (repeated measures ANOVA analysis) are shown next to the figure. *P*-values < 0.05 are shown by bold letters.

4.3.2. The plant water status under drought treatment varies with the provenance

Plant water status was characterized by the leaf relative water content (RWC) and leaf predawn water potential (Ψ). Both RWC and Ψ declined with proceeding drought exposure (P < 0.001, Time*Treatment, Fig. 4.2). Overall, in the drought-treated plants, RWC was significantly reduced compared with the RWC in well-watered plants (P < 0.001, F= 274.544, Fig. 4.2A). Control plants maintained a constant RWC of 85 % throughout the duration of the

experiment (P = 0.450, F = 1.089, Fig. 4.2A). The RWC decline started immediately after withholding irrigation (Fig. 4.2A). Within a 14-day period of water withholding, RWC significantly decreased relative to controls in the HP (P = 0.001, t-test) and IP (P = 0.003, t-test) provenances while the drought-treated LP provenance showed a reduced RWC only after 25 days of water withholding (Fig. 4.2A). The latter provenance maintained a high RWC of 75% throughout 45 days of drought. The strongest constraints of drought on RWC were registered in the IP provenance with values, which dropped below 70% already 16 days after withholding irrigation (Fig. 4.2A). The HP provenance showed a slow RCW decrement reaching values of about 75% to 60 % after a 50-day period of withholding water.

Withholding water resulted in a massive drop of Ψ, closely matching the trends observed in RWC changes under drought treatment (Fig. 4.2). The well-watered plants showed constant Ψ values of -0.328 ± 0.003 MPa throughout the experiment (Fig 4.2B). The drought treatment strongly lowered Ψ in the treated compared with control plants (P < 0.001, F= 1679, Fig. 4.2B).

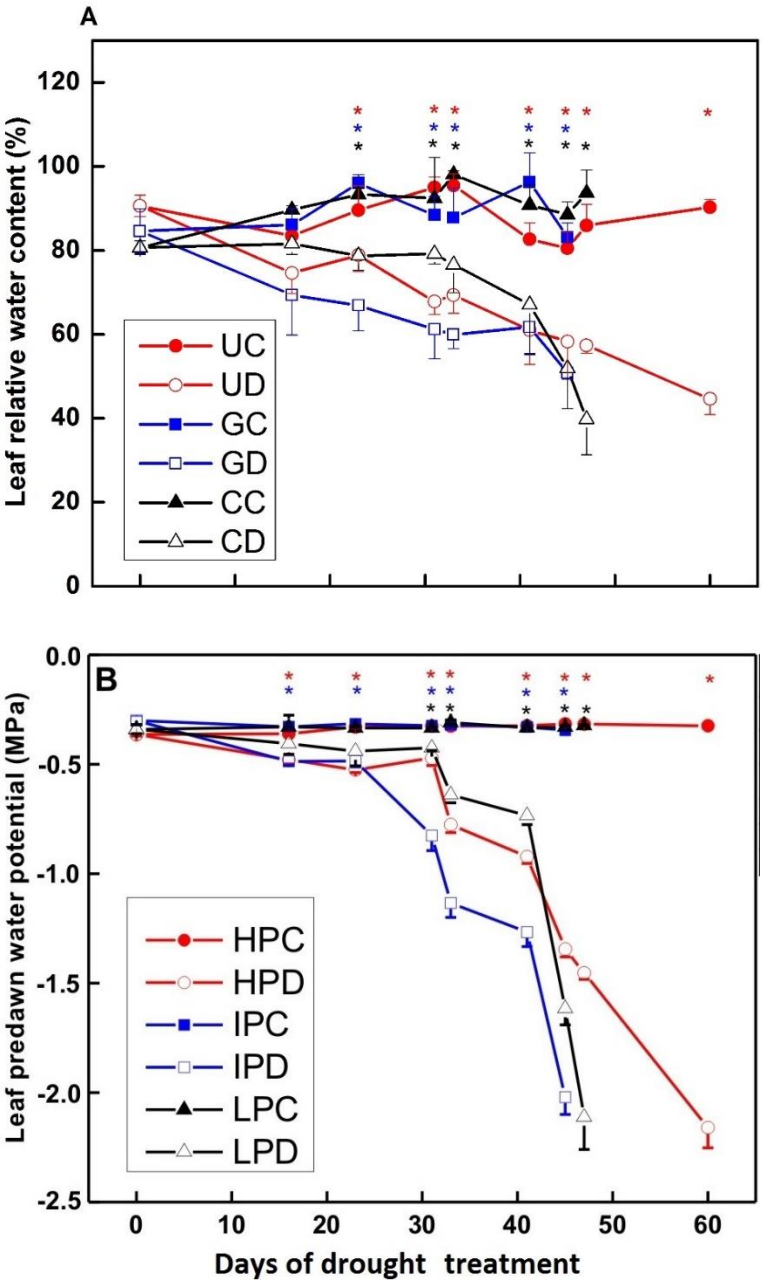
Within a 14-day drought period, Ψ significantly decreased in HP (P < 0.001, t-test) and IP (P < 0.001, t-test) provenances compared with Ψ in well-watered plants. The LP provenance maintained Ψ values similar to those of control plants for a 31-day period after drought inception (Fig. 4.2B). The provenances showed not only differences among the time points when Ψ declined, but also in the magnitude of the drought effect. The strongest Ψ decline was found in the HP provenance, which suffered a sharp Ψ drop from -0.445 ± 0.026 MPa to - 0.825 ± 0.07 MPa within a 23-day drought period. At this point time, the LP and IP provenances still maintained Ψ above -0.466 ± 0.063 MPa (Fig. 4.2B). When the predawn water potential dropped below the cavitation-inducing threshold of Ψcav = -1.9 MPa, the trees are susceptible to hydraulic failure and consequently death. The decline in the predawn water potential was in the order HP (lowest), LP and IP (highest).

