Environmental and genetic control of leaf traits in an oak provenance trial (*Quercus robur*)

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Statement of Authorship

I hereby declare that I am the sole author of this thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

Eidesstaatliche Erklärung

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt habe. Es wurden keine anderen als die angegebenen Hilfsmittel benutzt. Die aus fremden Quellen direkt oder indirekt übernommenen Formulierungen und Gedanken sind als solche kenntlich gemacht. Diese schriftliche Arbeit wurde noch an keiner Stelle vorgelegt.

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Abstract

The present pace of climate change seems rapid compared to present migration rates of trees. This might be a threat to *Quercus robur*, an economically and ecologically valuable deciduous tree in Central Europe. However, fast adaptation, amongst other processes, allowed for European oaks to cope with climatic warming during past interglacial periods. The aim of this study was to determine, whether there was any significant genetic or environmental variation in stomatal density (SD), vein density (VD), and carbon isotope composition ($\delta^{13}C$) between different provenances of *Q. robur*. All these leaf traits are closely related to the water status of a plant. To investigate potential genotypic or phenotypic adaptation in water relations to different climates, trees from ten European provenances, planted in a provenance trial at three different sites in Austria were sampled. Significant variations among provenances could mean a possible source for adaptation to climate change. Two-way ANOVAs yielded evidence for a significant site effect in vein density, as well as in carbon isotope composition. Further vein density showed a significant provenance effect and carbon isotope composition a significant interaction effect. Surprisingly, stomatal density did not show any significant differences among provenances or sites. A correlation matrix revealed some relationships between selected leaf and wood traits within *Q. robur*. $\delta^{13}C$ was negatively correlated with specific leaf area (SLA) and positively with leaf dry matter content (LDMC). Leaf nitrogen content was positively correlated with LDMC and diameter at breast height (DBH). The results of this study can help to understand how leaf traits vary with genotypes and environment. This information can be used by forest managers in their selection of reproductive material and in optimizing conservation measures.

Zusammenfassung

Die aktuelle Geschwindigkeit des Klimawandels ist hoch verglichen mit den aktuellen Migrationsraten von Bäumen. Dies kann eine potenzielle Gefahr für *Quercus robur* darstellen, eine ökonomisch und ökologisch wertvolle Laubbaumart Mitteleuropas. Allerdings erlaubte die schnelle Anpassung europäischen Eichen mit der Klimaerwärmung während vergangener Zwischeneiszeiten zurechtzukommen. Ziel dieser Arbeit war es herauszufinden, ob es bei *Quercus robur* signifikante Unterschiede in der Variation der Stomatadichte (SD), Dichte der Blattadern (VD) und der Kohlenstoffisotopenzusammensetzung ($\delta^{13}C$) zwischen Bäumen verschiedener Provenienzen oder Standorte gibt. Diese Blatteigenschaften sind eng an den Wasserhaushalt von Pflanzen gekoppelt. Zur Untersuchung potenzieller genetischer oder phänotypischer Anpassungen an das Klima, wurden Blattproben von 11-jährigen Bäumen zehn europäischer Provenienzen, die in einem Herkunftsversuch an drei verschiedenen Orten in Österreich gepflanzt wurden, gesammelt. Signifikante Variationen zwischen den

Provenienzen können eine Ressource für die Anpassung an den Klimawandel sein. Zweifaktorielle ANOVAs zeigten einen signifikanten Standortseffekt auf VD und $\delta^{13}C$. Ein signifikanter Provenienzeffekt konnte für VD nachgewiesen werden und eine signifikante Interaktion, Umwelt x Provenienz, für $\delta^{13}C$. Ein erwarteter Effekt in der Variation der SD wurde nicht gefunden. Eine Korrelationsmatrix untersuchte Beziehungen zwischen ausgesuchten Blatt- und Holzeigenschaften. $\delta^{13}C$ ist negativ mit der spezifischen Blattfläche (SLA) und positiv mit dem Blatttrockenmassegehalt (LDMC) korreliert. Der Blattstickstoffgehalt korreliert positiv mit LDMC und dem Brusthöhendurchmesser (DBH). Die Ergebnisse der Arbeit zeigen wie wichtige Blatteigenschaften mit dem Genotyp und der Umwelt variieren. Diese Information kann von der Forstwirtschaft genutzt werden, um die Auswahl von Vermehrungsgut und Maßnahmen zum Schutz der innerartlichen genetischen Vielfalt zu optimieren.

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Abbreviations

Table 1. Abbreviations and units of plant functional traits investigated

Abbr.	Trait	Unit
VD	vein density	mm mm^{-2}
SD	stomatal density	stomata mm^{-2}
g_s	stomatal conductance for water vapor	mol m^{-2} s^{-1}
K_h	theoretical hydraulic conductivity	kg m s^{-1} MPa^{-1} x 10^5
VA	vessel area	μm^2
PV	proportion of cross-sectional area occupied by vessels	%
W_i	intrinsic water-use efficiency	µmol CO_2 mol^{-1} H_2O
$\delta^{13}C$	carbon isotope composition	‰
$\delta^{15}N$	nitrogen isotope composition	‰
Leaf N	leaf nitrogen per leaf dry mass	g per 100g
Leaf C	leaf carbon per leaf dry mass	g per 100g
AR	leaf aspect ratio	/
LA	leaf area	mm^2
SLA	specific leaf area	mm^2 g^{-1}
LDMC	leaf dry matter content	mg g^{-1}
DBH	tree diameter at breast height	cm
Height	tree height	cm
WD	wood density	g cm^{-3}
WC	wood water content	g cm^{-3}

Introduction

In the past, oak and pine were valuable timbers for shipbuilding. In the 18th century, out of the need of suitable material for masts for large naval wessels, the Inspector-General of the French Navy and prominent botanist H. L. Duhamel du Monceau initiated comparative cultures of pine from different countries. This initiative to enhance the quality of cultivated forests made him a pioneer in provenance trials (Langlet 1971).

In the 19th century further provenance trials were arranged to cope with unsuccessful plantations caused by imported seeds (Langlet 1971).

Already in 1877 Kienitz worked on a sowing experiment of oak provenances, which unfortunately was not followed after sowing. Kienitz thereby was the first person to touch upon the importance of provenance of *Quercus robur* and *Quercus petraea (Krahl-Urban 1959)*.

Cieslar (1899) extrapolated that within botanical species and even within acknowledged morphological varieties there are physiological varieties which are provoked by the heredity of specific location factors.

The first Austrian oak provenance trial was conducted at BOKU. In 1905 Adolf Cieslar, at the time member on the board of the Institute for Forestry at BOKU, set an oak provenance trial in the forestry district Neuwaldegg in the Vienna Woods (Krahl-Urban and Joachim 1959). Cieslar detected that northern provenances flush later in spring than southern ones and therefore are less susceptible to late frosts. In addition, northern provenances develop a smaller number of Lammas shoots, grow slower and have tougher leaves. These trends have later been confirmed (Langlet 1971).

Krahl-Urban (1956) noted that previous oak provenance trials showed intraspecific differences in phenology, growth and interaction with the environment. This confirmed the existence of genetically different provenances in *Quercus robur*.

Quercus robur

The genus *Quercus* belongs to the family of Fagaceae and includes about 400 tree and shrub species (Peguero-Pina et al. 2015). In many European countries oak forests hold a good share of the total woodland cover (Kleinschmit 1993). *Quercus robur* L., commonly known as pedunculate oak, is a species of large and long-lived deciduous trees. This species can grow as tall as 30 to 40 m and live as long as for 1000 years. *Q. robur* is a temperate species native to most of Europe (Eaton et al. 2016). The genus *Quercus* is characterized by great intraspecific variability and known to hybridize interspecifically (Masarovlčová 1991). Asymmetric hybridization, where *Q. petraea* preferentially pollinates *Q. robur*, occurs naturally (Ducousso and Bordacs 2004).

Q. robur is monoecious and wind-pollinated (Eaton et al. 2016). Birds and mammals are important seed dispersers of the acorns, especially the Eurasian jay (*Garrulus glandarius*) (Bossema 1979). In forest management coppicing *Q. robur* trees has been widely used for their vegetative regeneration (Ducousso and Bordacs 2004).

Looking at the distribution map (*Figure 1*), the natural distribution of *Q. robur* reaches from southern Norway and Sweden southwards to northern Spain and Portugal and from Ireland eastwards into continental Russia. In a large proportion of its range it occurs sympatrically with *Q. petraea* (Aas 2014).

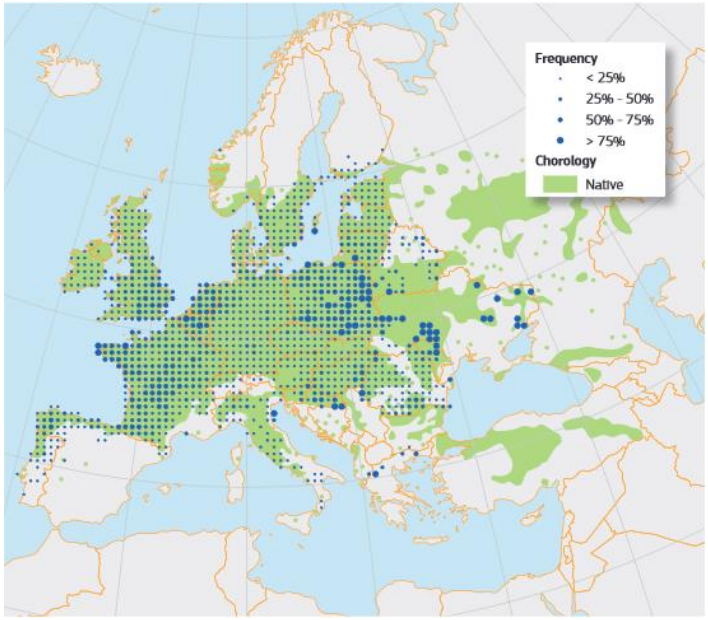

Figure 1. Natural distribution area of *Quercus robur;* (Source: Eaton et al. 2016).

Q. robur is a meso-hygrophilous species that prefers to grow on heavy soils in wet lowlands and damp areas by streams and rivers under continental climate. Mature trees even tolerate periodic flooding. *Q. robur* is a pioneer species in plains, plateaus and hills (Eaton et al. 2016, Ducousso and Bordacs 2004). However, they rarely grow in pure stands under natural conditions. *Fagus sylvatica* (beech) is the main competitor of *Q. robur*, since *F. sylvatica* is a shade-tolerant tree species, whereas *Q. robur* is shade-intolerant (Eaton et al. 2016).

The leaves vary in size and shape. However, leaves are pinnately lobed and length mostly ranges between 6 and 16 cm (Aas 2014). Leaf petiole and blade of *Q. robur* are mostly hairless. Simple hairs sometimes occur on the veins and leaf margins (Batič, Sinkovič and Javornik 1995).

Q. robur is one of the most abundant and ecologically as well as economically most important deciduous trees of Central Europe. Nevertheless, due to conversion into agricultural land many oak forests, which inhabited low elevations with rich soil, were lost

(Kleinschmit 1993). In earlier times oaks have been promoted by humans for several reasons. Young bark was used to obtain tanning bark. Tan-bark oak forests were used for coppice with a rotation of 20 to 30 years. Nowadays tanning bark gets imported from tropical regions or gets replaced by synthetic tanning agents. Ever since, the wood of *Q. robur* was versatile in its use and known for its sturdiness and decorative value. Wood from fast-growing trees with wide growth rings is used in building and construction because of its hardness and durability. Historically, oak was a popular material for wooden piles, as well as for building foundations. Until the 19th century it was agricultural practice to mast pigs with acorns in oak wood pastures. In gardening and landscaping oak still shows for example in fences and wood-block paving. Further, oak is of value in folk medicine, used for example as an external application against skin diseases in the form of bark poultices (Aas 2014).

Postglacial period

The last glacial period lasted for over 100,000 years and is believed to have evoked nuclear genetic differentiation among refugial gene pools of deciduous oaks and thereby triggered subdivision. This nuclear differentiation existing at the beginning of recolonization is absent today. The forces of gene flow via pollen and local selection pressures may have promoted this disappearance. Chloroplastic divergence deriving from the three major refugial zones, that is still present today, implies that different genetic divisions were formed during the last glacial period due to genetic isolation in separate refugia (Kremer et al. 2002).

Within a paternity analysis of Streiff et al. (1999) a descriptive model for pollen dispersal indicated an average pollen flow of 333 m for *Q. robur* in a French oak stand. According to Brewer et al. (2002) today's distribution and limits of European oak species were already set 6000 years ago, and later changes may have been due to anthropogenic influences. *Q. robur* therefore had the possibility to exchange pollen between eastern and western ranges, which will have influenced homogenization of the nuclear genetic pattern of the species (Kremer et al. 2002). Kremer et al. (2002) stated that the extended distribution of *Q. robur*, tolerating different environmental conditions, builds a good foundation for selection in terms of population diversity. Indeed, considerable clinal and ecotypic variation was found among European oak populations (Kleinschmit 1993).

Kremer et al. (2002) divided the origin of the recent chloroplastic diversity and phenotypic trait variation into four phases. The first phase included the building of three major refugia of oaks with supposedly associated chloroplastic and nuclear divergence after the end of the glacial period. The second phase happened during recolonization, when oaks migrated, the chloroplastic spatial structure was soon set and kept due to limited seed

migration. In the third phase pollen flow led to the reduction of the supposedly nuclear differentiation after the glacial period. The fourth phase included the selection pressures, which evolved genetic differentiation between populations as indicated by phenotypic traits.

Local Adaptation

Local adaptation typically results in a local population having the highest fitness at its home site, as opposed to other locations within the species' range (Savolainen, Pyhäjärvi and Knürr 2007).

Local adaptation is affected by the genotype and the environment. Several genes control the phenotypes, which are also under the influence of the environment (de Villemereuil et al. 2016). Population differentiation can be caused by adaptive and by stochastic processes, the relative contribution of which are of special interest (Leinonen et al. 2013). Environmental heterogeneity and the ratio between gene flow and selection can promote genetic differentiation (Savolainen et al. 2007).

Many factors can influence local adaptations, such as herbivores, climate and soil conditions (Sork, Stowe and Hochwender 1993, Rellstab et al. 2016, Burgess et al. 2015). Populations from different provenances can show variable responses (Savolainen et al. 2007). Savolainen et al. (2007) also suggested that local adaptation at the margins of tree populations may break down. They showed that at the population's margins gene flow from the center leads to genetic variation in the margin's individuals, which thereby are hindered to adapt to their local conditions.

As described above, local adaptation can be hampered by gene flow. It can also be confused by genetic drift, which can lead to genetic differentiation in a non-adaptive way. Local adaptation might be prevented by natural selection considering temporally heterogenous environmental conditions, which typically favor generalist phenotypes and increased dispersal. Furthermore, local adaptation can be restrained by low genetic variation of traits, as through variation, e.g. by occasional migration or mutation, local adaptation can be replenished (Kawecki and Ebert 2004). To study the genetic basis of adaptation, quantitative trait loci (QTL) mapping (Tanksley 1993) or genome-wide association studies (GWAS) are used (Savolainen et al. 2007). The main aim of GWAS in plants is to link genomic variants to phenotypes via specific alleles (Anderson et al. 2020). Adaptive traits are thought to show most variation when located on loci with small effects. Furthermore, traits linked to adaptation are often found to be in polymorphic genes (Savolainen et al. 2007).

To study local adaptation using reciprocal transplant experiments, these need to fulfil the following prerequisites. First, they should include the home sites of the populations. Second, relative fitness of transferred and local populations should be observed. Third, the traits studied should be adaptive traits. In forestry local adaptation is studied with the help of provenance trials, where different provenances are grown outside their original location. Such experiments allow to find the most appropriate provenances for the given conditions (Savolainen et al. 2007).

Provenance trials

The OECD Scheme (2018) uses the term “origin” to reference:

“For an autochthonous seed source or stand, the origin is the place in which the trees are growing. For a non-autochthonous seed source or stand, the origin is the place from which the seed or plants were originally introduced. The origin of a seed source or stand may be unknown”.

Further the OECD Scheme defines the term “provenance” as:

“The place in which any seed source or stand of trees is growing”.

These are two terms that must not be used synonymously. For my own results, I will be solely talking about provenances, as it is unknown if the place where the seed material for the *Q. robur* plantations was taken from are the origins as well.

According to Langlet (1971) provenance trials are a set-up of comparative cultivation, under comparable field conditions, of plant material from geographically or environmentally different sites. Provenance trials allow to identify stands or populations which show anticipated traits and economically desired characteristics at the trial location in trees (Matyas 1996). As outlined above, provenance trials often follow practice-oriented and commercial motives of silviculture. Another use is to analyze intraspecific diversity in traits such as allozymic, chemical, phenotypical, and morphological variation (Matyas 1996).

Provenance trials are thus a valuable tool to study intraspecific genetic variation in trees (Matyas 1996). Comparing trials of annual species with trials of trees, the latter, due to their long life-span include the effects of annual weather variations and possibly rare events in the outcome (Matyas 1996).

Common garden experiments and reciprocal transplant experiments are similar concepts but differ in some aspects. Reciprocal transplants want to test for local adaptation where the average fitness of local individuals is compared to that of aliens. Often the locals will

survive better. Common gardens, in contrast, investigate the genetic bases of both adaptive and non-adaptive traits (de Villemereuil et al. 2016).