Table 4.2. Biomass of the well-watered and drought-treated beech (*Fagus sylvatica*) plants throughout drought experiment. Beech (*Fagus sylvatica*) nuts provenances were collected in locations differing in mean annual precipitation: 766 mm Unterlüss (HP), 665mm Göhrde (IP), and 544 mm Calvörde (LP) and exposed to drought.

Drought time		0 days	33 days	45 days	47 days	60 days
Provenance	Treatment	Dry biomass (g plant^{-1})				
HP	Control	1.376 ± 0.070	1.574 ± 0.102	2.190 ± 0.091	2.077 ± 0.088	1.811 ± 0.101
	Drought	-	1.807 ± 0.082	1.987 ± 0.084	1.901 ± 0.064	2.103 ± 0.117
IP	Control	1.560 ± 0.134	1.584 ± 0.071	1.806 ± 0.066	na	na
	Drought	-	1.792 ± 0.063	1.689 ± 0.076	pd	pd
LP	Control	1.237 ± 0.104	1.831 ± 0.131	1.850 ± 0.114	2.132 ± 0.097	na
	Drought	-	1.760 ± 0.077	1.596 ± 0.072	1.921 ± 0.076	pd

Significances (multi-factor ANOVA analysis)*: Provenance ($F = 0.983$, $P = 0.376$); Treatment ($F = 0.001$, $P = 0.997$); Time ($F = 7.058$, $P < 0.001$)

A number of days in parenthesis represent the length of the drought stress. Values represent the means ± standard errors ($8<n<9$).
pd, plants died by 100% during drought stress.
na, data not available
* Calculated for the total plant biomass for the variables: Provenance, Treatment and Time.



Variables	*F*-Ratio	*P*-Value
Provenance	3.249	**0.042**
Time	10.315	< **0.001**
Treatment	274.544	< **0.001**
Provenance* Time	1.429	0.153
Time* Treatment	7.830	< **0.001**

Variables	*F*-Ratio	*P*-Value
Provenance	7.010	**0.001**
Time	33.951	< **0.001**
Treatment	1679.011	< **0.001**
Provenance* Time	3.379	< **0.001**
Time* Treatment	42.408	< **0.001**

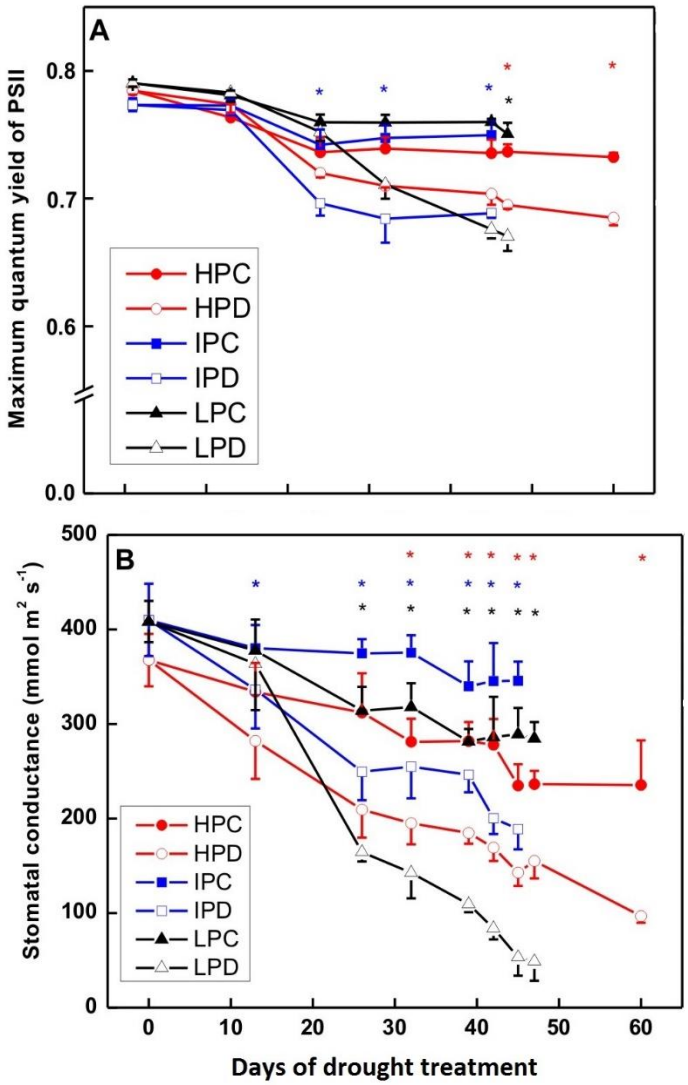
Figure 4.2. Relative leaf water content (RWC, A) and predawn leaf water potential (Ψ, B) of young beech (*Fagus sylvatica* L.) trees exposed to drought by withholding water (D) and well-watered control plants (C). Beech nuts provenances were collected in locations differing in mean annual precipitation: 766 mm Unterlüss (HP), 665 mm Göhrde (IP), and 544 mm Calvörde (LP) and exposed to drought. Symbols indicate the means ± SE standard errors (n=5). F- and P-values (multi-factor ANOVA analysis) are shown next to the figure. P-values < 0.05 are shown by bold letters. Asterisks indicate significant differences (t-test, P < 0.05) between means of the control and drought-treated plants.

4.3.3. Minor injury to PSII performance under drought treatment

To investigate whether drought treatment caused photo-inhibitory damage, we determined the maximum photochemical efficiency of PSII (Φ_{PSII}) in dark-adapted leaves of control and drought-treated beeches. Control plants showed a slight Φ_{PSII} variation of 3 to 7%. These alterations might indicate fluctuations in environmental conditions because the plants were kept outdoors. The impairment of Φ_{PSII} by drought was about 10% in comparison with controls ($P < 0.001$, $F = 95.030$, Fig. 4.3A). The three provenances varied in the ΦPSII response to drought (Fig. 4.3A). The IP provenance, which showed a depressed plant water status, was the first among the three provenances displaying reduced Φ_{PSII} relative to control plants after a 25-day period of drought treatment (Fig. 4.3A). At the same time, the HP provenance maintained Φ_{PSII} levels similar to those found in control plants for a 47-day period of drought treatment. The LP provenance experienced, in comparison to control plants, a 6% drop in Φ_{PSII} after a 33-day drought treatment period. In contrast to IP and HP provenances, which maintained a fairly stable Φ_{PSII} after the initial decrement, Φ_{PSII} decreased continuously in the LP provenance; when the LP plants reached Ψ_{cav} ΦPSII was 10% lower than that of controls (Fig. 4.3A).

4.3.4. Stomatal responses to drought vary with the provenance

Stomatal conductance (g_s) was regularly measured in drought-treated and well-watered plants. Control plants showed significant changes in stomatal conductance among provenances ($P < 0.001$, $F = 16.396$) and among the measuring days ($P < 0.001$, $F = 4.619$, Fig. 4.3B). In the drought-treated plants, g_s significantly decreased compared with control plants ($P < 0.001$, $F = 321.980$) with a high variation among the provenances (Fig. 4.3B). The LP provenance showed an abrupt decline in g_s between 14 and 20 days of drought and thereafter steadily continued to decrease (Fig. 4.3B). When Ψ_{cav} was reached in LP plants, g_s dropped almost to zero. In contrast to LP, the stomatal conductance decreased more slowly in the HP provenance and about 30% of the initial g_s was maintained almost until the end of treatment (Fig. 4.3B). At the time when Ψ_{cav} was reached, the HP provenance had reduced its stomatal conductance only by 59% (Fig. 4.3B). The reduction of g_s was least pronounced in the IP provenance among the three progenies in response drought (Fig. 4.3B). At the time when Ψ_{cav} was reached, the LP provenance still showed about 50% of the initial stomatal conductance (Fig. 4.3B).



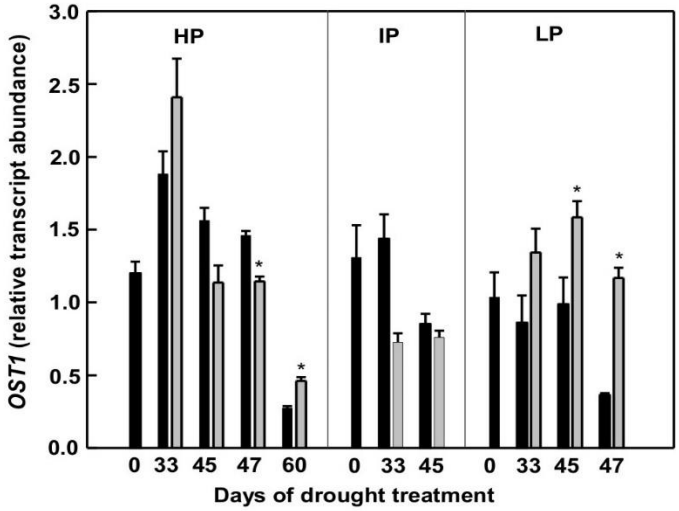
Variables	*F*-Ratio	*P*-Value
Provenance	9.100	**0.001**
Time	43.400	< **0.001**
Treatment	95.030	< **0.001**
Provenance* Time	1.210	0.286
Time* Treatment	9.670	< **0.001**

Variables	*F*-Ratio	*P*-Value
Provenance	59.550	< **0.001**
Time	49.320	< **0.001**
Treatment	321.980	< **0.001**
Provenance* Time	3.190	**0.001**
Time* Treatment	11.890	< **0.001**

Figure 4.3. Photochemical efficiency of PSII (Φ_{PSII}, A) in dark-adapted leaves and stomatal conductance (g_s, B) in young beech (*Fagus sylvatica* L.) exposed to drought by withholding water (D) and in well-watered and well-watered control plants (C). Beech (*Fagus sylvatica*) nuts provenances were collected in locations differing in mean annual precipitation: 766 mm Unterlüss (HP), 665 mm Göhrde (IP), and 544 mm Calvörde (LP) and exposed to drought. Symbols indicate the means ± SE standard errors (n=8). F- and P-values (repeated measures ANOVA analysis) are shown next to the figure. P-values < 0.05 are shown by bold letters. Asterisks indicate significant differences (t-test, P < 0.05) between means of the control and drought-treated plants.