My thesis made use of a common garden experiment, where the same design was implemented in different environments, which allows to study genotype-by-environment interactions. The operating principle of this experimental method is simultaneously observing genotypes, phenotypes and environments and thereby controlling for phenotypic plasticity and concomitantly distinguishing this plasticity from local adaptation (de Villemereuil et al. 2016). Provenance trials can be used to identify the most suitable provenances for different environments. These insights are of great value in finding genotypes suited for a future climate.

Climate change

Greenhouse gas and aerosol concentrations, as well as variations in solar activity can alter the climate, by changing the Earth's radiation budget. The Industrial Era created a dramatic increase in CO_2 concentration, compared to constant levels (280 ±10 ppm) in the preceding millennia. Anthropogenic emissions, mainly in the form of fossil fuel burning and deforestation, are the main reason for the rise in CO_2 concentration. Other agents such as methane (CH_4), nitrous oxide (N_2O) and halocarbons and related compounds have also directly or indirectly increased because of anthropogenic activities and play a role in climate change. Most of the warming that occurred during the last 50 years, is likely to be attributed to the increase of greenhouse gas concentrations (Houghton et al. 2001).

Air temperature over land has increased by 1.53 °C from 1850-1900 to 2006-2015. The corresponding average global temperature across land and ocean surface area has risen by 0.66 °C. The IPCC conclusion stated with high confidence, that warmer temperatures and changing precipitation patterns have changed vegetation periods and increased tree mortality (Shukla et al. 2019).

International meta-analyses, combining data of long-term records of animals and plants, suggested a significant range shift with an average of 6.1 km per decade poleward and a significant mean advancement of 2.3 days per decade for spring timing events (Parmesan and Yohe 2003).

Biologists are interested if and how species and populations adapt to the new environments that the observed climate change creates (Aitken et al. 2008). A challenge in these studies is to separate climatic induced changes from land-use change and natural fluxes of species abundance and distribution patterns, which predominantly contribute to short-term local changes (Parmesan and Yohe 2003).

The pace of ongoing climate change seems rapid compared to present migration rates of trees (Savolainen et al. 2007). In order for trees to adapt to climate change, genetic variation, dispersal as well as the possibility to establish are essential (Savolainen et al. 2007). Hamrick et al. (1992) surveyed genetic variation of woody taxa within species, within populations and among populations within species. Woody species have shown to have higher genetic variation within populations than other plant life forms, but variation among populations was lower. However, evolutionary history is also considered to play a big role in genetic variation of species and populations (Hamrick et al. 1992). Evolutionary reactions to climate change may be hindered by limited dispersal and establishment rates (Savolainen et al. 2007). Competition between different species might also influence adaptational processes (Kellomäki et al. 2001, Savolainen et al. 2007).

A review by Kremer (2010) concluded that rapid migration, extensive gene flow, fast adaptation and hybridization allowed European oaks to cope with climate warming during past interglacial periods. Even if the recent climate change is going at faster rates than in the previous phases of natural warming, evolutionary responses might be similar (Kremer 2010).

Migration of oak after the last glacial period happened at an average speed of about 500 m per year (Brewer et al. 2002, Huntley and Birks 1983, cited in: Kremer 2010), which was faster than theoretical dispersal models predicted (Kremer 2010). Further, this event led to genetic homogenization (Aitken et al. 2008) and to low genetic differentiation among current European oak populations (Mariette et al. 2002). Populations of *Q. robur* were strongly influenced by extensive pollen dispersal (Streiff et al. 1999), which maintained genetic diversity throughout the entire population and facilitated fast adaptation of populations to newly available territories (Kremer 2010). Rapid adaptation was achieved in an interplay with diversifying selection, whereby novel allelic combinations lead to population differentiation (Kremer 2010). Another process observed in the post glacial period was the hybridization of *Q. robur* and *Q. petraea*, this interspecific gene exchange accelerated species migration and succession rates (Kremer 2010).

The processes described above will likely determine how *Q. robur* will respond to climate change in the future. Theoretical models predict a shift towards the pole, although the speed of temperature changes is faster than the European oaks can cope with considering migration through seed dispersal. Accelerated species migration and facilitated establishment on new environmental conditions are ways in which hybridization could contribute to increased adaptation. Concerning gene flow, pollen dispersal from southern to northern populations is thought to raise the fitness of oak populations exposed to a warmer climate (Kremer 2010).

Especially widespread species dispose over a high genetic within-population variation (Hamrick et al. 1992). *Q. robur* is believed to be able to adapt to climate change in rather few generations because of its status as a potential pioneer and widespread species (Eaton et al. 2016) with high fecundity (Kremer 2010) occurring in large populations. It will possibly experience some adaptational lag, but should be able to compete at least for the interim period, as all competitors need to adapt as well (Aitken et al. 2008).

Translocations of populations can be used as a tool to integrate pre-adapted alleles into conservation populations. Through reforestation from milder to colder conditions short and long term predictions of adaptive traits can be made, which could be adopted into adequate seed transfer guidelines (Aitken et al. 2008).

Leaf functional traits

Plant functional traits reflect different adaptations to the environment, including temperature and drought. They are thought to influence plants' evolution of fitness and life history, which still lacks comprehensive testing (Adler et al. 2014). In general, plant functional traits are commonly studied using species' mean trait values to interpret community composition and ecosystem functioning (Fajardo and Siefert 2016). Thereby many studies show, that distances between functional traits are kept throughout different environments (Mudrák et al. 2019). Wright et al. (2004) studied key leaf traits that reflect many basics of leaf economics and discovered that most leaf investment strategies can be displayed along one spectrum. Adaptation of these leaf traits and their relationships were, in general, found to be under modest influence of climatic change. The process of averaging, however, conceals intraspecific trait variability (ITV), thus some studies include ITV to improve ecological significance of their results (Fajardo and Siefert 2016). Widely used leaf functional traits are height, LDMC, SLA, leaf $\delta^{13}C$ content, leaf carbon content, leaf $\delta^{15}N$ content, and leaf nitrogen content (Mudrák et al. 2019).

Stomatal Density (SD)

Stomata provide openings in the cuticle and epidermis, that allow for CO_2 uptake into and transpiration out of leaves. A balance between CO_2 uptake for mesophyll photosynthesis and water loss through transpiration for an appropriate plant water status needs to be achieved (Adams and Terashima 2018). Since the occurrence of vascular plants about 400 million years ago, declining concentration of atmospheric CO_2 as well as changing moisture patterns forced stomata to increase epidermal conductance to CO_2 diffusion and to enhance transpiration efficiency (CO_2 fixed per unit water transpired) (Franks and Farquhar 2007).

The amount, pattern, size, shape and speed of the stomatal movement are features that vary according to site adaptation and also show individual differences (Larcher 1994). Stomatal characteristics like stomata size (Raven 2014), shape of guard cells (Franks and Farquhar 2007) and presence of subsidiary cells (Bertolino, Caine and Gray 2019) impact stomatal responses, such as the reaction time for closure.

Abaxial (lower) and adaxial (upper) leaf surfaces may differ in density of stomata and may give different responses to environmental stresses (Lu 1989). Stomatal density is defined as the number of stomata per unit leaf area. The common unit for stomatal density is stomata mm^{-2}.

Vein Density (VD)

Veins in leaves play a major role for water supply and thus photosynthetic capacity (Sack and Holbrook 2006, Roth-Nebelsick et al. 2001). The xylem moves water from the roots up the plant into the leaves' veins before it evaporates and exits the plant through living tissues to the sites of evaporation (De Boer et al. 2012, Sack and Holbrook 2006).

Vein density is defined as the length of veins per leaf area. The common unit is mm mm^{-2}. Typical vein densities of angiosperms range from 5 to 25 mm mm^{-2} (Boyce et al. 2009). De Boer et al. (2012) showed that rapid angiosperm evolution during the Cretaceous was likely due to the decrease of the leaf interior path way for water beyond the leaf interior transport path way for CO_2 at a crucial vein density of 2.5 to 5 mm mm^{-2}. Increasing vein density means shorter distances between veins and evaporation sites, which helps to reduce hydraulic resistance where it is the highest in a leaf (Simonin and Roddy 2018, Brodribb, Feild and Jordan 2007, Buckley et al. 2015). This permitted to maintain equal water loss at higher carbon returns through evolving leaves with smaller and more stomata (De Boer et al. 2012).

Homogeneous water distribution over the leaf blade is greatly influenced by the arrangement of major veins (Roth-Nebelsick et al. 2001), but minor veins predominate the distributive network of mesomorphic leaves (Plymale and Wylie 1944). Leaf venation patterns vary greatly among species. The path by which water flows from the xylem into the bundle sheath and further is still unresolved. In general water flux can be apoplastic, transcellular or symplastic, though actual water movement might differ among species (Roth-Nebelsick et al. 2001).

Measurement methods of leaf vein density depend on magnification, field of view and image resolution. When comparing vein density, consistency of these factors should be considered to increase confidence and facilitate comparisons between measures across leaves (Price, Munro and Weitz 2014).

Stable Carbon Isotopes

Stable isotopes are non-radioactive, thus persistent versions of atoms bearing a different number of neutrons. In the case of carbon, two stable isotopes, ^{12}C and ^{13}C, occur on Earth in a ratio of about 98.9 to 1.1 (Rounick and Winterbourn 1986).

Isotopic carbon compositions are specified as $\delta^{13}C$ values:

$$\delta\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \text{x } 1000$$

where R_{sample} and $R_{standard}$ are the abundance ratios, $^{13}C/^{12}C$, of the sample and the standard, PDB, respectively.

Isotopic discrimination, d, is defined by

$$d = 1000 \text{ x } \left(1 - \frac{R_{product}}{R_{source}}\right).$$

Using the above definition of δ, this is equivalent to

$$d = \frac{\delta_{source} - \delta_{product}}{1 + \delta_{source}/1000}$$

(Farquhar, O'Leary and Berry 1982).

The type of photosynthetic cycle used is correlated with the carbon isotope composition of the plant material (Deines 1980). In higher C_3-plants carbon isotopic composition differs by about 20 ‰ between plant tissue and atmospheric CO_2, whereas C_4-plants discriminate less (Farquhar et al. 1982, after: Bender 1968). It was found that plants systematically discriminate against ^{13}C (Baertschi 1953). Park and Epstein (1960) suggested that preferential use of ^{12}C in CO_2 uptake from the atmosphere and in the carboxylation step of photosynthesis is responsible for the discrimination. Different plant organs vary in their carbon isotopic composition (O'Leary 1981).

Increasing ratios between CO_2 concentration inside the leaf and concentration in the air, c_i/c_a, will decrease a plant's $\delta^{13}C$. Circumstances that restrict CO_2 uptake, like drought stress, lead to low c_i/c_a ratios and therefore higher $\delta^{13}C$ of a plant (Tieszen and Boutton 1989). Carbon isotopic composition of a leaf can therefore serve as a proxy of plant water-use efficiency (WUE), and $\delta^{13}C$ relates to photosynthetic activity and water loss (Farquhar et al. 1989). Leaf $\delta^{13}C$ can be used as an integrated measure of intrinsic water-use efficiency (W_i) as it incorporates the c_i/c_a ratio during the formation of leaf tissue (Gouveia and Freitas 2009).

Roussel et al. (2009, after: Farquhar and Richards 1984) model the relationship between $\delta^{13}C$ and W_i as follows:

$\delta^{13}C_{sample} = b - (b - a)\frac{1.6\,W_i}{c_a}$

"with: *a*, discrimination during diffusion of CO_2 through stomata (4.4‰) and *b*,

discrimination during carboxylation of RuBP (27‰ taking into account 10% C fixed by PEPC; Farquhar and Richards, 1984) and c_a, atmospheric CO_2 mole fraction (continuously monitored in the greenhouse with a mean value of 394 ± 12 µmol mol^{-1}). The resulting equation is then $\Delta^{13}C_{sample} \approx 27 - 0.092W_i$." (Roussel et al. 2009)

Water-use efficiency is used in different contexts. This thesis refers to W_i, which is defined as the ratio between net photosynthesis (A) and leaf conductance for water vapor (g_s). It is a complex trait linked to a number of physiological and structural leaf traits that affect A or g_s (Roussel et al. 2009).

Carbon isotope compositions can be determined through mass spectrometers, which compare an isotope ratio of a sample to a standard (O'Leary 1981).

Aims and objectives

The aim of this study was to determine to which extent intraspecific variation of *Q. robur* is controlled by genotype, environment and genotype x environment.

Objectives:

- Data of stomatal density, vein density, $\delta^{13}C$, leaf C content, $\delta^{15}N$ and leaf N content will be collected from leaf material of a *Q. robur* provenance trial
- The influence of environment (site), genotype (provenance) and their interaction on the leaf traits will be analyzed and tested for their effect size
- Possible correlations between the leaf traits, wood traits, growth traits and climate variables will be determined

Using a provenance trial of *Q. robur* with trees from ten provenances planted in three sites of contrasting temperature and precipitation regimes, I tested if stomatal density, vein density, $\delta^{13}C$, leaf C content, $\delta^{15}N$, and leaf N content are controlled more by genes or environment. A look on intraspecific variation of *Q. robur* might also give insightful information on local adaptation and phenotypic plasticity, which could be used for future-oriented forest strategies to cope with climate change.

Material and methods

Experimental design

The Bundesforschungszentrum für Wald (BFW) initiated the provenance trial in the frame of their project “PROEICHE” in autumn 2006. Five experimental sites with trees from the same 22 provenances were installed. Among the provenances were fourteen Austrian and eight foreign ones (Germany, Czech Republic, Croatia, Slovenia, Hungary). The experimental plans of all study sites followed a randomized block design. Each site was divided into three blocks of equal size containing all 22 provenances of *Q. robur* and *Q. petraea*. Trees within these blocks were planted with a distance of one meter within the rows and two meters between rows. To omit the edge effect the oak experimental sites were surrounded by other tree species functioning as a buffer zone.

Provenances and experimental sites were selected for a gradient in mean annual temperature and mean annual precipitation (*Table 2*, *Table 3*). The work for this thesis was carried out at the University of Life Sciences Vienna (BOKU).

Samples used for this thesis are from

3 trial sites,

10 provenances, and

9 trees per provenance and trial site.

This leads to 270 samples in total. The trees sampled from the same provenance within a trial site came from nine different mother trees, but trees from the same provenance between the different sites were of the same nine mother trees, i.e. they were half-siblings.

Figure 2. Location of the trial sites (star symbols) and the provenance sites (grey circles). WL = Wels, WR = Weistrach, WB = Weyerburg; Source: Google Maps.

The three trial sites are located in Weyerburg (WB), Wels (WL), and Weistrach (WR) as shown in *Figure 2*. Weyerburg and Weistrach are located in Lower Austria, whereas Wels is situated in Upper Austria. Mean annual temperature (MAT) differs marginally between the three trial sites spanning from 8.5 to 8.8 °C. In contrast, mean annual precipitation (MAP) varies more with Weyerburg having the lowest (641 mm), Weistrach intermediate (890 mm) and Wels the highest (1005 mm) MAP. A similar pattern can be found in the climate data of the *Q. robur* provenances in *Table 3*. The range of MAT lies between 7.4 and 10.0 °C, whereas MAP lies between 638 and 1066 mm. The bioclimatic variables of MAT and MAP represent annual trends and are derived from monthly temperature and rainfall values. Data was taken from WorldClim (http://www.worldclim.org/bioclim) using the BIO1 (MAT) and BIO12 (MAP) datasets with a resolution of 1 km.

Table 2. Climate data of the experimental sites including mean annual temperature (MAT) and mean annual precipitation (MAP).

Experimental site	**Code**	**Federal state**	**Latitude (N°)**	**Longitude (E°)**	**MAT (°C)**	**MAP (mm/y)**
Wels	WL	Upper Austria	48.185	13.989	8.5	1005
Weistrach	WR	Lower Austria	48.053	14.562	8.4	890
Weyerburg	WB	Lower Austria	48.557	16.171	8.8	641

Coordinate system: Geographic coordinates (degree/min/sec); Geodetic date: World Geodetic System 84 (WGS84)

Table 3. Provenances of *Quercus robur* arranged by country including latitude, longitude, mean annual temperature (MAT) and mean annual precipitation (MAP).

Provenance	Country	Code	Latitude (N°)	Longitude (E°)	MAT (°C)	MAP (mm/y)
Geinberg	AUT	1	48.277	13.307	8.7	1066
Linz	AUT	2	48.326	14.294	9.2	841
Braunsberger Wald	AUT	6	48.473	16.333	9.1	638
Rainfeld	AUT	8	48.042	15.731	7.4	785
Luising	AUT	12	47.023	16.477	9.7	696
Klagenfurt	AUT	14	46.63	14.35	8.0	988
Hluboka	CZ	17	49.09	14.444	7.4	764
Kutina	HR	18	45.433	16.683	10.9	915
Murska šuma	SLO	19	46.498	16.511	10.1	810
Velika Gorica	HR	21	45.674	16.161	10.5	920

Leaf material

Leaf samples of *Q. robur* from all three trial sites were collected on June 18 and 19, 2018. Upper leading branches of *Q. robur* carrying mature sun-exposed leaves were cut with telescopic pruning shears. Branches in good condition with over ten healthy and whole leaves, free from herbivore damage and fungal infection, were picked. On the Weyerburg (WB) experimental site some samples did not meet these conditions and the least damaged leaves were collected. After cutting, the branches were put into plastic bags and kept moist until put in cool storage. While this thesis focused on stomatal and vein density as well as C and N isotopes, I also used data derived from the same trees (Momirović 2019) for comparison.

For stable isotope composition ($\delta^{13}C$, $\delta^{15}N$), leaf C content and leaf N content, nine to eleven leaves per tree were carefully selected. It was paid special attention to homogeneity of pooled samples. Specific leaf area (SLA), leaf dry matter content (LDMC), leaf area (LA) and aspect ratio (AR) were calculated from the same sample material.

LDMC and LA needed measurements of fresh and therefore hydrated leaves. After, leaves were dried in the oven at 60 °C for at least 72 hours, dry weight was determined to 0.1 mg to calculate SLA as fresh leaf area / dry weight ($mm^2\ mg^{-1}$) and LDMC as dry weight / saturation weight ($mg\ g^{-1}$).

For stomatal density and vein density one additional fresh leaf was chosen. This means stomatal density and vein density were determined using the same single leaves, whereas stable isotope data was measured by pooling material from leaves used for leaf area.

Additional data was included in this thesis to compare effect sizes and to determine bivariate correlations among traits and between traits and the climate of origin: specific leaf area (SLA), leaf dry matter content (LDMC), leaf area (LA), aspect ratio (AR), stomatal conductance for water vapour (K_h), theoretical hydraulic conductivity (g_s), vessel

area (VA), proportion of cross-sectional area occupied by vessels (PV), diameter at breast height (DBH), tree height (height), wood density (WD), wood water content (WC) (Momirović 2019).

Measurement of stomatal density

Stomatal density is defined as the number of stomata per unit area. Fresh leaves were pressed into acrylic slides using butanone as a solvent to obtain permanent imprints. Stomatal density was measured by taking microscopic images of these imprints.

Image acquisition

Imprints were imaged using a Leica DM5500 B microscope with the suitable software LAS X. The illumination and exposure settings were set as follows:

Illumination:

- Intensity: 64
- Aperture: 22
- Field: 28
- Axis: TL

Exposure (ms): 9.56

These settings were adjusted when necessary, e.g. depth of imprints, for heterogenous imprints.

An area with the least possible disturbances, for example fungal hyphae and gaps, was chosen to acquire a big enough frame size. The LAS X software allowed to vary the frame size through the number of single tiles chosen. The regular frame size consisted of 3x4 adjacent single tiles at a magnification factor of 20x, for a resolution of 3438.32 pixel mm^{-1}. If the sample's imprint quality was insufficient to gather the pursued frame size at least five single tile images were taken, which in some cases lied scattered over the imprint. After selecting the frame, the position of focus points was marked. This was done by applying the "Use Focus Map" option, setting the "Predictive Focus Mode" to "9 Point Rectangle". The foci were set according to visual perception. If the result was insufficient, image acquisition was rerun with manually selected focus points. All single tiles of a sample as well as one merged image of these tiles were captured and saved as JPEG images.

Image quality was varying according to leaf and imprint quality of the samples. Unevenness of the imprints and software bugs led to more blurred images.

Recognition of stomata

In order to calculate stomatal density, the images were uploaded to StomataCounter (Fetter et al. 2019). StomataCounter is an online tool that automatically estimates stomatal density from epidermal micrographs.

Between five to thirteen single tiles per sample were compressed into a zip-file. These zip-files were uploaded to StomataCounter with stomata size set to “Small (50px to 100px)”.

StomataCounter automatically annotates stomata on the images. Tiles that showed gaps, where fungi covered stomata or where blurriness made it impossible to detect stomata, were excluded.

At least five single tiles of every sample were corrected manually. Only single tiles which showed intact leaf material were selected for manual corrections. The tool lies red circles around the stomata detected (*Figure 3*). Automatic annotations were manually corrected by adding missing stomata or deleting false annotations by respectively clicking stomata or unclicking the red circles. To determine stomatal density only stomata that were positioned within the lighter area or on the transition from lighter area to frame were considered.

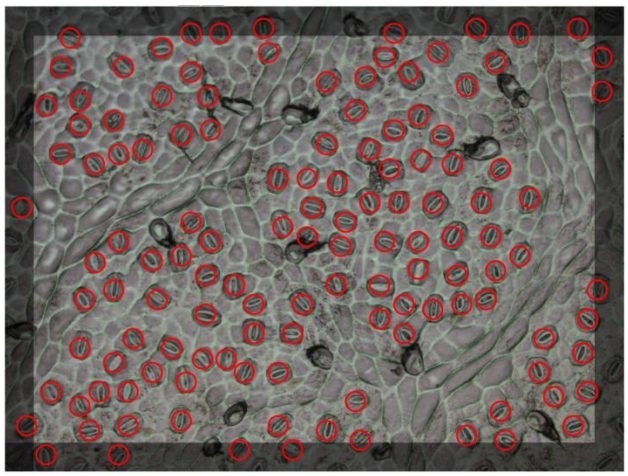

Figure 3. Annotation mode in StomataCounter; Source: stomata.science.

A summary of all data could be downloaded as a csv-file. Stomatal density per mm² was then calculated in Excel. In total samples from 267 trees were considered for the trait of stomatal density. Three samples needed to be excluded either for poor quality or suspicion of confusion with other samples.

Correlation of manual and automatic stomata counts

During image evaluation it appeared that images of higher quality achieved higher accuracy in automatic stomata annotation. *Figure 1* In an attempt to find a correlation between high image quality and accuracy of automatic counts the image score frequency

domain measure of standard-deviation (fSTD) was used. According to Koho et. al (2016) fSTD produces good separation of blurred images from sharp ones. It is calculated from the high-frequency tail of the power-spectrum, which allows to focus on finer image details (Koho et al. 2016). Human counts, automatic counts, as well as fSTD were derived from StomataCounter and are presented in a correlation graph in *Figure 4*. As for fSTD the median was determined and used to group image quality. fSTD above the median, colored in blue, is considered as good image quality and fSTD below the median, colored in red, is considered more blurred and out-of-focus.

The correlation between image quality and automatic counts cannot be observed in the graph. A simple linear regression was computed to assess the relationship between the number of human and the number of automatic counts of the frames (595.64 x 446.73 µm) of stomata. A significant regression equation was found, with $R^2 = 0.60$, $p < 0.001$. Automatic counts predicted were equal to 36.855 + 0.613 (human counts). Automatic annotation slightly underestimated the actual number of stomata, considering the manual counts as the correct reference. Therefore, in this thesis only manual counts were used to calculate stomatal density.

Dittberner et. al (2018) found a correlation of $R^2 = 0.81$, $p < 0.01$ when correlating manual and automatic results of stomata density of 32 independently grown individuals of *Arabidopsis thaliana*. The discrepancy of R^2 values in Dittberner et. al (2018) and my thesis might lie in the difference of methods. Dittberner et al. used quality filters in their high-throughput microscopy pipeline, whereas no image pre-processing was done for the *Q. robur* images. Homogeneity in image quality of my samples might have been influenced by a bug in the Leica LAS X software, which impeded flawless focus of the images. Furthermore, Dittberner et. al (2018) correlated manual and automatic results of only 32 samples using microscopic images of 0.15 mm^2, whereas I correlated 267 samples using microscopic images of 0.27 mm^2.

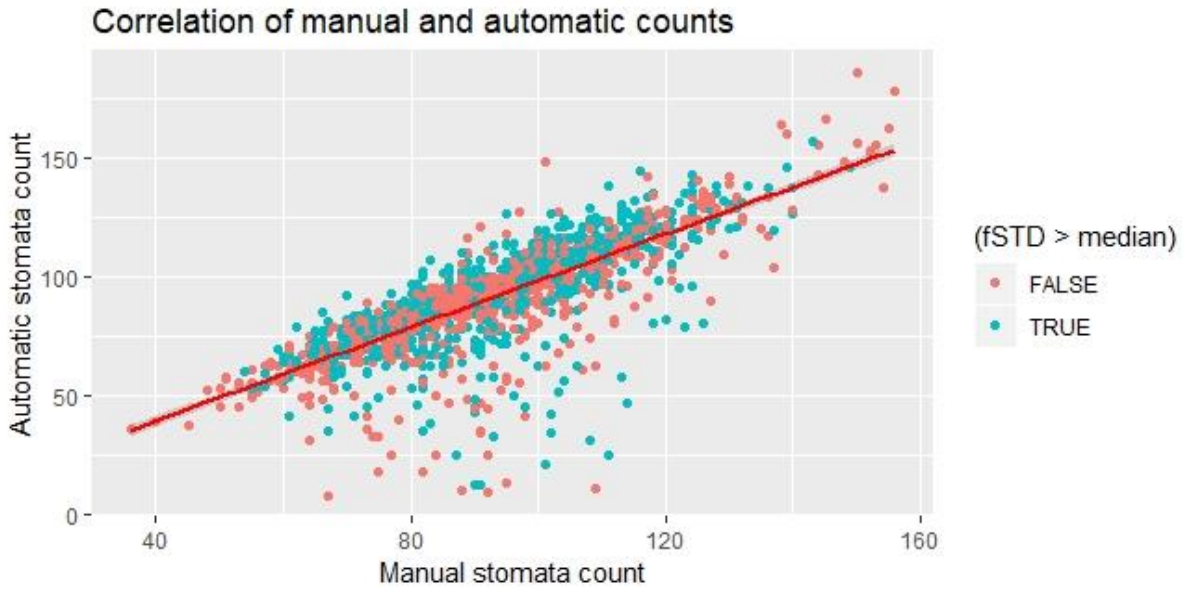

Figure 4. Correlation between human and automatic count for images with image quality (measure as fSTD) above or below the median. The red line illustrates a linear model fit of the data.

Measurement of vein density

Vein density is defined as the total length of veins per unit area. The measurement of vein density was determined by first bleaching discs of the leaf samples and then taking microscopic images. Measures of vein lengths were computed semi-automatically in imageJ and subsequently vein density was calculated.

Vein density measurements were based on Pérez-Harguindeguy (2013).

Bleaching

Leaf discs of 15.9 mm diameter were conserved in Karnowsky fixative (glutaraldehyde-paraformadehyde fixative solution) until bleaching. In a next step this was replaced by a 5% NaOH solution. To fasten the process of bleaching, the samples were put into an oven at 60 °C. On day two and day three the 5% NaOH solution of all samples was renewed. Between day four and day seven visual control took place, and samples that were considered clear enough for the second phase of bleaching were removed from the oven and stored in 50% EtOH. If the solution of the samples that needed a longer period of soaking turned to an opaque brown color, the solution was renewed. On day seven all leaf samples were put into a 50% EtOH solution and thus ready to proceed.

Dying

The next step involved a second phase of bleaching and subsequently a phase of dying.

First the samples were taken out of the 50% EtOH solution, in which they were conserved in. After, samples passed five minutes each in distilled water, 14% sodium hypochlorite (NaClO), and again distilled water. Next, the leaf discs were cut in half and only the half

with fewer major veins was further processed. Then, disc halves were put into safranin (1:5 with distilled water), distilled water, and 50% EtOH for five minutes in each. After the last step samples were transferred onto microscope slides.

Image acquisition

Images were digitized using a Leica DM5500 B microscope with the software LAS X. The adaxial side of the sample faced up to achieve the best visibility of the veins. The requirements for the image acquisition were a frame of at least 8 mm^2 with mostly homogeneous bleaching. The quality of the leaves as well as the degree of bleaching differed among the samples, therefore illumination, exposure and focus were set individually. Images were captured at 1719.17 pixel mm^{-1}. After image acquisition the dyed halves of the samples were put back into the 50% EtOH tubes with their bleached pair.

Image evaluation

Vein density was measured using the software ImageJ (US National Institutes of Health; http://www.nih.gov/). Images of dyed leaves were analyzed for vein density using a customized macro. At first vein images were reduced to 16 bits and a Gaussian blur of 2 was laid over the image (*Figure 5a)*. After manually setting contrast and brightness, a manual threshold was applied to create a binary image (red area in *Figure 5b*). Using the BoneJ plugin in ImageJ (Doube et al. 2010) a skeleton of that binary image was created (*Figure 5c*), and analyzed to measure the lengths of all branches with the “analyze skeleton” function. Images of the skeletons were saved. Branch length data was transferred into Excel, where vein length in pixels was converted into vein density as mm mm^{-2}.

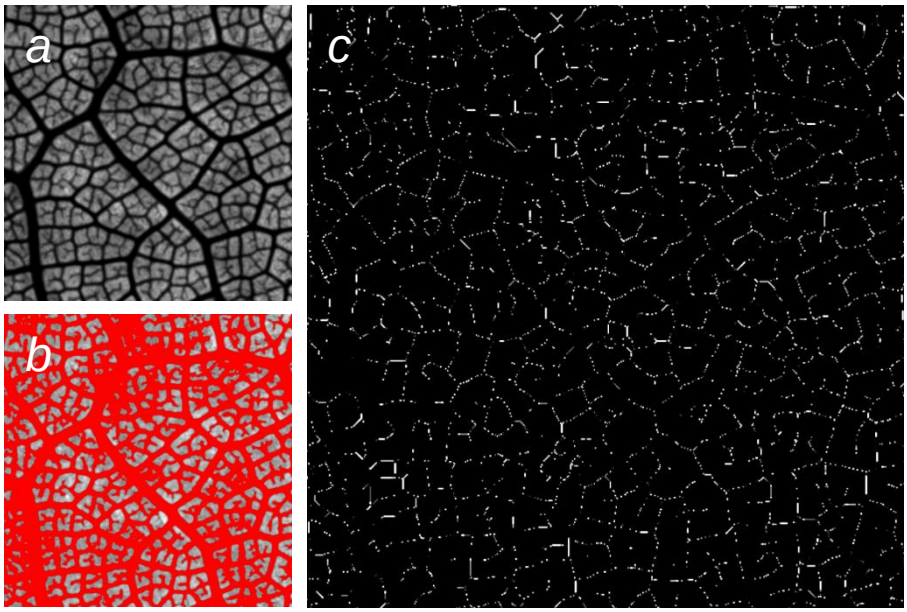

Figure 5. Image evaluation of WL_12_03 in imageJ. (a) gaussian blur, 16 bit, brightness and contrast, (b) setting the threshold, (c) skeleton.

To test the accuracy of the semi-automatic vein length measurements, ten images of 3 mm² size each, were measured manually in ImageJ. The results were compared to the semi-automatic results with $R^2 = 0.67$ and $p = 0.004$. Semi-automatic measurements predicted are equal to 3.535 + 0.682 (human measurements). As *Figure 6* shows, the correlation between manual and semi-automatic measures is acceptable for the majority of images, despite some images with higher or lower than average semi-automatic vein length compared to manual measurements. These outliers resulted from more blurry images, which were intentionally kept to assess the quality of the semi-automatic method. If those three outliers are removed, semi-automatic measurements predicted are equal to 1.916 + 0.809 (human measurements), $R^2 = 0.978$ and $p = 2.457e^{-05}$. The semi-automatic method was thus reliable for the majority of images and was used for analysis as it substantially saved time (it took for one image 0.5 h by hand but 2 minutes with the methods).

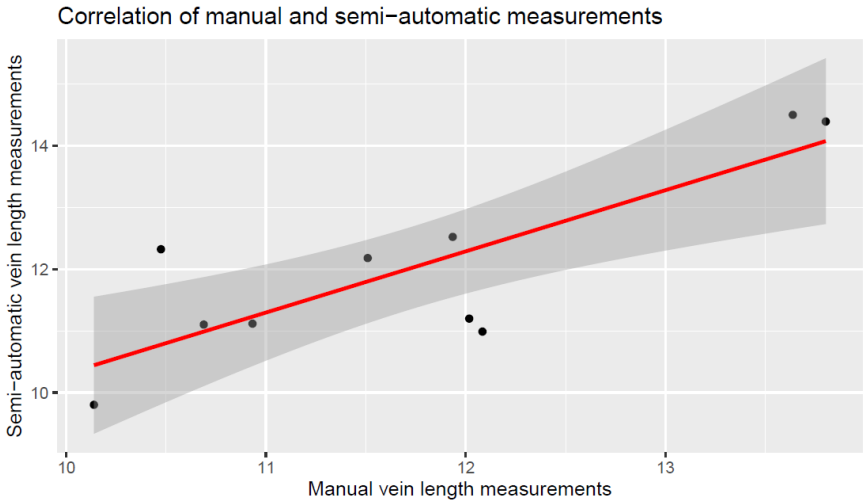

Figure 6. Correlation between manual and automatic measurements of vein density.

Vein densities were measured on 262 samples and eight samples were excluded due to poor image quality.

Measurement of stable isotopes

The delta notation (symbol: δ) expresses the variation of an isotopic ratio of an element R (e.g., $\delta^{13}C = {}^{13}C/{}^{12}C$), relative to the isotopic ratio of a standard R_{std} (e.g., $\delta^{13}C_{PDB} = {}^{13}C/{}^{12}C = 11237.2 \pm 9.0$, where PDB stands for Pee Dee Belemnite). The delta notation is expressed per mil (parts per thousand, ‰) for carbon and nitrogen (Pinti 2011).

Preparing samples

For each of the 270 trees, eight to eleven leaves were pooled by punching 9 mm diameter discs out of each leaf. Major veins were omitted. To ensure homogenous sample weight

between the different samples, in tree samples with a total of eight leaves, two of them were punched out twice and in tree samples with a total of nine leaves one of them was punched out twice.