4.3.5. OST1 gene expression

To catch a glimpse of the molecular sensing and signaling in stomatal guard cells that might explain the variation in stomatal adjustment among the three provenances, *OST1* was analyzed. OST1 functions as a positive regulator of ABA-induced stomatal closure (Schroeder et al. 2001). The expression of *OST1* showed a large variation among the three beech provenances, in both well-watered and drought-treated plants ($P < 0.001$, $F = 31.365$, Fig. 4.4). In the LP provenance, drought treatment induced a significant increase in the *OST1* transcript levels by 160% and 322% relative to control plants after a 45-day and 47-day period of drought, respectively (Fig. 4.4); in the same period, stomatal conductance decreased by 82% (Fig 4.3B). The HP provenance showed a significant *OST1* up-regulation under drought treatment compared with control plants only at the time point of Ψ_{cav} while an *OST1* down-regulation occurred before, at 47-day of drought (Fig. 4.4). *OST1* transcript abundance remained unchanged in drought-treated and well-watered plants of the IP provenance throughout the experiment (Fig. 4.4). The interaction between the factors Drought*Time was not significant ($P = 0.447$, $F = 0.774$) indicating that the *OST1* expression level under drought stress was less dependent on the time scale and subsequently drought severity (Fig. 4.4).



Variables	*F*-Ratio	*P*-Value
Provenance	31.365	< **0.001**
Time	36.416	< **0.001**
Treatment	2.538	0.118
Provenance* Time	11.471	< **0.001**
Time* Treatment	0.447	0.774

Figure 4.4. Relative transcript abundance of *OST1* in leaves of young beech (*Fagus sylvatica* L.) trees of three provenances grown under control (black bars) and drought treatments (grey bars). Beech nuts provenances were collected in locations differing in mean annual precipitation: 766 mm Unterlüss (HP), 665mm Göhrde (IP), and 544 mm Calvörde (LP) and exposed to drought. Symbols indicate the means ± SE standard errors (n=5). F- and P-values (multi-factor ANOVA analysis) are shown next to the figure. P-values < 0.05 are shown by bold letters. Asterisks indicate significant differences (t-test, P < 0.05) between means of the control and drought-treated plants.

4.4. Discussion

4.4.1. Beech provenances differ in drought resistance strategies

The main question addressed in this study was whether there is intraspecific variation in the drought resistance mechanisms employed by beech progenies from different habitats. The beech populations from which the progenies of the current study originated show very high genetic variation within (97%) and very little variation (3%) among the populations (Carsjens et al., 2014). This genetic structure with high within- and low among-population variation is typical for beech (Jump et al., 2006, Pluess and Weber, 2012). Therefore, it was unclear if the variation in drought adaptedness of different genotypes within the population was higher than the differences between the populations. The strongly diverging drought responses among these populations, which we detected here for stomatal conductance and those found in earlier studies for antioxidative systems, drought-signaling related genes (Carsjens et al., 2014) and cell wall elasticity (Knutzen et al., 2015) indicate that high genetic relatedness does not preclude local adaptation.

Recently, the concept classifying drought responses of species according to their stomatal behavior as isohydric or anisohydric functional types has been advanced (Brodribb and McAdam, 2013). Isohydric and anisohydric stomatal behavior indicate divergent drought resistance mechanisms. Our data support that the within-species drought responses of beech can also vary between isohydric or anisohydric stomatal behavior. The beech provenance LP from the dry habitat exhibited an isohydric phenotype because the plants showed more rapid stomatal closure and maintained higher leaf RWC and predawn water potentials than those from mesic conditions. Thereby, the population from the dry habitat clearly displayed a drought avoidance strategy. In contrast, the HP progenies showed a slow decline in stomatal conductance, but a stronger fall in the predawn water potential upon water limitation. These findings suggest that the progenies from mesic conditions displayed an anisohydric functional type and a drought tolerance strategy. The latter suggestion is also supported by the finding that the HP provenances activated antioxidant systems (Carsjens et al., 2014) and exhibited higher cell wall elasticity (Knutzen et al., 2015) under drought than the LP provenances.

The observation that beech provenances from different areas show differences in stomatal responses to drought agrees with previous findings (Tognetti et al., 1995, Peuke et al., 2002, Sánchez-Gómez et al., 2013, Knutzen et al., 2015). Therefore, it is possible that intraspecific variation from aniso- to isohydric functional types forms a continuum in beech,

similar to that reported for different forest tree species (Klein, 2014). This suggestion is speculative and should be investigated in future.

Furthermore, the genetic basis of intraspecific variation of isohydric or anisohydric functional types in beech is currently unknown. Differences in *OST1* (this study), *ERD* and *PP2C* expression (Carsjens et al., 2014) suggest that differences in ABA regulation, which are responsible for the between-species variation of stomatal behavior (Brodribb et al., 2014), could also lead to divergent drought resistance mechanisms among the beech progenies. We found lower *OST1* relative transcript abundance in the mesic than in the xeric provenance, but the up-regulation occurred exclusively under severe drought stress, at the time when stomatal closure reached the maximum value while before no pattern was observed. Overall, our results demonstrate that variation in the mechanism of drought responsiveness of stomatal behavior exists within an important forest tree species, even in the absence of strong genetic divergence among populations. Likewise, the reliance of stomatal regulation mechanism on drought intensity and duration, transcription levels of ABA-related genes were also impacted by drought characteristics and seasonality (Carsjens et al., 2014).