The leaf discs of the 270 tree samples were ground in tubes with a TissueLyser 2 (Qiagen) automated shaker. The tubes were shaken for 30 minutes at a frequency of 30 Hz. This step was to finely grind the leaf discs to homogenous leaf powder. After the shaking the metal beads were removed from the tubes.

Sample weigh-in

According to the protocol (see Appendix) isotopic standards, reference material and sample material were weighed in on a M5 micro balance (Sartorius) with a resolution of 1 µg. The tin capsules (3 x 5 mm) were tared and then filled with 3 mg of leaf powder. Tin capsules were closed wrapping the edges and stored in a labeled container for analysis.

Mass spectrometer

The analysis was conducted at the isotope laboratory of the Institute of Soil Sciences (BOKU). An Elemental Analyzer - Isotope Ratio Mass Spectrometer (EA-IRMS) coupling was used to simultaneously assess the abundance of C and N isotopes ($\delta^{13}C$, $\delta^{15}N$) as well as C and N content of the leaf material. *Figure 7* shows the exact set-up of the EA-IRMS used for these measurements.

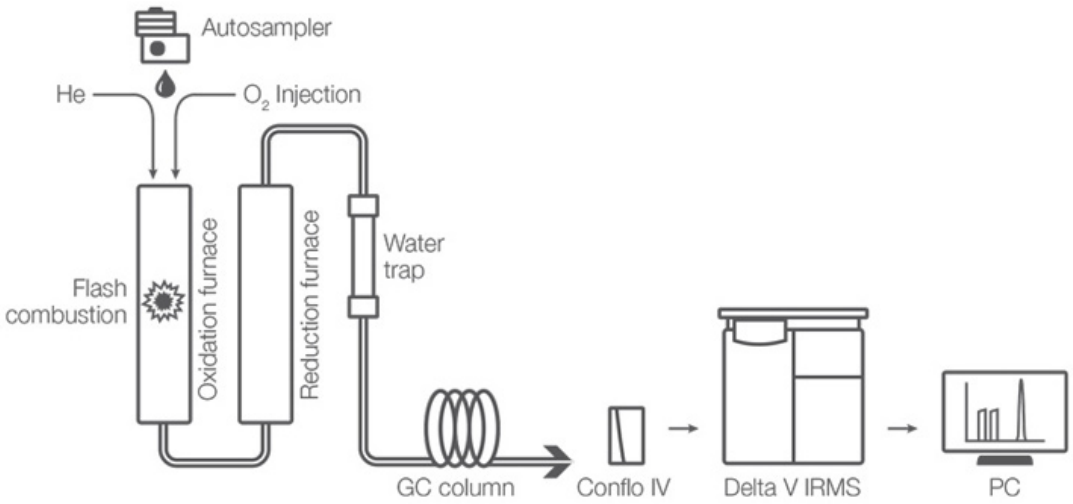

Figure 7. Set-up of EA-IRMS; Source: https://www.thermofisher.com/at/en/home/industrial/mass-spectrometry/isotope-ratio-mass-spectrometry-irms/gas-isotope-ratio-mass-spectrometry-irms/gas-irms-peripherals.html.

At first tin capsules were loaded into an automatic sampler of an elemental analyzer (Thermo Scientific Flash 2000). In the presence of an excess of oxygen the bulk material was flash combusted in an oxidation furnace, producing gaseous CO_2 and N_2/NO_x. Nitrous

oxides and oxygen surplus were reduced in a reduction furnace. Furthermore, water was removed by a chemical trap. CO_2 and N_2 were separated by a gas chromatographic column. Helium was used as a carrier gas. In a continuous flow gas interface (Thermo Scientific ConFlo IV) CO_2 and N_2 standard gases with known isotope abundances were added into the gas stream (Gebauer 2011). The relative abundances of the isotopes ^{13}C and ^{12}C as well as ^{15}N and ^{14}N, were analyzed by isotope ratio mass spectrometry (Thermo Scientific Delta V Advantage) with an accuracy of ≤0.2 ‰ for C and N.

Measurement sequences included blanks, standards and samples (see protocol in the Appendix). The following standards were used:

- Acetanilide
- IVA Protein
- IVA Urea
- IVA Sorghum
- IVA Wheat

Statistical analysis

All statistical calculations were performed with R Studio using R version 3.5.3 (RStudio Team 2018). Two-way linear mixed effect model ANOVAs were calculated on the effects of site, provenance and their interaction for all traits using the “lmer” function. The factor “site” contained three levels and the factor “provenance” contained ten levels. An error term for the mother tree was included, to account for variation possibly caused by genetic relatedness. Post-hoc tests were carried out using Tukey's HSD (honestly significant difference) based on simple two-way ANOVAs.

Eta-squared (η2) tests were used to attribute the proportion of traits’ variance to site, provenance and their interaction.

Pearson correlation coefficients for pairwise comparisons were used to create a correlation matrix of selected traits. An alpha level of .05 was used for all statistical tests.

Results

First the significance of site and provenance on the traits measured are presented, as well as specific groups of significant effects are observed for differences. Then the variance explained by site and provenance on these and on other traits, that were measured previously on the same trees, are shown. Furthermore, the relationship among traits and between traits and the climate of origin as bivariate correlation and as a PCA are calculated.

Stomatal density

In a total of 267 samples, stomatal density ranged from 201 to 552 stomata mm^{-2} (mean: 352 stomata mm^{-2}).

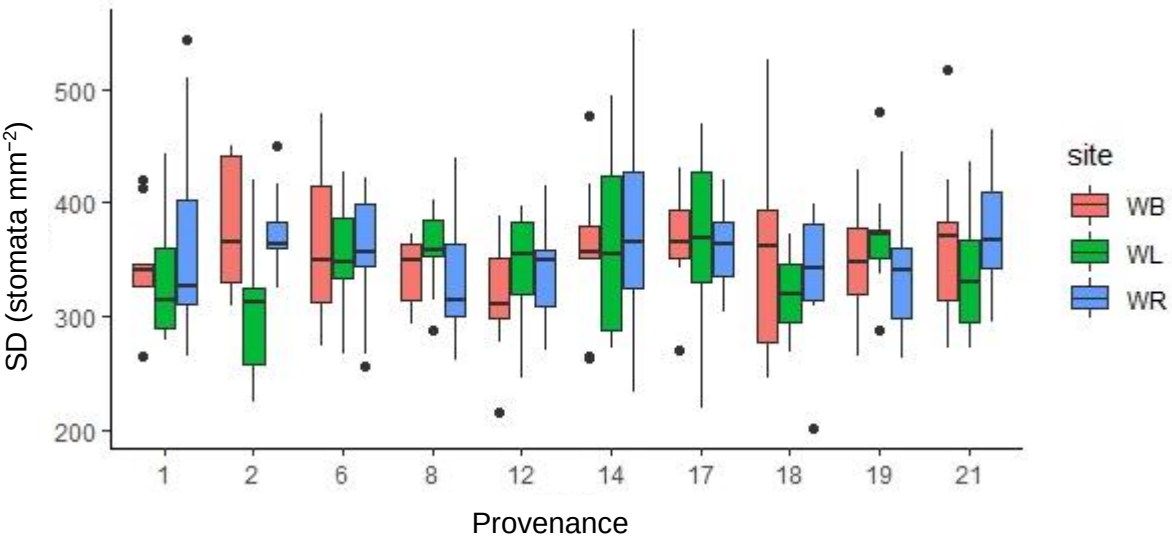

Figure 8. Boxplot showing the distribution of stomatal density depending on site and provenance.

Neither site, nor provenance or interaction effect were statistically significant (*Table 4*). The sites showed means of WL (345.76), WB (353.42) and WR (356.05) (*Figure 8*). The highest mean of provenances was displayed by provenance 17 (366.19) and provenance 14 (364.83), the lowest mean had provenance 12 (335.13).

Table 4. ANOVA results for stomatal density.

	SS	Df	P	F
Site	5481	2	0.455	0.791
Provenance	20900	9	0.733	0.670
Site : Prov	59140	18	0.522	0.948

Vein density

In 262 samples, vein density ranged from 9.34 to 18.32 mm mm^{-2} (mean: 13.33 mm mm^{-2}).

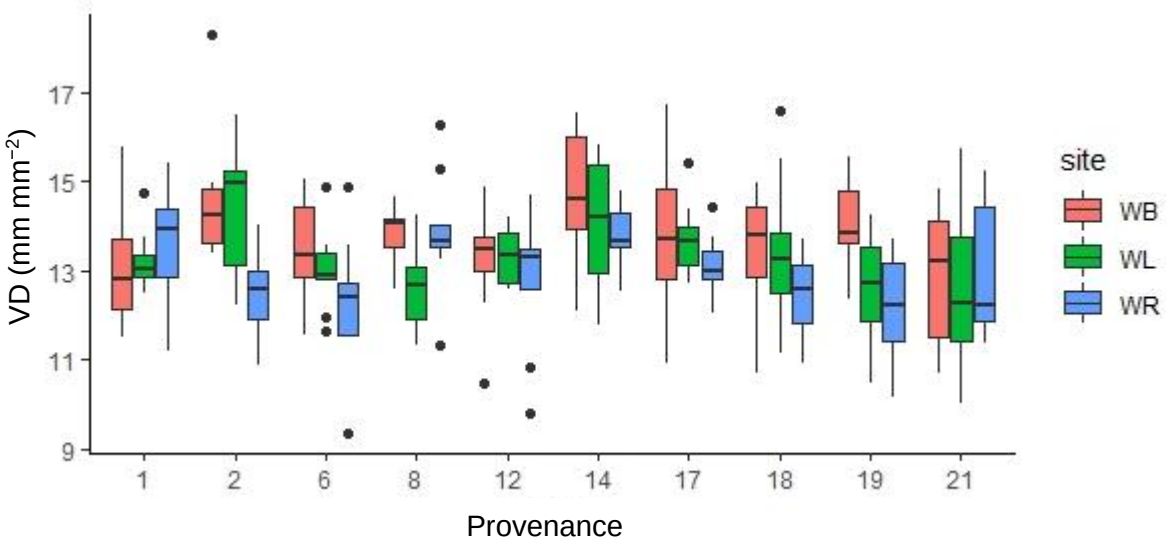

Figure 9. Boxplot showing the distribution of vein density depending on site and provenance.

The factor site, as well as the factor provenance showed a significant effect. There was no significant interaction between the two factors (*Table 5*).

Mean vein density was highest in WB (13.72), followed by WL (13.33) and WR (13.00). Provenances 14, 17, 18 and 19 reflected this pattern quite clearly (*Figure 9)*. However, WL, with intermediate vein density, was the site with the highest MAP (1005 mm) as well as with the most fertile soil. The highest means were found in provenance 14 (14.15) and provenance 2 (13.87), the lowest mean had provenance 21 (12.82). Whereas provenance 14 showed a high MAP (988 mm), that of provenance 2 (841 mm) was very close to the provenances' mean (842 mm). Provenance 12 had the second lowest MAP (696 mm) of all provenances.

Post-hoc testing amongst the three sites revealed significant differences between WB and WR. The site WL did not significantly differ from the other two sites. Furthermore provenance 14 was significantly different from provenances 19, 6 and 21 (*Figure 10*).

Table 5. ANOVA results for vein density.

	SS	Df	P	F
Site	25.2	2	< 0.001	7.892
Provenance	37.8	9	0.010	2.635
Site : Prov	44.2	18	0.083	1.539

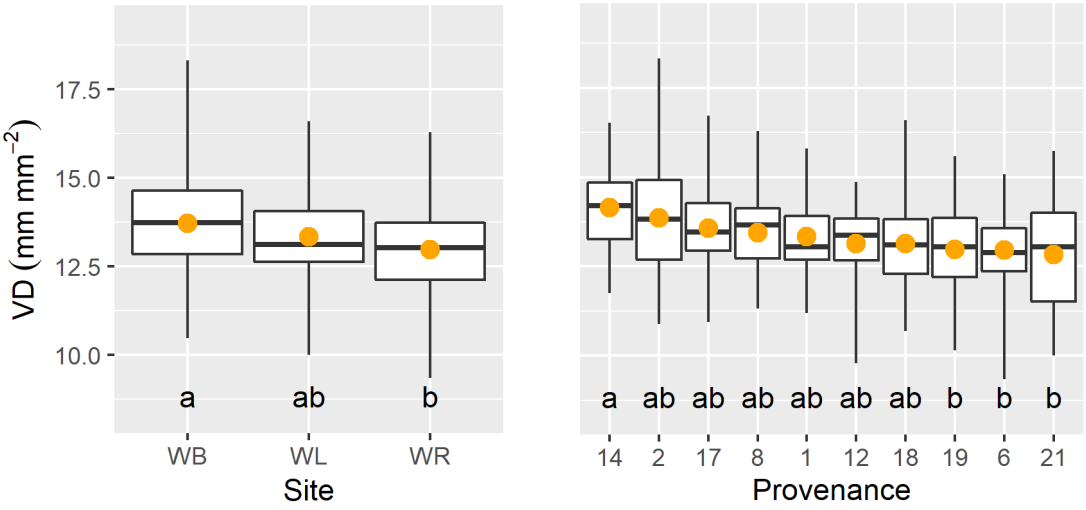

Figure 10. Tukey post-hoc test for site and for provenance of vein density.

Carbon Isotope Composition ($\delta^{13}C$)

In total, 270 samples were analyzed with $\delta^{13}C$ ranging from -31.98 to -26.09 ‰ (mean: -28.69 ‰).

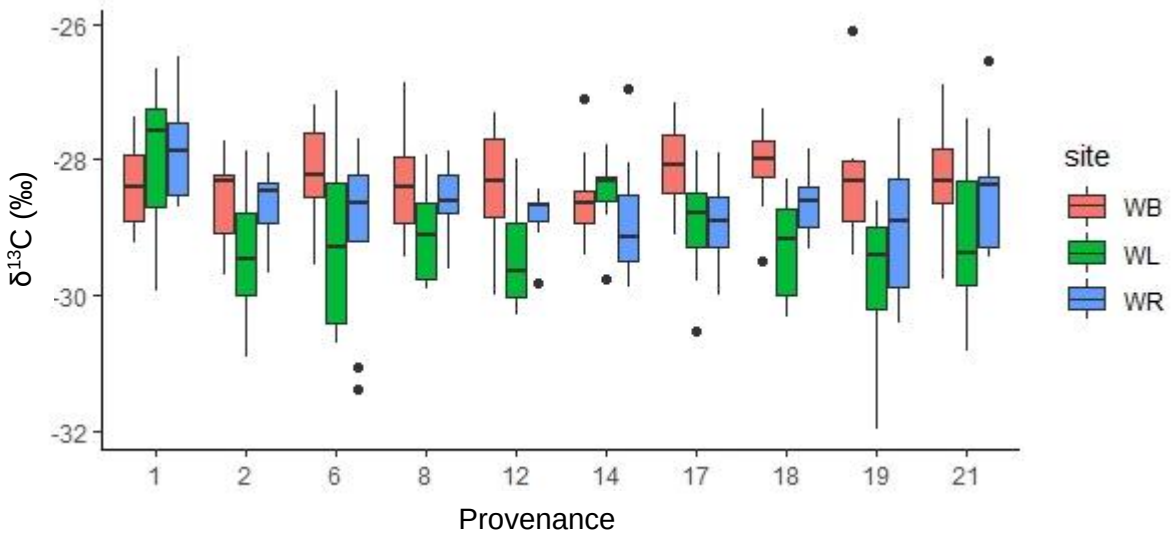

Figure 11. Boxplot showing the distribution of carbon isotope composition on site and provenance.

The analysis was highly significant for the main factor site, but only marginally significant at $p < 0.1$ for provenance. There was a significant interaction between the two factors at $p < 0.5$ (*Table 6*).

The mean of site WB with -28.33, was higher than that of WR with -28.68 and WL with -29.07. The highest mean among provenances had provenance 1 with -28.07, followed by provenance 14 with -28.63, the lowest mean was found in provenance 19 with -28.97 (*Figure 11*).

To display the interaction term an interaction plot was created (*Figure 12*). Provenances 2, 8, 12, 18, 19, 21 showed a more negative $\delta^{13}C$ for WL than for both WB and WR, while provenances 1, 6, 14 and 17 had a similar $\delta^{13}C$ between sites.

Table 6. ANOVA results for carbon isotope composition.

	SS	Df	P	F
Site	24.8	2	< 0.001	21.325
Provenance	9.0	9	0.096	1.726
Site : Prov	17.8	18	0.043	1.706

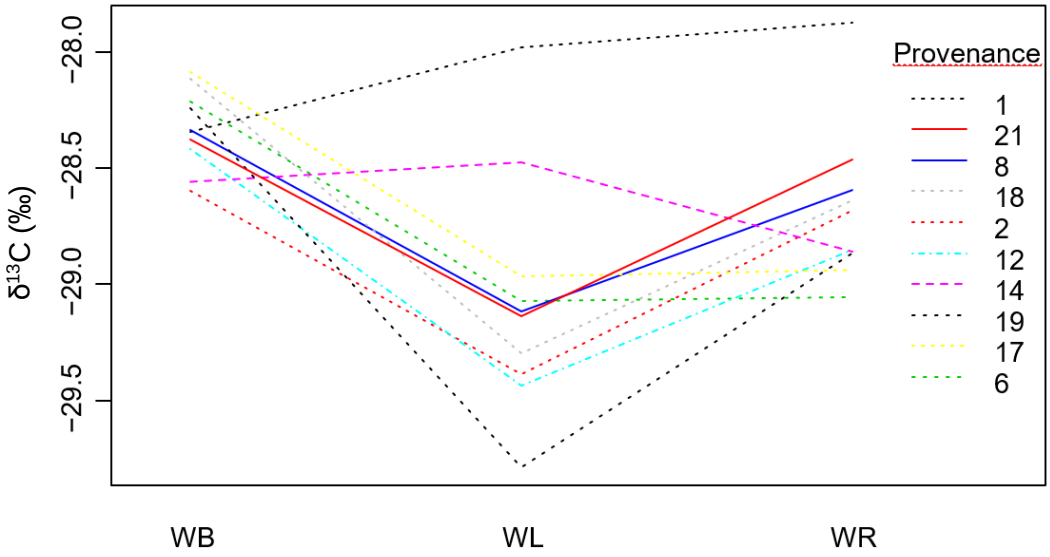

Figure 12. Interaction plot for site x provenance effect of carbon isotope composition.

Nitrogen Isotope Composition ($\delta^{15}N$)

In 270 samples, $\delta^{15}N$ ranged from -9.44 to -2.00 ‰ (mean: -5.82 ‰).

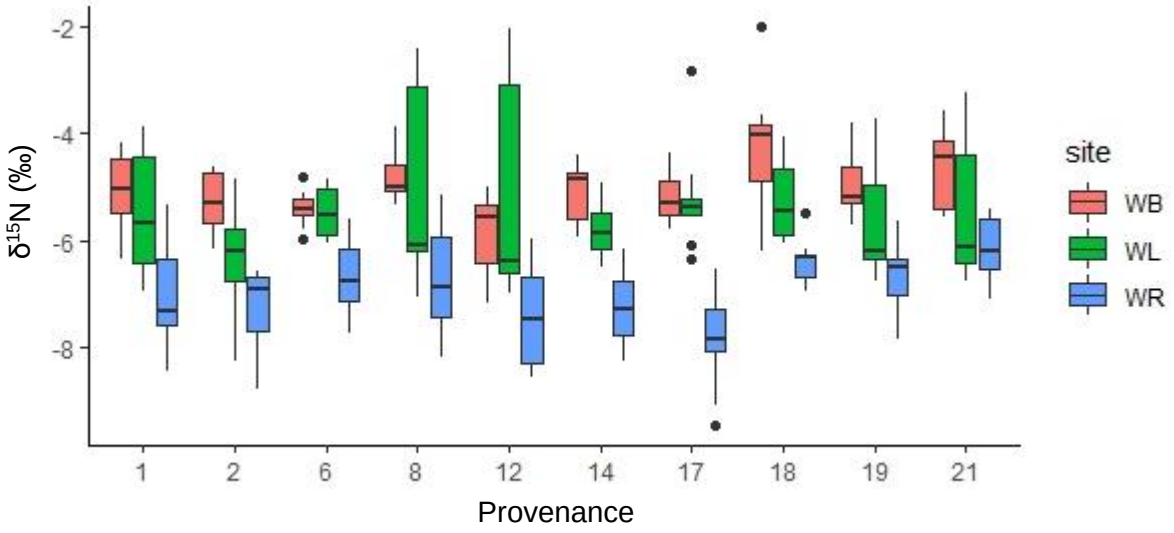

Figure 13. Boxplot showing the distribution of nitrogen isotope composition on site and provenance.

The analysis was highly significant for the main factor site and significant for the main factor provenance. There was no significant interaction between the two factors (*Table 7*).

The mean $\delta^{15}N$ of WR (-6.92) differed by more than 1 from WB (-5.05) and WL (-5.47) (*Figure 13*). The order from highest to lowest MAP, however, was WL > WR > WB. This order also described the degree of soil water availability and fertility of soil.

A Tukey post-hoc test showed that the all sites (WB, WL, WR) differed significantly from each other at $p < 0.05$. Provenance 18 and 21 were different from provenance 2 (*Figure 14*). Provenances 18 and 21 are the two provenances from Hungary, whereas provenance 2 comes from Austria, Linz. The Hungarian provenances have higher MAT than the Austrian one.

Table 7. ANOVA results for nitrogen isotope composition.

	SS	Df	P	F
Site	174.0	2	< 0.001	101.086
Provenance	24.7	9	< 0.01	3.190
Site : Prov	21.0	18	0.154	1.357

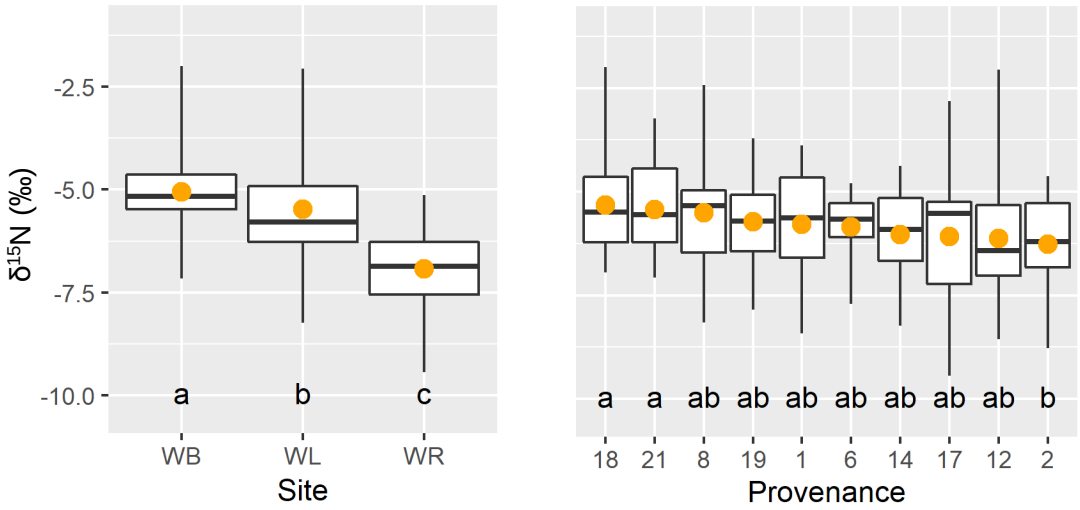

Figure 14. Tukey post-hoc test for site and provenance of nitrogen isotope composition.

Leaf carbon content

270 samples, with a range of 44.29 to 51.23 % (mean: 47.37 %) in leaf C content, were analyzed.

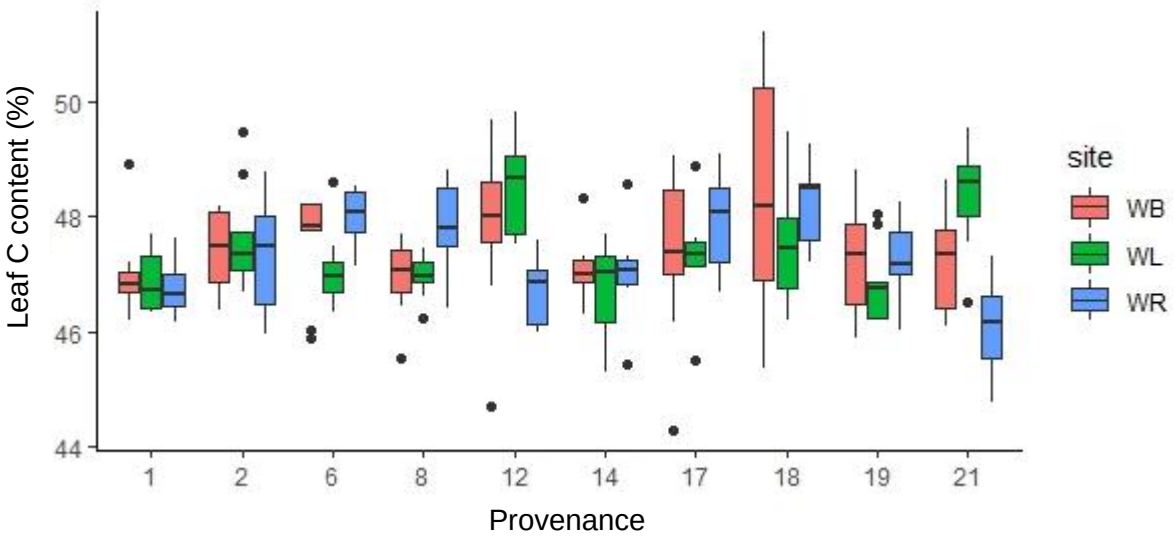

Figure 15. Boxplot showing the distribution of leaf carbon content on site and provenance.

The highest mean of sites was observed in WB (47.41), followed by WL (47.40) and WR (47.31), differences between means were not significant. The highest mean of provenances had provenance 18 (48.08), followed by provenance 12 (47.67) and lowest was found in provenance 1 (46.91) (*Figure 15)*. Provenance 1 is the westernmost among all provenances with a rather low MAT (8.7), whereas provenance 12 and 18 are found more in the east and show higher MATs (9.7 and 10.9, respectively).

An analysis of variance on leaf C content yielded significant variation among provenances and for the interaction term, but no significant variation among sites (*Table 8*).

Table 8. ANOVA results for leaf carbon content.

	SS	Df	P	F
Site	0.7	2	0.612	0.492
Provenance	19.7	9	< 0.01	3.251
Site : Prov	54.2	18	< 0.001	4.473

Leaf nitrogen content

In 270 samples, leaf nitrogen content ranged from 1.61 to 4.20 % (mean: 2.87 %).

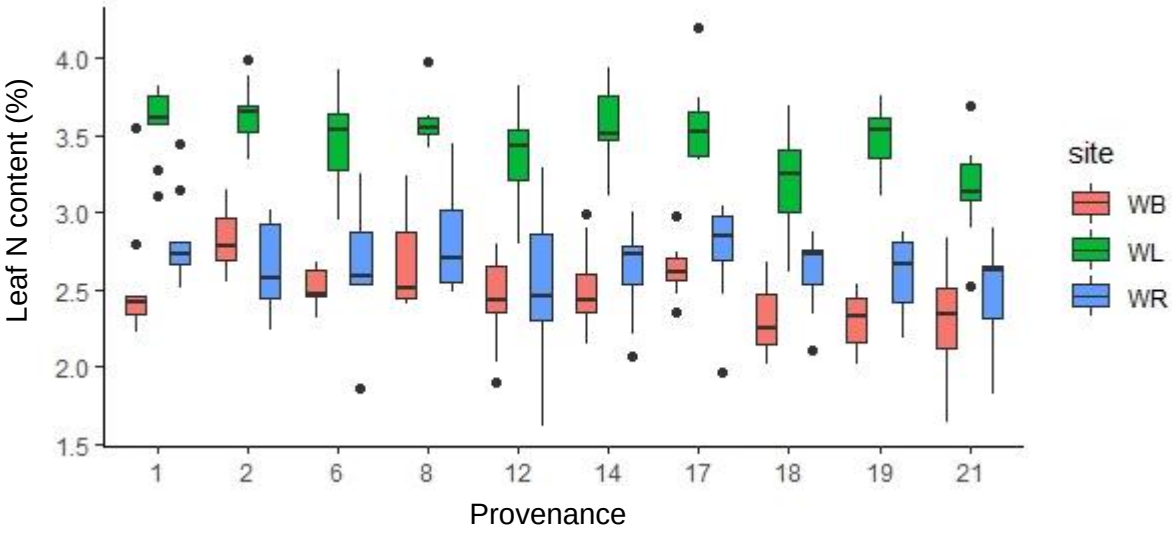

Figure 16. Boxplot showing the distribution of nitrogen content on site and provenance.

The mean of WL (3.46) was substantially higher than for WR (2.66) and WB (2.50). This reflected the pattern of highest to lowest MAP. Among provenances, the highest means showed provenance 2 and 8 (3.04 each) and the lowest mean was observed in provenance 21 (2.64) (*Figure 16*).

The analysis of variance showed a highly significant effect for both main factors, site and provenance. No interaction effect between site and provenance was observed (*Table 9*).

A Tukey post-hoc test showed that all sites (WB, WL, WR) differed significantly from each other at $p < 0.05$. The ten provenances showed two significantly differing groups at $p < 0.05$. Provenances 12, 18 and 21 differed significantly from provenances 2 and 8 (*Figure 17*). Provenance 19 did not differ significantly from any other provenance. Tukey post-hoc tests for provenance of $\delta^{15}N$ and leaf N content showed similar results (*Figure 14*).

Table 9. ANOVA results for leaf nitrogen content.

	SS	Df	P	F
Site	47.9	2	< 0.001	277.198
Provenance	4.9	9	< 0.001	6.262
Site : Prov	1.3	18	0.655	0.838

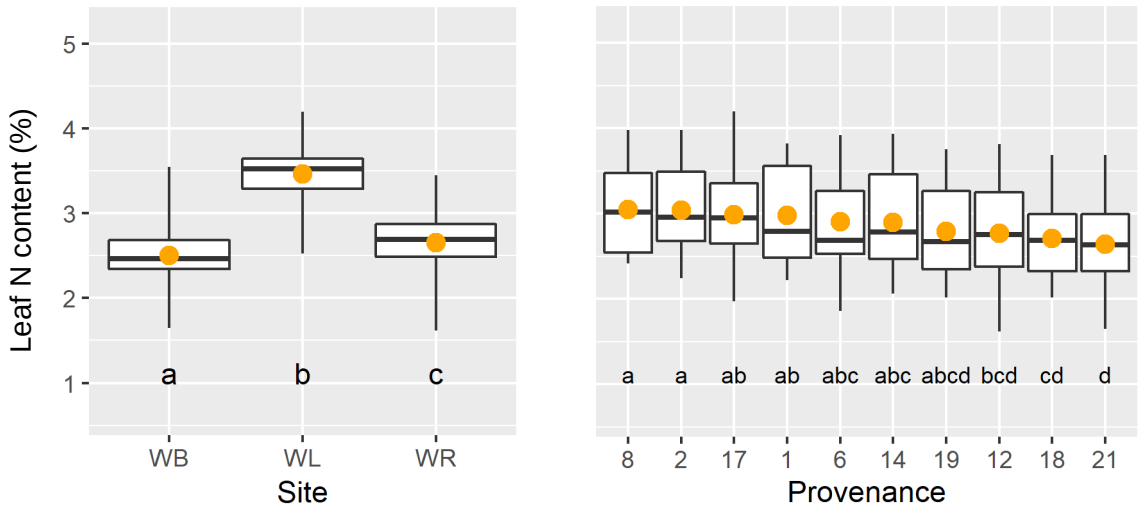

Figure 17. Tukey post-hoc test for site and provenance of leaf nitrogen content.

Effect size of site and provenance on traits

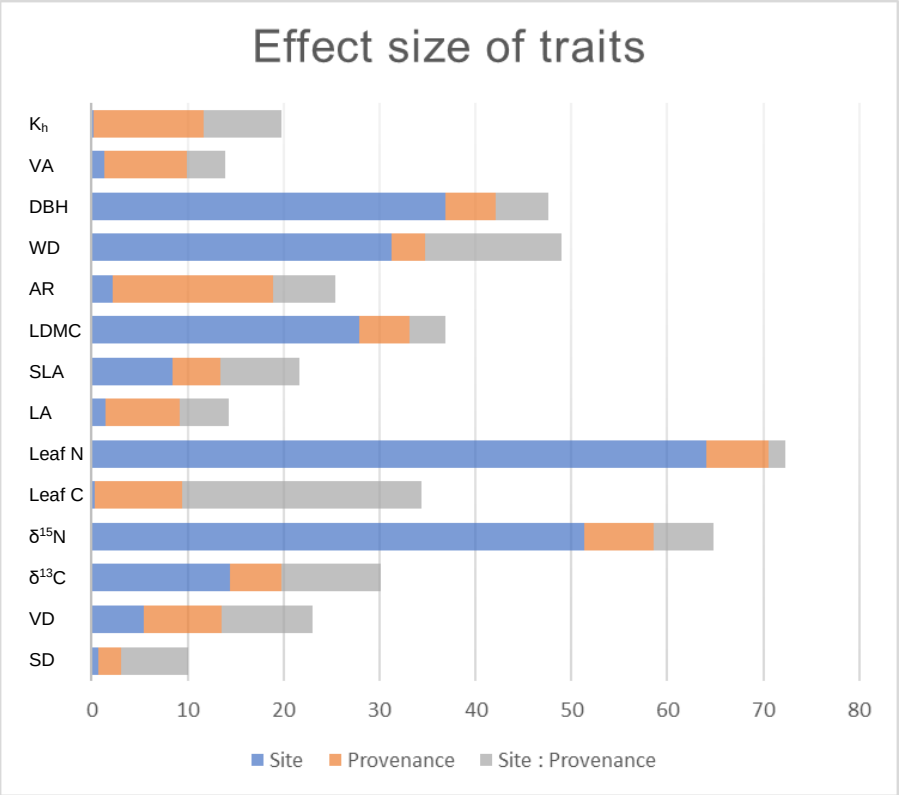

Figure 18. Effect size (η^2-test**)** of site, provenance and interaction term of leaf and wood traits.

Effect size was tested with an η^2-test (eta squared). The total variance explained was highest in leaf N content (72 %) and $\delta^{15}N$ (65 %), the lowest in stomatal density (10 %). Site explained more variance than provenance and the interaction term. Site explained 64 % of the change in leaf N content and 51 % in $\delta^{15}N$. Provenance explained most variance in AR, K_h and VA and the interaction term explained most in leaf C content with 25 % and SD (*Figure 18*).