4.4.2. Fitness of beech provenances in relation to drought avoidance and drought tolerance

An important question is whether drought avoidance or tolerance mechanisms afford higher fitness to young beech trees. Among the fitness traits studied here Φ_{PSII} and biomass loss, which integrate the negative impact of drought on the primary processes of photosynthesis and growth, were not useful to answer this question. Only severe stress indicated by water potentials below -1.7 to 1.9 MPa (Hacke and Sauter, 1995, Leuzinger et al., 2005) resulted in a moderate decline in Φ_{PSII}. High resistance of Φ_{PSII} to drought has also been found in other studies with beech (García-Plazaola and Becerril, 2000, Valladares et al., 2002). Similarly, no differences in Φ_{PSII} were found among drought-avoiding and drought–tolerant tree species in Mediterranean climate (Martínez-Ferri et al., 2000). These observations underpin high tolerance of the primary processes of photosynthesis to drought across beech provenances from a large precipitation gradient.

Young beech trees display a high plasticity on growth and biomass allocation under low water availability (Tognetti et al., 1995, Meier and Leuschner, 2008, Schall et al., 2012), but here we found no drought influence, either on growth or biomass allocation. The reason for this finding is that beech has a determinate growth pattern, where the main growth phase of the

leader shoot occurs early in the season (Heilmann-Clausen et al., 2007). Drought applied after termination of leader shoot growth, as in the present study, has no effect on whole plant biomass of young, drought-exposed beech trees (this study, Peuke et al., 2002, Knutzen et al., 2015).

Once the soil became exhausted of available water, the severity of drought overcame the plant capacity to maintain their water content above the lethal threshold. Hydraulic failure was probably the primary cause of mortality since production of new xylem or repair of embolized xylem under the progressive severe drought are less possible (McDowell et al., 2008). The provenances were distinguished by the time point when they reached Ψ_{cav}. According to this, the fitness decreased in the order HP > LP ≥ IP. This result is in an apparent contrast to other studies showing that beech from drought-prone habitats perform better under drought that beeches from mesic climates (Peuke et al., 2002, Robson et al., 2012, Thiel et al., 2014, Aranda et al., 2015), though some exceptions have been noted (Peuke et al., 2002, Baudis et al., 2014). Here, the HP provenance with the longest time maintaining Ψ above Ψ_{cav} came from the site with the highest precipitation, whereas LP from the lowest and IP from an intermediate precipitation level suddenly dropped to Ψ_{cav}. The evolutionary history of the beech forests in these areas is not entirely clear but is unlikely that entirely unadapted beech trees would have survived for more than 100 years. In general, resident populations have an advantage of higher fitness under their local environmental conditions than plants originating from other habitats (Kawecki and Ebert, 2004). Under the current climatic conditions very long-lasting drought periods of more than six weeks during summer, which have been applied in our study, are unlikely. Therefore, drought avoidance, which instantaneously protects the water status of the more isohydric plants, might be a necessary safety strategy for beech trees often exposed to moderate drought because the metabolic costs incurred by this behavior are probably low, and maintenance of the water status has high priority. In contrast, beech trees from mesic climate, where drought events are rare, tolerate a moderate decline in their water status but activate other metabolic protection measures(Carsjens et al., 2014, Knutzen et al., 2015), whose production and maintenance may be more costly. Therefore, the beech progenies might be well adapted to the current climatic conditions. Based on the results of this study, we suggest that the anisohydric functional type of beech is better endowed to cope with the predicted future climate extremes than the isohydric type.

In conclusion, beech exhibited intraspecific variation in drought resistance strategies characterized by anisohydric or isohydric behavior. In future studies, it will be important to investigate the magnitude of variation between these functional types, their presence in matures

trees and the underlying molecular mechanisms. The latter is critical for the development of marker genes to distinguish these functional types at an early stage. The finding of a higher adaptedness of the functional tolerance type to severe drought must also be tested in mature beech trees and related to their hydraulic architecture.

4.5. References

Ambrose AR, Baxter WL, Wong CS, Næsborg RR, Williams CB, Dawson TE (2015) Contrasting drought-response strategies in California redwoods. Tree Physiol: tpv016.

Anjum SA, Farooq M, Xie X, Liu X, Ijaz MF (2012) Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. Sci Hortic 140:66–73.

Aranda I, Cano FJ, Gascó A, Cochard H, Nardini A, Mancha JA, López R, Sánchez-Gómez D (2015) Variation in photosynthetic performance and hydraulic architecture across European beech (*Fagus sylvatica* L.) populations supports the case for local adaptation to water stress. Tree Physiol 35:34–46.

Baudis M, Ellerbrock RH, Felsmann K, Gessler A, Gimbel K, Kayler Z, Puhlmann H, Ulrich A, Weiler M, Welk E, Bruelheide H (2014) Intraspecific differences in responses to rainshelter-induced drought and competition of *Fagus sylvatica* L. across Germany. For Ecol Manag 330:283–293.

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann For Sci 63:20.

Brodribb TJ, McAdam SAM (2013) Abscisic Acid Mediates a Divergence in the Drought Response of Two Conifers. Plant Physiol 162:1370–1377.