Correlation matrix of selected traits and climate

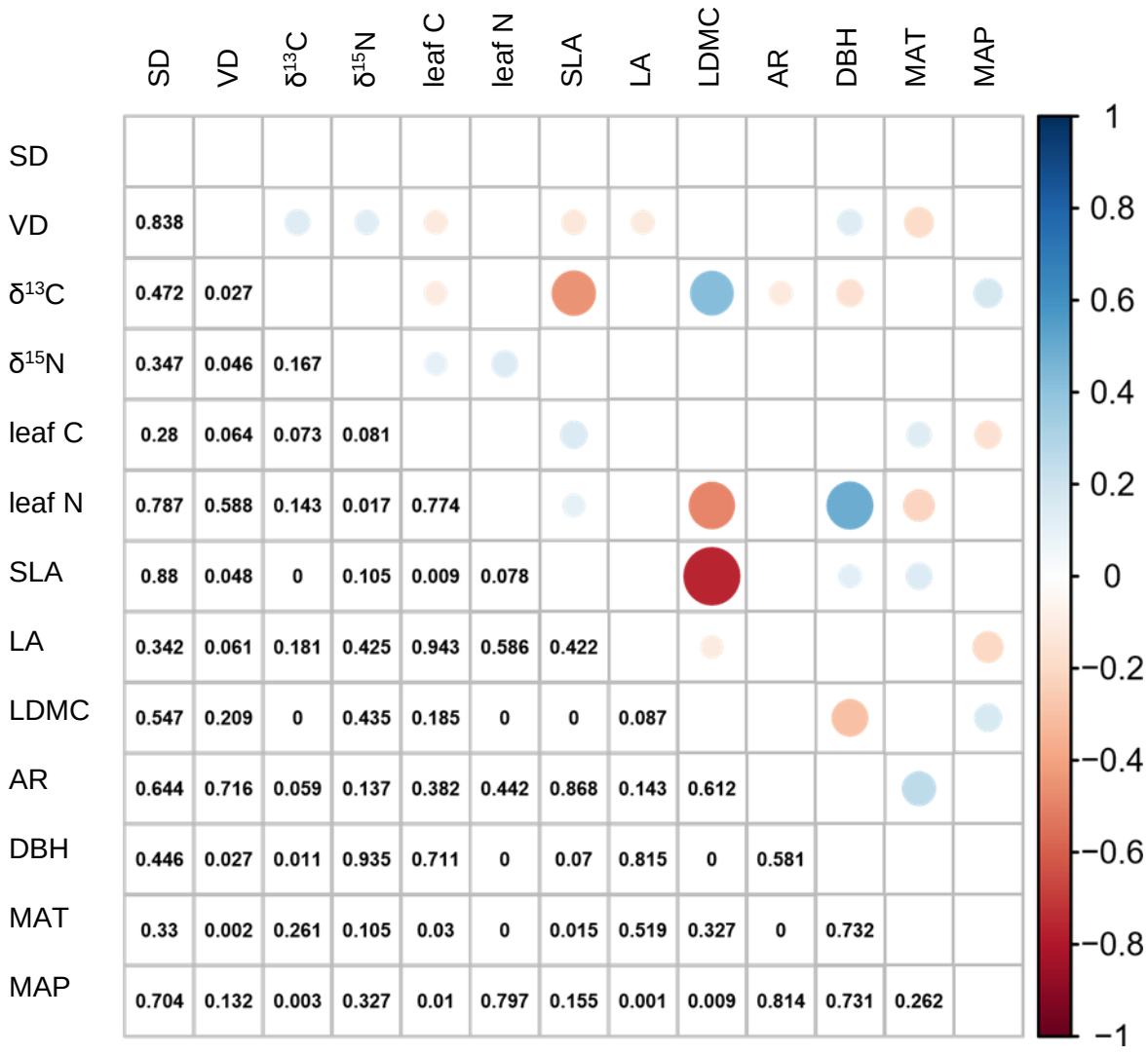

Figure 19. Correlation matrix of selected leaf traits, diameter at breast height and climate data of *Q. robur*. Correlation coefficient shown by symbol size and color intensity. Numbers in the lower diagonal are p-values.

Figure 19 shows that stronger bivariate correlations were found among leaf traits than between leaf traits and climate of origin. The strongest relationship was negative and found between SLA and LDMC, which had a correlation coefficient of r = -0.756 ($p < 0.001$). Further $\delta^{13}C$ correlated positively with LDMC and negatively with SLA. Leaf N content showed a positive correlation with DBH and a negative one with LDMC. Leaf trait data of $\delta^{13}C$, leaf C content, $\delta^{15}N$ and leaf N content, SLA, LA, LDMC and AR originated from the same leaves while a different leaf from the same branch was used for SD and VD. No bivariate correlation was observed between SD and other traits. VD, $\delta^{15}N$, leaf C content leaf C content, LA, AR, MAT and MAP only showed very weak correlations with other traits.

Principal Component Analysis (PCA)

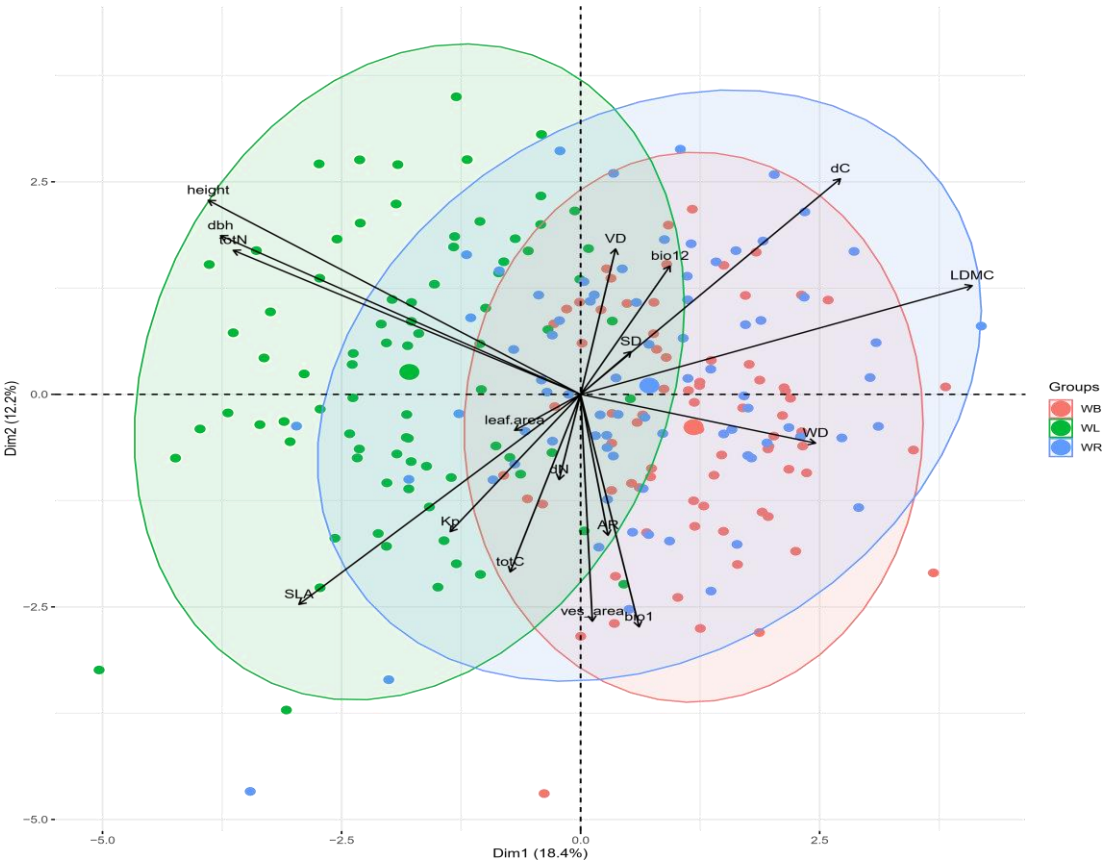

Figure 20. PCA biplot showing the first two principal components grouped by sites. Dim1 = first principal component, Dim2 = second principal component. Stomatal density (SD), vein density (VD), carbon isotope composition (dC), nitrogen isotope composition (dN), leaf C content (totC), leaf N content (totN), specific leaf area (SLA), leaf area (leaf.area), leaf dry matter content (LDMC), leaf aspect ratio (AR), vessel area (ves_area), K_p (theoretical hydraulic conductivity), diameter at breast height (dbh), tree height (height), wood density (WD), mean annual temperature (bio1) and mean annual precipitation (bio12).

The PCA in *Figure 20* shows the first two principal components explaining for 30.6% of the variance of the data. The first principal component was strongly correlated with five of the original variables. PC1 was most strongly correlated with LDMC, with a correlation of -0.42. The first principal component increases with increasing LDMC and decreases with DBH, tree height, leaf N and SLA. This suggests that these variables vary together. The second component mostly scaled with AR, leaf C content, MAP (bio 12 in *Figure 20*), MAT (bio 1 in *Figure 20*), VA, and VD. However, these variables accounted for less than 10% each for the total variance in PC2. Furthermore, AR, MAT and VA showed a strong positive correlation. Another strong positive correlation was found among LA, K_p and SLA. A third strong positive correlation can be observed between SD, $\delta^{13}C$ and MAP.

Discussion

Stomatal density

Stomatal density is thought to be of polygenic inheritance (Gailing et al. 2008). Next to the genetic factors, also environmental conditions can have an impact on variation of stomatal density (Bertolino et al. 2019). Stomatal density shows flexible responses to short term changes in environment, but is also strongly controlled by genetic factors in oaks (Gailing et al. 2008).

Recent studies advanced in uncovering the genetic and molecular mechanisms of stomatal density, especially in the model plant *Arabidopsis thaliana*. Genes regulating stomatal patterning were found. For example, overexpression of the genes *EPF1* (Hara et al. 2007) and EPF2 (Hunt and Gray 2009) were shown to decrease the number of stomata drastically. However, the functionality of such genes in *A. thaliana* remain to be verified within *Q. robur*.

Analysis of quantitative trait loci (QTL) is a tool that estimates number and effect of genes involved in complex traits like stomatal density (Gailing et al. 2008). Gailing et al. (2008) studied QTLs in a full-sib family of a cross between *Q. robur* and *Q. robur* subsp. *slavonica*. The seedlings examined were grown under greenhouse conditions. A great amount of genetically determined variation, in contrast to phenotypic variance, could be explained by the QTLs for stomatal density (from 63.6 % to 94.4 %).These findings disprove the results of Brendel et al. (2008), who could not find any QTLs for stomatal density in *Q. robur*. However, heritability (H^2) of stomatal density as a trait seems to be low to moderate (Keller et al. 2011, McKown et al. 2014, Dittberner et al. 2018).

The results of this current thesis failed to show significant differences in stomatal density in the ten provenances examined (*Table 4*). Cortan et al. (2017) similarly could not find significant differences in stomatal density in an experiment with four native populations of *Populus nigra* on different locations with uniform environmental conditions, but they did find inter-individual variation. Nevertheless, Pearce et al. (2006) were able to find significant differences in stomatal density among genotypes in a common garden experiment of riparian poplar species.

It is widely known that stomatal density can change according to environmental conditions during leaf development (Pearce et al. 2006). Changes in atmospheric CO_2 concentration and light can cause new developing leaves to adjust stomatal development to these changing conditions (Lake et al. 2001). In *Q. robur* this can already show within a one-year cycle, as trees produce two sets of leaves. Beerling and Chaloner (1993) made comparisons of these two sets of leaves and found that temperature had a greater

influence on stomatal density of *Q. robur* than CO_2 and light. Dittberner et al. (2018) found correlations of stomatal density and climatic principal components (PC), which included precipitation, temperature, water vapor pressure, wind speed and soil water content in *A. thaliana*.

Leaf material that was collected for my samples originated from sun exposed branches. Unfortunately, it was not possible to only use branches with the same orientation to light. This might have had an effect on stomatal density, as it can increase with increasing light intensity (Batos et al. 2010, Lake et al. 2001). A meta-analysis studied the responses of stomatal density to the daily light integral in 65 species. Stomatal density, and to a similar extent stomatal conductance, increased during growth with increasing daily light integral (Poorter et al. 2019).

Given the strong rainfall gradient (638-1066 mm/y) and moderate differences in mean temperature (8.4-8.8 °C/y) in the three sites studied (*Table 2*), a significant site effect was expected, but was not confirmed by the analysis of variance

Changes in stomata can lead to affect W_i, as they play a key role in the water and carbon dynamics of plants (Bertolino et al. 2019). Especially in case of droughts high W_i is of advantage. Drought stress and low water availability in deciduous trees (Pearce et al. 2006, Dunlap and Stettler 2001) and other species (Xu and Zhou 2008) has often been correlated with high stomatal densities and small stomata. A strong negative relationship between stomatal density and stomata size can be observed. Interestingly, Pearce et. al (2006) found in a common garden experiment of 51 genotypes of poplar species that adaxial stomatal size was not correlated with stomatal density, whereas abaxial size and density were correlated. Adaptations of maximum stomatal conductance to water vapor (g_{wmax}) to environmental factors can be achieved through increasing stomatal density paired with decreasing stomatal size (Franks and Beerling 2009, Franks, Drake and Beerling 2009).

Roussel et. al (2009) found that in a greenhouse experiment of a full-sib family, *Q. robur* genotypes with higher stomatal density also had higher stomatal conductance. It can be concluded that correlations between stomatal density, stomatal size and stomatal conductance have been found and all of them impact W_i. An increase in W_i together with high stomatal density might result in the ability to adjust to a gradually increasing water deficit and might indicate an evolutionary adaptation to changing environments (Xu and Zhou 2008, Bertolino et al. 2019, Dittberner et al. 2018).

Reflecting on W_i of *Q. robur* in this experiment, it seems the anatomical trait of stomatal density played a less important role than the physiological traits related to $\delta^{13}C$ in adapting to different water availabilities. Whereas I could not find any significance of site or

provenance in stomatal density (*Table 4*), $\delta^{13}C$ showed significant effects for site and for site x provenance. These effects will be discussed in the discussion of $\delta^{13}C$.

Vein density

The hydraulic pathway in plants sets major limitations to gas and water exchange (Brodribb, Feild and Sack 2010, Sack and Holbrook 2006). The leaf is pervaded with veins of different branching orders. This allows for a high volume of apoplastic flow approaching close to the sites of evaporation (Brodribb et al. 2007). The venation architecture of leaves shows great variability, which can be explained by the need for different water transport capacities. The resistance of vapor, diffusing from the living mesophyll cells and the epidermal cells inside the leaf to the atmosphere via the stomata, is often hundreds of times greater than the resistance to liquid moving through the dead xylem cells (Sack and Holbrook 2006, after: Cowan 1972).

The density of veins is an important trait of leaf venation, as it is directly linked to vein functions (Zhu et al. 2012). Vein density has a considerable influence on leaf hydraulic conductance (K_{leaf}), rates of gas exchange (g_s), stomatal conductance and stomatal density (Sack and Scoffoni 2013). K_{leaf} indicates how efficiently water is transported through the leaf (Sack and Holbrook 2006), which has consequences on stomatal opening and photosynthesis (Franks 2006). Recent studies suggested that vein density is positively correlated to K_{leaf} and g_s (Boyce et al. 2009)

Vein density within and among species have frequently shown to vary (Uhl and Mosbrugger 1999, Roth-Nebelsick et al. 2001, Dunbar-Co, Sporck and Sack 2009, Zhang et al. 2018). A greenhouse experiment showed that interspecific variations of vein density can be observed in *Quercus* species, with *Q. faginea* having significantly higher vein densities than *Q. robur* (Peguero-Pina et al. 2015).

The relationship of vein density and climatic conditions of ten populations of *Quercus variabilis* were studied by Zhu et. al (2012) in one common garden and several *in situ* experiments. Their results implied that vein density in *Q. variabilis* is genotypically adapted to long-term environmental conditions and therefore not responsive to short-term changes. This lends support to the significant difference found between the ten provenances of *Q. robur* in my thesis (*Table 5*), which can be interpreted as a genetical adaptation to their home sites. However, only 8.2 % of the variation can be explained by provenance (*Figure 18*). Zhu et. al (2012) showed that densities of minor and major veins show different reactions to environmental conditions. Minor vein density shows a close relationship to long-term environmental conditions like MAT and MAP in both *in situ* and common garden experiments. This to some extent contradicts results from the present study where site only explained 5.4 % of the variation in vein density. The conclusion

should be treated with caution, as the seedlings of the common garden were only observed in the first two years. Given that our findings are based on leaf material that was conserved with a delay, vein quality and as a consequence image quality varied and does not reliably permit to distinguish vein orders. Manual measurements could have provided more accurate results here but were considered too labor intensive.

By contrast, Zhang et. al (2018) found intraspecific differences in vein density between different environmental conditions. Strong associations between vein density and MAT, MAP, and elevation were found in *in situ* experiments of fourteen *Parrotia subaequalis* populations in eastern China. My results share similarities with Zhang et. al (2018), as a site effect could be found and as vein density correlates negatively with MAT of the provenance.