Brodribb TJ, McAdam SAM, Jordan GJ, Martins SCV (2014) Conifer species adapt to low-rainfall climates by following one of two divergent pathways. Proc Natl Acad Sci 111:14489–14493.

Cano FJ, Sánchez-Gómez D, RodríGuez-Calcerrada J, Warren CR, Gil L, Aranda I (2013) Effects of drought on mesophyll conductance and photosynthetic limitations at different tree canopy layers: Limitations to carbon uptake into the canopy. Plant Cell Environ 36:1961–1980.

Carsjens C, Ngoc QN, Guzy J, Knutzen F, Meier IC, Müller M, Finkeldey R, Leuschner C, Polle A (2014) Intra-specific variations in expression of stress-related genes in beech progenies are stronger than drought-induced responses. Tree Physiol: tpu093.

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Report 11:113–116.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide

reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533.

Ellenberg H (1988) Vegetation Ecology of Central Europe. Cambridge University Press.

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5th edn. Ulmer, Stuttgart, Germany.

Fang J, Lechowicz MJ (2006) Climatic limits for the present distribution of beech (*Fagus* L.) species in the world. J Biogeogr 33:1804–1819.

García-Plazaola JI, Becerril JM (2000) Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. Trees 14:485–490.

Gessler A, Keitel C, Kreuzwieser J, Matyssek R, Seiler W, Rennenberg H (2007) Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21:1–11.

Granier A, Reichstein M, Bréda N, Janssens IA, Falge E, Ciais P, Grünwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Köstner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala T, Wang Q (2007) Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. Agric For Meteorol 143:123–145.

Hacke U, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica* f. purpurea and *Populus balsamifera*. J Exp Bot 46:1177–1183.

Heilmann-Clausen J, Bradshaw RHW, Emborg J, Hannon G (2007) The History and Present Conditions of Suserup Skov: A Nemoral, Deciduous Forest Reserve in a Cultural Landscape. Ecol Bull: 7–17.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jones, H. G. (1993) Drought tolerance and water-use efficiency. Eds. J.A.C Smith and H. Griffiths. BIOS Scientific Publishers, Oxford, UK: 193-203.

Jump AS, Hunt JM, Peñuelas J (2006) Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. Glob Change Biol 12:2163–2174.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241.

Klein T (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct Ecol 28:1313–1320.

Knutzen F, Meier IC, Leuschner C (2015) Does reduced precipitation trigger physiological and morphological drought adaptations in European beech (*Fagus sylvatica* L.)? Comparing provenances across a precipitation gradient. Tree Physiol: tpv057.

Leuzinger S, Zotz G, Asshoff R, Körner C (2005) Responses of deciduous forest trees to severe drought in Central Europe. Tree Physiol 25:641–650.

Levitt J (1972) Responses of Plants to environmental stresses. Academic Press: New York.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manag 259:698–709.

Martínez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. Tree Physiol 20:131–138.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence-a practical guide. J Exp Bot 51:659–668.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739.

Meier IC, Leuschner C (2008) Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. Glob Change Biol 14:2081–2095.

Mustilli A-C, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002) Arabidopsis OST1 Protein Kinase Mediates the Regulation of Stomatal Aperture by Abscisic Acid and Acts Upstream of Reactive Oxygen Species Production. Plant Cell Online 14:3089–3099.

Müller-Haubold H, Hertel D, Seidel D, Knutzen F, Leuschner C (2013) Climate Responses of Aboveground Productivity and Allocation in *Fagus sylvatica*: A Transect Study in Mature Forests. Ecosystems 16:1498–1516.

Olbrich M, Gerstner E, Welzl G, Fleischmann F, Osswald W, Bahnweg G, Ernst D (2008) Quantification of mRNAs and housekeeping gene selection for quantitative real-time RT-PCR normalization in European beech (*Fagus sylvatica* L.) during abiotic and biotic stress. Z Für Naturforschung C J Biosci 63:574.

Peuke AD, Schraml C, Hartung W, Rennenberg H (2002) Identification of drought-sensitive beech ecotypes by physiological parameters. New Phytol 154:373–387.

Pfaffl MW (2004) Quantification strategies in real-time PCR. AZ Quant PCR 1:89–113.

Picon C, Guehl JM, Ferhi A (1996) Leaf gas exchange and carbon isotope composition responses to drought in a drought-avoiding (*Pinus pinaster*) and a drought-tolerant

(*Quercus petraea*) species under present and elevated atmospheric CO2 concentrations. Plant Cell Environ 19:182–190.

Pluess AR, Weber P (2012) Drought-Adaptation Potential in *Fagus sylvatica*: Linking Moisture Availability with Genetic Diversity and Dendrochronology. PLoS ONE 7:e33636.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological Responses of Forest Trees to Heat and Drought. Plant Biol 8:556–571.

Robson TM, Sánchez-Gómez D, Cano FJ, Aranda I (2012) Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. Tree Genet Genomes 8:1111–1121.

Sánchez-Gómez D, Robson TM, Gascó A, Gil-Pelegrín E, Aranda I (2013) Differences in the leaf functional traits of six beech (*Fagus sylvatica* L.) populations are reflected in their response to water limitation. Environ Exp Bot 87:110–119.

Schall P, Lödige C, Beck M, Ammer C (2012) Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. For Ecol Manag 266:246–253.