Uhl and Mosbrugger (1999) found similar measures of vein density in leaves of *Quercus petraea* and *Acer monspessulanum* grown under differing carbon dioxide concentrations, which were conducted with herbarium material and fresh material in gas chamber experiments. Stomatal density showed significantly lower numbers at 700 ppm carbon dioxide than at 350 ppm. Studies among species (Zhang et al. 2012, Oguro, Hinata and Tsunoda 1985) and within species (Brodribb and Jordan 2011) showed linear correlations of stomatal density and vein density. Assuming that carbon dioxide concentrations on the three experimental sites of my thesis were quite similar, such an expected correlation was not observed. Strong correlations between vein density and stomatal density support the idea that their development and function are coordinated (Brodribb and Jordan 2011, Zhang et al. 2012) to optimize photosynthetic yield relative to carbon investment in leaf venation.

Sun leaves compensate their typically smaller size, compared to shade leaves, by rising vein density in correspondence with the growing evaporative and photosynthetic demand for water (Brodribb 2009). In a trial of *Quercus rubra* it was investigated how hydraulic limitations impact leaf traits of different crown placement (Zwieniecki, Boyce and Holbrook 2004). Mature leaves had higher vein densities on the top of the canopy than on the bottom. At both canopy layers leaves showed a heterogenous distribution of vein density across the blade. Vein density was higher close to the margin and at the lobe tips, near to main veins it was the lowest. As already mentioned in the discussion of stomatal density, it was not always possible to use branches of the exact same orientation to light, which could have impacted my results.

Carbon isotope composition ($\delta^{13}C$)

An experiment with juvenile *Q. robur* clones (i.e., a full-sib family) of a controlled cross in a randomized complete block design at one site found medium to high heritability for $\delta^{13}C$ (Brendel et al. 2008). Within the same experiment quantitative trait loci (QTL) for $\delta^{13}C$ were detected in ten genomic regions. The major part of the clonal mean variance was controlled by few QTL, with one QTL contributing to more than 20 % of the phenotypic variance of $\delta^{13}C$ (Brendel et al. 2008). This result should be interpreted with caution, as Beavis (1994) showed in his simulations that small numbers of progeny (< 500) can lead to overestimations of QTL. Brendel et al. (2008) and Roussel et al. (2009) worked with the same progeny of *Q. robur* and found that QTL for $\delta^{13}C$ and W_i measured by gas exchange were colocalized and that these two traits were closely related, which confirms the model of Farquhar and Richards (1984) linking $\delta^{13}C$ to W_i. Furthermore, variability of both traits was mostly independent from different temporal frames and different environments (Roussel et al. 2009).

In situ experiments of adult co-occurring *Q. petraea* and *Q. robur* trees, displayed that the latter showed persistently higher isotopic discrimination and therefore lower W_i (Ponton et al. 2001). This implies genetic determination of $\delta^{13}C$, which I found imbedded in a site x provenance effect (*Table 6*). A significant interaction effect at $p = 0.043$ was found. This is further supported by the large intraspecies variability of water-use efficiency found in the mixed stands of Ponton et al. (2001). Farquhar and Richards (1984) expected that the greatest W_i may be found in genotypes of provenances with low water availability and high nutrient availability. However, this was not found in the present data, which showed no significant correlation between W_i and MAP at the provenances' home sites.

Different genotypes of forest trees growing under common environmental conditions showed that trees with higher W_i lose less water and/or preserve higher assimilation rates than trees with lower W_i (Guehl et al. 1995, cited in Brendel et al. 2008).

In naturally mixed Mediterranean *Quercus suber* and *Quercus ilex* plots in Portugal, intraspecific differences in $\delta^{13}C$ of the evergreen *Q. suber* were observed in trees growing in three different densities (Gouveia and Freitas 2008). Differences of 1.25 ‰ could be found between mean $\delta^{13}C$ of the different density plots. Gouveia and Freitas (2008) suggested that optimum density was a trade-off between increased retention and intraspecific competition of water resources and could therefore discriminate most against ^{13}C. This was observed in the tree density of 40 trees ha^{-1}, while densities of 20 and 60 trees ha^{-1} were less enriched in $\delta^{13}C$, likely because of lower water retention for low densities and due to increased competition for water in high densities.

With samples from a randomized block design, my results are not affected by variations in tree density. However, differences in rainfall between the three experimental sites, with a span of 428 mm in MAP, was substantial. This was reflected by the $\delta^{13}C$ values of the sites: drier locations, that are under more drought stress, showed less discrimination and higher W_i. Observed $\delta^{13}C$ site means in ascending order were WL < WR < WB. Gouveia and Freitas (2009) conducted another experiment with *Q. suber* plots to investigate the influence of precipitation on water-use efficiency. A difference of 4.2 ‰ in $\delta^{13}C$, with the higher discrimination at the wetter end, was found along an 800 mm rainfall gradient. This result suggests that leaf gas exchange at leaf level is strongly influenced by water availability. My samples showed a highly significant site effect for $\delta^{13}C$ at $p = 6.09e^{-09}$ (*Table 6*), which is likely due to the rainfall gradient.

Another influence to consider is leaf position, where leaves lower in the canopy have lower values of $\delta^{13}C$, which may be caused by higher c_i/c_a under low light (Farquhar et al. 1982). This is unlikely to have had a substantial effect on my data, where branches were generally collected from the sunlit part of the crown and the stands were all not very dense and low.

My results revealed a significant interaction effect of site and provenance of $\delta^{13}C$ in *Q. robur* at a rather high but significant p-value, $p = 0.043$. In a common garden experiment *Q. robur* and *Q. petraea* seedlings were subjected to different irradiance and water regimes. Water stress did not show strong environment × genotype interaction on $\delta^{13}C$ (Ponton et al. 2002), which concurs with the findings of this study (*Table 10*).

As mentioned above, $\delta^{13}C$ presents a trade-off among traits regulating assimilation rate and stomatal conductance (Gouveia and Freitas 2009). Consequently, W_i derived from $\delta^{13}C$ can be changed through systematic variation among genotypes of these traits (Roussel et al. 2009). The higher nitrogen availability at the site of WL should drive net CO_2 assimilation rate (A) and thereby increase $\delta^{13}C$, which could not be observed. A strong correlation of g_s with W_i and $\delta^{13}C$ of starch in *Q. robur* leaves suggested that stomatal conductance is an agent for variation in W_i, whereas A did not relate to the variability in $\delta^{13}C$ (Roussel et al. 2009). Apparently, the reduction in g_s had a greater effect on W_i and $\delta^{13}C$ than a likely increase in A. Furthermore, a correlation of stomatal density and $\delta^{13}C$ of bulk leaf samples of *Q. robur* was found. At the same time, genotypes with higher g_s had higher stomatal densities. Roussel et al. (2009) therefore suggested that a positive correlation of stomatal density with stomatal conductance could be the connection to the positive correlation detected of $\delta^{13}C$ with stomatal density and as a consequence lead to diversity of W_i among genotypes in *Q. robur*.

Nitrogen isotope composition ($\delta^{15}N$) and leaf nitrogen content

Nitrogen isotope composition ($\delta^{15}N$) in leaves is the result of many different physiological processes (Kolb and Evans 2002). $\delta^{15}N$ as a tracer of different source N pools can be inconsistent, because mixing pools and fractionation of ^{15}N and ^{14}N isotopes can distort differences in $\delta^{15}N$ among source N pools. In order to get reliable results, $\delta^{15}N$ should only be used as a tracer, if there are clear differences in $\delta^{15}N$ of the source N pools (Robinson 2001). $\delta^{15}N$ was measured in this study, because it could be detected in an Elemental Analyzer in the same run as $\delta^{13}C$ without additional expense.

The site of WB has rich Cambisols (Braunerde) that are moderately fresh to fresh on the lower slopes. The site WL has periodically wet Stagnosols (Hangpseudogley) in a sloping side. The site WR has a Stagnosol (Pseudogley), which is also periodically wet, but more stagnating than WL, because the area is flatter. The results for total nitrogen content in my leaf samples were highly significant for site and for provenance effect. The site of WL had a mean of 3.5 % N, whereas WR showed a mean of 2.7 % N and WB a mean of 2.5 % N (*Figure 16*). With the nitrogen availability differing between sites, it is likely that the $\delta^{15}N$ source signal also does. The provenance effect in leaf N content could indicate different uptake capacity and uptake mechanisms of the provenances, which might likely have an effect on ^{15}N discrimination during uptake. In addition, trees could also differ in the soil layers they preferentially absorb N from, which might also impact $\delta^{15}N$. Post-hoc Tukey tests on provenances of leaf N content (*Figure 17*) and $\delta^{15}N$ (*Figure 14*) showed similar patterns, where provenance 18 and 21 differed significantly from provenance 2.

In a study of a young *Q. robur* population living under a canopy of pines in Spain, leaf N content did not correlate with any soil variable (Gallardo and Covelo 2005). However, their study demonstrated spatial dependence in leaf N contents, which can also be the reason for the site effect of my results. Total variance explained in leaf N content (72 %) and $\delta^{15}N$ (65 %) was best described by site (*Figure 18*). Gallardo and Covelo (2005) also suggest, that the remaining variance of leaf N content to be explained, might be due to genotypical variability between *Q. robur* individuals. A study on *Q. acutissima* provenances in China (Wu et al. 2014) showed that leaf N content of provenances grown in a common garden with high extractable soil N concentration varied more strongly than that of *in situ Q. acutissima* and other *Quercus* species in China (Wu et al. 2012). Furthermore, it was found that leaf N content had a positive correlation with latitude and a negative correlation with MAT at the geographic origins. This could not be observed in this study, where significantly differing provenances did not show such obvious patterns.

Correlation matrix of selected traits and climate

Looking at *Figure 19*, LDMC and SLA showed a strong inverse relationship. SLA is a function of LDMC and leaf thickness and as a consequence can possibly relate in a simple way. This strong relationship was also found in a study, that observed a worldwide leaf economics spectrum (Wright et al. 2004).

A strong correlation between $\delta^{13}C$ and precipitation was found in a study on *Quercus suber*, where higher precipitation led to higher discrimination against ^{13}C. Sites that received over 1000 mm rainfall showed 20 % higher discrimination against ^{13}C, compared to the driest sites with only 500 mm (Gouveia and Freitas 2009). An ANOVA found significant differences in $\delta^{13}C$ among the three sites of *Q. robur*, where sites with higher means of $\delta^{13}C$ had lower MAP. In contrast, the direct correlation between $\delta^{13}C$ and MAP of provenances' home sites was positive, which would mean higher discrimination at lower rainfall. Gouveia and Freitas (2009) also found that leaves with higher SLA discriminated more against ^{13}C at higher rainfall. This is in line with the strong negative correlation found between SLA and $\delta^{13}C$ in this thesis (*Figure 19*). Further studies concurred with this finding (Ramírez-Valiente et al. 2010, Marchin, Sage and Ward 2008, Bussotti et al. 2002).

In a *Quercus petraea* common garden experiment SLA was found to relate negatively to MAP as well as MAT at the provenances' home sites (Torres-Ruiz et al. 2019). The current study does not support this finding, as a slightly positive correlation was found between SLA and MAT and no correlation between SLA and MAP.

Leaf N content among and within different species and different scales showed different relationships to LDMC. In an *in situ* study conducted with the grass *Phragmites australis* the relationship between leaf N content and LDMC, as well as SLA, were significant at inter-regional scale, but not significant at regional scale (Hu et al. 2015). High leaf N content in light-demanding trees improves plant metabolism and is positively correlated with respiration and assimilation (Keeling et al. 2008 after:, Wright et al. 2004, Poorter and Bongers 2006, Reich et al. 2006). Since the wetter site (WL) has more fertile soils, with higher nitrogen content, trees on that site grow faster and this affects leaf N content, as well as DBH positively.

Principal Component Analysis

The first principal component, which explained for 18.4 % of the variance, was more controlled by site, whereas the second principal component, which explained for 12.2 % of the variance, was more controlled by provenance.

The PCA showed that the site of WL compared to WR and WB tended to have higher growth (height, DBH), leaf N and SLA, as well as lower VD, LDMC and $\delta^{13}C$. This was also reflected in the ANOVA results, where all of the above mentioned traits showed a significant site effect (*Table 10)*. A post-hoc test showed that many of the provenances on WL had significantly more negative $\delta^{13}C$ than WB and WR (*Figure 12*) and that leaf N content had significantly higher means than the other sites (*Figure 17*).

The second principal component scaled the most with AR, leaf C, MAP, MAT, VA and VD. These parameters are mainly explained by provenance.

Environmental vs genetic control of trait variation

Table 10. ANOVA results for selected leaf, wood and growth traits.

Trait			Site	Provenance	Site x Prov	n
leaf	leaf area	LA	0.157	**0.024**	0.766	270
	leaf aspect ratio	AR	**0.044**	**1.10e^{-05}**	0.443	270
	specific leaf area	SLA	**7.67e^{-06}**	0.106	0.148	270
	leaf dry matter content	LDMC	**2.00e^{-16}**	**0.038**	0.753	265
	stomatal density	SD	0.455	0.733	0.522	267
	vein density	VD	**5.40e^{-04}**	**0.010**	0.083	262
	carbon isotope comp.	$\delta^{13}C$	**6.09e^{-09}**	0.096	**0.043**	270
	nitrogen isotope comp.	$\delta^{15}N$	**2.20e^{-16}**	**0.001**	0.154	270
	leaf carbon content	leaf C	0.612	**0.002**	**8.29e^{-08}**	270
	leaf nitrogen content	leaf N	**2.20e^{-16}**	**6.01e^{-08}**	0.655	270
wood	vessel area	VA	0.201	**0.02**	0.928	252
	theor. hydr. conduct.	K_h	0.768	**0.001**	0.244	252
	wood water content	WC	**4.64e^{-06}**	**0.038**	0.164	252
	prop. of cross-sectional area occupied by vessels	PV	**0.003**	**0.001**	0.053	252
	wood density	WD	**2.20e^{-16}**	0.125	**3.30e^{-05}**	252
growth	diam. at breast height	DBH	**2.00e^{-16}**	**0.014**	0.191	271
	tree height	H	**2.20e^{-16}**	**3.72e^{-07}**	**0.009**	271

Some leaf traits (LA, AR, SLA, LDMC), the wood traits (VA, K_h, WC, PV, WD) and the growth traits (DBH, H) were taken from Momirović (2019).

To integrate the provenance x site effect over the whole plant, I compared ANOVA results for leaf, wood, and growth traits. Among the leaf traits, significant site and provenance effect prevailed over the interaction effect (*Table 10*). A highly significant interaction effect was only observed for leaf C content, a far less significant interaction effect was observed in $\delta^{13}C$. Wood traits showed slightly more significant provenance than site effects. A significant interaction effect was only detected in wood density (WD). It seems wood anatomy traits (VA, K_h and PV) are more determined by provenance effects than leaf traits. WC was stronger affected by site, which likely relates to local water availability. Growth traits showed equal influence by site and provenance, whereas height in

comparison to diameter at breast height (DBH) also showed a significant interaction effect. Leaf shape and size are more controlled by the genetic factor and leaf anatomy traits (SLA, LDMC, VD) by the environment factor. Except for leaf C, where the site x provenance interaction dominated, all traits are mainly controlled by either site or provenance.

Torres-Ruiz et. al (2019) used a *Q. petraea* common garden experiment to quantify genetic variation in several leaf and woody traits. They observed that growth and phenology traits had higher genetic differentiation than traits linked to physiology, xylem anatomy and hydraulics. Growth and phenology traits were leaf unfolding, leaf senescence and tree height, which showed significant differences between provenances. Also, the leaf trait SLA was significantly different between provenances. No genetic differentiation could be observed in $\delta^{13}C$, $\delta^{15}N$, leaf C, leaf N, SD, VD and WD, which contrasts with the data presented here for $\delta^{15}N$, leaf C, leaf N, VD and WD. The number of significant results in leaf traits, however, was equally distributed among site and provenance. Since seeds were collected at the site of mother trees, epigenetic effects of the climatic conditions during seed development might have impacted estimates of population differentiation (Aitken et al. 2008).

The study of Torres-Ruiz et. al (2019) related the cline in leaf unfolding to differences in the climate of provenance origin and thereby indicated local adaptation to the environmental conditions in phenological traits for *Q. petraea*. Similarly, Hajek et al. (2016) found that most wood traits and some leaf traits in a *Fagus sylvatica* common garden experiment with ten provenances were related to the climate at provenance origin. This intraspecific genetic variability in the two species could facilitate adaptation to the ongoing climate change. However, the correlation matrix in *Figure 19* only found weak correlations of $R^2 < 0.1$ between the selected traits and the climate variables of MAT and MAP at the provenances' home sites. The difference between my results and others might be partially explained by the different selection of provenances and study designs.

Marker-based heritability estimates of *Populus balsamifera* in a greenhouse experiment, showed low heritability ($\hat{h}^2 < 0.5$) values, with no significance for $\delta^{13}C$, SD, g_s and with significance for SLA, W_i and leaf N (Keller et al. 2011). Height increment showed high and significant heritability ($\hat{h}^2 > 0.8$) in *P. balsamifera*. Results from mixed-model ANOVA showed that provenances differed significantly (after Bonferroni correction) in growth, but not in $\delta^{13}C$, SD and g_s. An exception was water-use efficiency estimated as the ratio of A/g_s, which also showed a significant provenance effect. Their study design did not allow to observe for site effects. However, their results regarding provenances contradicts with some of my findings. In *Q. robur* SLA did not show a provenance effect and $\delta^{13}C$ did in the

form of a significant interaction effect (*Table 10*). In regards of $\delta^{13}C$ the results indicate genetic variation in plasticity to adapt to different environments. This finding needs to be interpreted with caution, as the significance level of p = 0.043 is rather on the edge of significance. Q_{ST}-F_{ST} comparisons of growth and phenology (bud set, bud flush) traits indicated local adaptation to climate variation (Keller et al. 2011). Q_{ST}-F_{ST} comparisons are a means to track back the source of genetic variance in traits. By relating the genetic variance between populations to the total genetic variance of a trait, Q_{ST}, as well as to the variance at a specific locus, F_{ST}. If $Q_{ST} \approx F_{ST}$, this indicates that divergence could result from genetic drift alone. If $Q_{ST} > F_{ST}$, trait divergence likely origins from directional selection. If $Q_{ST} < F_{ST}$, trait divergence likely resulted from uniform or stabilizing selection (Leinonen et al. 2013).