Scholander P, Hammel H, Bradstreet E, Hemmingsen E (1965) Sap pressure in vascular plants. Science 148:339–346.

Schroeder J. I., Gethyn J A., Veronique H., June M. K., and Waner, D. (2001) Guard Cell Signal Transduction. Annual Review of Plant Physiology and Plant Molecular Biology 52, 627–658.

Schwanz P, Häberle K-H, Polle A (1996) Interactive Effects of Elevated CO2, Ozone and Drought Stress on the Activities of Antioxidative Enzymes in Needles of Norway Spruce Trees (*Picea abies*, [L] Karsten) Grown with Luxurious N-Supply. J Plant Physiol 148:351–355.

Sirichandra C, Davanture M, Turk BE, Zivy M, Valot B, Leung J, Merlot S (2010) The Arabidopsis ABA-Activated Kinase OST1 Phosphorylates the bZIP Transcription Factor ABF3 and Creates a 14-3-3 Binding Site Involved in Its Turnover Peer WA (ed). PLoS ONE 5: e13935.

Skelton RP, West AG, Dawson TE (2015) Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proc Natl Acad Sci 112:5744–5749.

Stojanović DB, Kržič A, Matović B, Orlović S, Duputie A, Djurdjević V, Galić Z, Stojnić S (2013) Prediction of the European beech (*Fagus sylvatica* L.) xeric limit using a regional climate model: An example from southeast Europe. Agric For Meteorol 176:94–103.

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49:419–432.

Thiel D, Kreyling J, Backhaus S, Beierkuhnlein C, Buhk C, Egen K, Huber G, Konnert M, Nagy L, Jentsch A (2014) Different reactions of central and marginal provenances of *Fagus sylvatica* to experimental drought. Eur J For Res 133:247–260.

Tognetti R, Johnson JD, Michelozzi M (1995) The response of European beech (*Fagus sylvatica* L.) seedlings from two Italian populations to drought and recovery. Trees 9:348–354.

Valladares F, Skillman JB, Pearcy RW (2002) Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architectures: a case of morphological compensation. Am J Bot 89:1275–1284.

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45:523–539.

Weber P, Bugmann H, Pluess AR, Walthert L, Rigling A (2012) Drought response and changing mean sensitivity of European beech close to the dry distribution limit. Trees 27:171–181.

Zang C, Hartl-Meier C, Dittmar C, Rothe A, Menzel A (2014) Patterns of drought tolerance in major European temperate forest trees: climatic drivers and levels of variability. Glob Change Biol 20:3767–3779.

4.6. Declaration

The following work in this chapter has been conducted by Ngoc Quynh Nguyen

- Molecular analysis
- Statistical analysis

The manuscript was written by Ngoc Quynh Nguyen, Rodica Pena and Andrea Polle

All authors commented on the final version.

Chapter 5: Conclusion and Outlook

5.1. Conclusion

This study was conducted to investigate the responses of European beech (*Fagus sylvatica* L.) to drought stress and different annual precipitation amounts. A field experiment in beech forests was carried out to characterize anatomical responses of mature beech trees along a gradient of precipitation. A common garden experiment was set out with three-year-old beech saplings to investigate the expression of genes related to ABA signaling and stress. To link gene expression with plant performance, progeny-and drought-related effects on leaf area and membrane integrity in the absence and presence of acute oxidative stress were determined. A drought stress experiment was conducted to explore intraspecific variation in the drought resistance mechanisms employed by 4-month-old beech seedlings from contrasting habitats.

The comparison of beech trees at the wet and the dry sites indicated that mature beech trees on the dry site changed their anatomical properties to balance between water uptake efficiency and avoidance of embolism in beech stems (chapter 2). This mechanism probably helped the trees to maintain the water status under dry condition. Another typical characteristic of drought avoidance mechanism was found for beech saplings exposed to drought stress (in chapter 3). Progenies from the drier site, generally, showed smaller leaf areas than those from the wetter sites under drought stress. Reduced leaf size is advantageous to restricted water use and to withstand water deficit conditions and is considered as a morphologic change to avoid drought (Micco and Aronne 2012). Carbon isotope analysis of beech trees on the dry site showed changes in carbon discrimination in the late period of the growing season. This finding suggests that beech trees probably closed their stomata to prevent water loss and to maintain their tissue water status. There were, however, differences in the behavior because beech provenance from low precipitation exhibited an isohydric phenotype showing more rapid stomatal closure and maintenance of higher leaf relative water contents and predawn water potentials than those from mesic conditions (chapter 4). These results suggest that mature beech trees on the dry site and young beech trees (seedlings and saplings) from dry site exhibit a drought avoidance strategy to cope with low water availability.

Another strategy to deal with drought is drought tolerance. The high precipitation progenies showed a slow decline in stomatal conductance, but a stronger decrease in the predawn water potential upon water limitation, which is typical for a drought tolerance strategy of anisohydric plants (in chapter 4). The mesic-precipitation provenance maintained its water potential above -2.0 MPa for a longer period than the other two provenances and consequently mortality was delayed in this population. In addition, expression levels of ABA- and stress-related genes (nine-cis-epoxy-dioxygenase (*NCED*), protein phosphatase 2C (*PP2C*), early responsive to dehydration (*ERD*), ascorbate peroxidase (*APX*), superoxide dismutase (*Cu/ZnSOD*), aldehyde dehydrogenase (*ALDH*), glutamine amido transferase (*GAT*) were higher in the progenies from moist than in those from drier sites (chapter 3). As described in the introduction part of chapter 3, these above genes are strongly involved in or mediate drought tolerance in plants. These results suggest that beech seedlings/saplings derived from mesic site have a drought tolerance strategy and genetic factors may regulate this mechanism.

In conclusion, beech exhibited intraspecific variation in drought resistance strategies characterized by drought avoidance or drought tolerance. Drought avoidant type of beech was able to suffer with the moderate drought. However, the drought tolerant type of beech was better endowed to cope with the predicted climate extremes than the drought avoidant type because it possess a drought tolerance strategy. Overall, the present study show that European beech (*Fagus sylvatica* L.) has the ability to deal with low water availability.

5.2. Outlook

Water availability strongly affected the wood anatomical features of European beech (*Fagus sylvatica* L.) to deal with low water availability. These changes converged in vessel properties but not in fibre features since vessels play the most important role of water conduit in the stem of hardwood species. Other anatomical characteristics of vessels such as the internal structure of the vessel walls, vessel wall composition and wood density of beech xylem should be studied in the future work. In addition, soil nutrients and other environmental factors of study locations should be considered for the evolution of beech drought persistence. Here, the adaptability of beech to drought was addressed in young saplings. Intraspecific differences in constitutive expression and drought responsiveness were explored by analyzing the transcriptional regulation of genes for drought signaling and defense throughout the growing season. The molecular reasons for the observed differentiation should be elucidated. An important finding was that different drought resistance strategies of beech was exhibited with anisohydric (drought tolerance) and isohydric (drought avoidance) behavior. However, the

magnitude of variation between these functional types, their presence in matures trees and the underlying molecular mechanisms are unknown and should be investigated. Moreover, the functional tolerance type to severe drought must also be tested in mature beech trees and related to the hydraulic architecture of the plants.

Declaration of originality and certificate of the authorship

I, Ngoc Quynh Nguyen, hereby declare that I the sole author of this dissertation entitled "*Anatomical, physiological and molecular responses of European beech (Fagus sylvatica* **L.***) to drought*". All references and data sources that I used in the dissertation have been appropriately acknowledged. Furthermore, I declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. Lastly, I declare that I have not applied for a Ph.D. at any other higher school of education.

Göttingen, February 2016

Acknowledgements

First of all, I would like to express my greatest appreciation to my supervisor Prof. Dr. Andrea Polle for giving me the chance to study in such excellent environment, the topic of this work and her unconditional support, guidance and useful advices. I was always impressed by her enthusiasm, staidness and the intelligence of the research. She always encouraged me all the time to think independently, scrupulously and comprehensively. I am sincerely thankful to Prof. Dr. Konstantin Krutovsky from the Department of Forest Genetics and Forest Tree Breeding for accepting to be my second supervisor, and Prof. Dr. Alexander Knohl from the Department of Bioclimatology for agreeing to be my third supervisor.

I would like to thank Dr. Caroline Carsjens, Dr. Rodica Pena for working together and providing perfect guidance, a lot of useful documents and uncountable assistance and valuable advices and comments during my PhD study. I sincerely thankful Heike Diekmann for her guidance of using Electronic Microscope; Dr. Dennis Janz for his help during data and image analyses; Viktoria Pfander for collecting samples, harvesting, using a sledge microtome and guidance of molecular work; Bern Kopka for helping me to solve my computer problems; Merle Fastenrath for preparing chemical solutions and FAE and ethanol liquids; Gisbert Langer-Kettner for carbon and nitrogen analysis and helpful technical support, Thomas Klein for his guidance of molecular works. Special thanks to Dr. Jens Dyckmans from the Centre for Isotope Stable Research and Analysis, KOSI, University of Göttingen for stable isotope composition analysis.

I would like to thank to those who friendly helped me so much: Dr. Bettina Otto, Dr. Henning Wildhagen, Dr. Nicole Brinkman, Silke Ammerschubert, Aljosa Zavisic, Josephine Sahner, Nan Yang, Nur Edy, Xu Cao, Shanty Paul, Nguyen Quang Dung, Mareike Kavka, Gerrit-Jan Strijkstra, Abdallah Awad, Lisa Kins and other colleagues for their kind support, contribution, discussion as well as useful advices during the time I studied here.

Many thanks are also extended to the Ministry of Education and Training of Viet Nam for providing the scholarship to pursue my PhD within the Molecular Science and Biotechnology of Crops and Trees (BIONUTZ), Georg-August-University Göttingen. Moreover, I would like to express my sincere gratitude to the Lower Saxony Ministry of Science and Culture for funding the work during my research (KLIFF project-Klimafolgenforschung in Niedersachsen or Climate impact and Adaptation research in Lower Saxony).

Finally, my deepest gratitude and love goes to my beloved family who supported me during whole PhD studies and always believed in me. My thanks will not be complete without thanking my beloved wife Bui Thi Hong Minh, my lovely sons (Nguyen Minh Vu and Nguyen Minh Khang) for their constant love, ever-continuous encouragement, patience and unlimited emotional support. I would like to indicate grateful thank to my parents and my parents-in-law, my grandmother and my grandmother-in-law, my sisters and my big family in Viet Nam, without their support, I cannot achieve this difficult work.

Last but not least, I really like to thank Hung Vuong University for giving a chance to go to Germany to pursue PhD study.