Breeding trees, due to their long life-spans, takes longer than in agricultural crops. Traditional genetic improvement methods might not be sufficient to cope timely with the ongoing climate change and new approaches need to be developed. Narrow-sense heritability, $h^2 = V_A/V_P$, is defined as the proportion of phenotypic trait variance that is caused by additive genetic variance (Falconer 1989). Additive genetic variation and narrow-sense heritability can be used in breeding programs to select for future climatic demands (Harfouche et al. 2012). This can be of interest especially for growth, but also for some of the wood traits related to stress resistance or wood quality. Another forest practice that has recently received more attention is assisted gene flow. Pre-adapted individuals are used for plantations to enable faster adaptation of forest trees to climate change. For this strategy, local adaptation to environmental factors, such as climate, as well as the pattern of gene flow of a species need to be well understood (Aitken and Bemmels 2016, Aitken and Whitlock 2013)

Conclusions

The aim of this study was to determine the extent of genetic and environmental control on the leaf traits of stomatal density, vein density and isotope composition using a provenance trial. The different sites of the trial, as well as the provenances' home sites, showed different water and temperature regimes. Isotopic composition of carbon and nitrogen, as well as vein density were strongly influenced by the environment. Stomatal density, in contrast, did not show any significant effects. More pronounced site effects as opposed to prevailing provenance effects is a sign of high phenotypic plasticity (Torres-Ruiz et al. 2019). Phenotypic plasticity allows for greater tolerance to climate change, but at the same time species with high phenotypic plasticity might need longer to adapt to changing environments (Aitken et al. 2008).

References

Aas, G. 2014. Quercus robur. In *Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie*, 1-14. Wiley Online Library.

Adams, W. W. & I. Terashima. 2018. *The Leaf: A Platform for Performing Photosynthesis*. Springer.

Adler, P. B., R. Salguero-Gómez, A. Compagnoni, J. S. Hsu, J. Ray-Mukherjee, C. Mbeau-Ache & M. Franco (2014) Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences,* 111**,** 740.

Aitken, S. N. & J. B. Bemmels (2016) Time to get moving: assisted gene flow of forest trees. *Evolutionary Applications,* 9**,** 271-290.

Aitken, S. N. & M. C. Whitlock (2013) Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics,* 44**,** 367-388.

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang & S. Curtis-McLane (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary applications,* 1**,** 95-111.

Anderson, R., D. Edwards, J. Batley & P. E. Bayer (2020) Genome-Wide Association Studies in Plants. *eLS, John Wiley & Sons, Ltd (Ed.)*.

Baertschi, P. (1953) Die Fraktionierung der natürlichen Kohlenstoffisotopen im Kohlendioxydstoffwechsel grüner Pflanzen. *Helvetica Chimica Acta,* 36**,** 773-781.

Batič, F., T. Sinkovič & B. Javornik (1995) Morphological and genetic variability of pedunculate oak (Quercus robur L.) populations in Slovenia. *Zbornik gozdarstva in lesarstva***,** 75-96.

Batos, B., D. Vilotić, S. Orlović & D. Miljković (2010) Inter and intra-population variation of leaf stomatal traits of Quercus robur L. in northern Serbia. *Archives of Biological Sciences,* 62**,** 1125-1136.

Beavis, W. D. 1994. The power and deceit of QTL experiments: lessons from comparative QTL studies. 250-266. Washington, DC.

Beerling, D. J. & W. G. Chaloner (1993) The impact of atmospheric CO2 and temperature changes on stomatal density: observation from Quercus robur lammas leaves. *Annals of Botany,* 71**,** 231-235.

Bender, M. M. (1968) Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon,* 10**,** 468-472.

Bertolino, L. T., R. S. Caine & J. E. Gray (2019) Impact of stomatal density and morphology on water-use efficiency in a changing world. *Frontiers in plant science,* 10.

Bossema, I. (1979) Jays and Oaks: an Eco-Ethological Study of a Symbiosis. *Behaviour,* 70**,** 1-116.

Boyce, C. K., T. J. Brodribb, T. S. Feild & M. A. Zwieniecki (2009) Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society B: Biological Sciences,* 276**,** 1771-1776.

Brendel, O., D. Le Thiec, C. Scotti-Saintagne, C. Bodénès, A. Kremer & J.-M. Guehl (2008) Quantitative trait loci controlling water use efficiency and related traits in Quercus robur L. *Tree Genetics & Genomes,* 4**,** 263-278.

Brewer, S., R. Cheddadi, J. L. De Beaulieu & M. Reille (2002) The spread of deciduous Quercus throughout Europe since the last glacial period. *Forest ecology and management,* 156**,** 27-48.

Brodribb, T. J. (2009) Xylem hydraulic physiology: the functional backbone of terrestrial plant productivity. *Plant Science,* 177**,** 245-251.

Brodribb, T. J., T. S. Feild & G. J. Jordan (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant physiology,* 144**,** 1890-1898.

Brodribb, T. J., T. S. Feild & L. Sack (2010) Viewing leaf structure and evolution from a hydraulic perspective. *Functional Plant Biology,* 37**,** 488-498.

Brodribb, T. J. & G. J. Jordan (2011) Water supply and demand remain balanced during leaf acclimation of Nothofagus cunninghamii trees. *New Phytologist,* 192**,** 437-448.

Buckley, T. N., G. P. John, C. Scoffoni & L. Sack (2015) How does leaf anatomy influence water transport outside the xylem? *Plant Physiology,* 168**,** 1616-1635.
Burgess, J., K. Szlavecz, N. Rajakaruna & C. Swan (2015) Ecotypic differentiation of mid-Atlantic *Quercus* species in response to ultramafic soils. *Australian Journal of Botany,* 63**,** 308-323.
Bussotti, F., D. Bettini, P. Grossoni, S. Mansuino, R. Nibbi, C. Soda & C. Tani (2002) Structural and functional traits of Quercus ilex in response to water availability. *Environmental and Experimental Botany,* 47**,** 11-23.
Cieslar, A. (1899) Neues aus dem Gebiete der forstlichen Zuchtwahl. *Centralblatt für das gesamte Forstwesen,* 25.
Cortan, D., D. Vilotic, M. Sijacic-Nikolic & D. Miljkovic (2017) Leaf stomatal traits variation within and among black poplar native populations in Serbia. *Bosque (Valdivia),* 38**,** 337-345.
Cowan, I. R. (1972) An electrical analogue of evaporation from, and flow of water in plants. *Planta,* 106**,** 221-226.
De Boer, H. J., M. B. Eppinga, M. J. Wassen & S. C. Dekker (2012) A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. *Nature Communications,* 3**,** 1221.
de Villemereuil, P., O. E. Gaggiotti, M. Mouterde & I. Till-Bottraud (2016) Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity,* 116**,** 249-254.
Deines, P. 1980. The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry,* eds. P. Fritz & J. C. Fontes, 329-406. Amsterdam: Elsevier.
Dittberner, H., A. Korte, T. Mettler-Altmann, A. P. M. Weber, G. Monroe & J. de Meaux (2018) Natural variation in stomata size contributes to the local adaptation of water-use efficiency in Arabidopsis thaliana. *Molecular ecology,* 27**,** 4052-4065.
Doube, M., M. M. Kłosowski, I. Arganda-Carreras, F. P. Cordelières, R. P. Dougherty, J. S. Jackson, B. Schmid, J. R. Hutchinson & S. J. Shefelbine (2010) BoneJ: free and extensible bone image analysis in ImageJ. *Bone,* 47**,** 1076-1079.
Ducousso, A. & S. Bordacs (2004) EUFORGEN Technical Guidelines for Genetic Conservation and Use for Pedunculate and Sessile oaks (Quercus robur and Q. petraea). *International Plant Genetic Resources Institute, Rome*.
Dunbar-Co, S., M. J. Sporck & L. Sack (2009) Leaf trait diversification and design in seven rare taxa of the Hawaiian Plantago radiation. *International Journal of Plant Sciences,* 170**,** 61-75.
Dunlap, J. M. & R. F. Stettler (2001) Variation in leaf epidermal and stomatal traits of Populus trichocarpa from two transects across the Washington Cascades. *Canadian Journal of Botany,* 79**,** 528-536.
Eaton, E., G. Caudullo, S. Oliveira & D. de Rigo (2016) Quercus robur and Quercus petraea in Europe: distribution, habitat, usage and threats In: San-Miguel-Ayanz. *J, de Rigo, D, Caudullo, G, Houston Durrant, T, Mauri, A (eds) European atlas of forest tree species, Publication Office of the European Union, Luxembourg*.
Fajardo, A. & A. Siefert (2016) Phenological variation of leaf functional traits within species. *Oecologia,* 180**,** 951-959.
Falconer, D. S. 1989. *Introduction to Quantitative Genetics*. London: Longman.
Farquhar, G. D., K. T. Hubick, A. G. Condon & R. A. Richards. 1989. Carbon Isotope Fractionation and Plant Water-Use Efficiency. In *Stable Isotopes in Ecological Research,* eds. P. W. Rundel, J. R. Ehleringer & K. A. Nagy, 21-40. New York, NY: Springer New York.
Farquhar, G. D., M. H. O'Leary & J. A. Berry (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology,* 9**,** 121-137.
Farquhar, G. D. & R. A. Richards (1984) Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. *Functional Plant Biology,* 11**,** 539-552.

Fetter, K. C., S. Eberhardt, R. S. Barclay, S. Wing & S. R. Keller (2019) StomataCounter: a neural network for automatic stomata identification and counting. *New Phytologist,* 223**,** 1671-1681.
Franks, P. J. (2006) Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients. *Plant, Cell & Environment,* 29**,** 584-592.
Franks, P. J. & D. J. Beerling (2009) CO2-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic. *Geobiology,* 7**,** 227-236.
Franks, P. J., P. L. Drake & D. J. Beerling (2009) Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using Eucalyptus globulus. *Plant, cell & environment,* 32**,** 1737-1748.
Franks, P. J. & G. D. Farquhar (2007) The Mechanical Diversity of Stomata and Its Significance in Gas-Exchange Control. *Plant Physiology,* 143**,** 78.
Gailing, O., R. Langenfeld-Heyser, A. Polle & R. Finkeldey (2008) Quantitative trait loci affecting stomatal density and growth in a Quercus robur progeny: implications for the adaptation to changing environments. *Global Change Biology,* 14**,** 1934-1946.
Gallardo, A. & F. Covelo (2005) Spatial pattern and scale of leaf N and P concentration in a Quercus robur population. *Plant and Soil,* 273**,** 269-277.
Gebauer, G. 2011. EA-IRMS coupling. Bayreuth: Universität Bayreuth.
Gouveia, A. C. & H. Freitas (2008) Intraspecific competition and water use efficiency in Quercus suber: evidence of an optimum tree density? *Trees,* 22**,** 521.
--- (2009) Modulation of leaf attributes and water use efficiency in Quercus suber along a rainfall gradient. *Trees,* 23**,** 267-275.
Guehl, J. M., A. Nguyen Queyrens, D. Loustau & A. Ferhi (1995) Genetic and environmental determinants of water use efficiency and carbon isotope discrimination in forest trees. *Colloques de l'INRA (France)*.
Hajek, P., D. Kurjak, G. von Wühlisch, S. Delzon & B. Schuldt (2016) Intraspecific variation in wood anatomical, hydraulic, and foliar traits in ten European beech provenances differing in growth yield. *Frontiers in plant science,* 7**,** 791.
Hamrick, J. L., M. J. W. Godt & S. L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. In *Population genetics of forest trees*, 95-124. Springer.
Hara, K., R. Kajita, K. U. Torii, D. C. Bergmann & T. Kakimoto (2007) The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. *Genes & development,* 21**,** 1720-1725.
Harfouche, A., R. Meilan, M. Kirst, M. Morgante, W. Boerjan, M. Sabatti & G. S. Mugnozza (2012) Accelerating the domestication of forest trees in a changing world. *Trends in plant science,* 17**,** 64-72.
Houghton, J. T., Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell & C. A. Johnson. 2001. *Climate change 2001: the scientific basis*. The Press Syndicate of the University of Cambridge.
Hu, Y.-K., X. Pan, G.-F. Liu, W.-B. Li, W.-H. Dai, S.-L. Tang, Y.-L. Zhang, T. Xiao, L.-Y. Chen, W. Xiong, M.-Y. Zhou, Y.-B. Song & M. Dong (2015) Novel evidence for within-species leaf economics spectrum at multiple spatial scales. *Frontiers in Plant Science,* 6**,** 901.
Hunt, L. & J. E. Gray (2009) The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. *Current Biology,* 19**,** 864-869.
Huntley, B. & H. J. B. Birks. 1983. *An atlas of past and present pollen maps for Europe: 0-13000 years ago*. London: Cambridge University Press.
Kawecki, T. J. & D. Ebert (2004) Conceptual issues in local adaptation. *Ecology letters,* 7**,** 1225-1241.
Keeling, H. C., T. R. Baker, R. V. Martinez, A. Monteagudo & O. L. Phillips (2008) Contrasting patterns of diameter and biomass increment across tree functional groups in Amazonian forests. *Oecologia,* 158**,** 521-534.
Keller, S. R., R. Y. Soolanayakanahally, R. D. Guy, S. N. Silim, M. S. Olson & P. Tiffin (2011) Climate-driven local adaptation of ecophysiology and phenology in balsam

poplar, Populus balsamifera L.(Salicaceae). *American Journal of Botany,* 98**,** 99-108.
Kellomäki, S., I. Rouvinen, H. Peltola, H. Strandman & R. Steinbrecher (2001) Impact of global warming on the tree species composition of boreal forests in Finland and effects on emissions of isoprenoids. *Global Change Biology,* 7**,** 531-544.
Kleinschmit, J. 1993. Intraspecific variation of growth and adaptive traits in European oak species. 166s-185s. EDP Sciences.
Koho, S., E. Fazeli, J. E. Eriksson & P. E. Hänninen (2016) Image quality ranking method for microscopy. *Scientific reports,* 6**,** 28962.
Kolb, K. J. & R. D. Evans (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. *New Phytologist,* 156**,** 57-64.
Krahl-Urban & Joachim (1956) Über Eichen-Provenienzversuche. *Silvae Genetica,* 6**,** 27.
---. 1959. Die Eichen. In *Forstliche Monographie der Traubeneiche und der Stieleiche*, 288. Hamburg und Berlin: Verlag Paul Parey.
Krahl-Urban, J. 1959. Die Eichen. In *Forstliche Monographie der Traubeneiche und der Stieleiche*, 288. Hamburg und Berlin: Verlag Paul Parey.
Kremer, A. (2010) Evolutionary responses of European oaks to climate change. *Irish Forestry*.
Kremer, A., J. Kleinschmit, J. Cottrell, E. P. Cundall, J. D. Deans, A. Ducousso, A. O. König, A. J. Lowe, R. C. Munro, R. J. Petit & B. R. Stephan (2002) Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection on genetic diversity in European oaks? *Forest Ecology and Management,* 156**,** 75-87.
Lake, J. A., W. P. Quick, D. J. Beerling & F. I. Woodward (2001) Signals from mature to new leaves. *Nature,* 411**,** 154-154.
Langlet, O. (1971) Two hundred years genecology. *Taxon,* 20**,** 653-721.
Larcher, W. 1994. *Ökophysiologie der pflanzen*. Eugen Ulmer Stuttgart.
Leinonen, T., R. J. S. McCairns, R. B. O'Hara & J. Merilä (2013) Q ST–F ST comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics,* 14**,** 179.
Lu, Z.-M. (1989) Ratio of stomatal resistance on two sides of wheat leaves as affected by soil water content. *Agricultural and forest meteorology,* 49**,** 1-7.
Marchin, R. M., E. L. Sage & J. K. Ward (2008) Population-level variation of Fraxinus americana (white ash) is influenced by precipitation differences across the native range. *Tree physiology,* 28**,** 151-159.
Mariette, S., J. Cottrell, U. M. Csaikl, P. Goikoechea, A. Konig, A. J. Lowe, B. C. Van Dam, T. Barreneche, C. Bodénès & R. Streiff (2002) Comparison of levels of genetic diversity detected with AFLP and microsatellite markers within and among mixed Q. petraea (Matt.) Liebl. and Q. robur L. stands. *Silvae Genetica,* 51**,** 72-79.
Masarovlčová, E. (1991) Leaf shape, stomata density and photosynthetic rate of the common oak leaves. *Biologia plantarum,* 33**,** 495-500.
Matyas, C. (1996) Climatic adaptation of trees: rediscovering provenance tests. *Euphytica,* 92**,** 45-54.
McKown, A. D., R. D. Guy, L. Quamme, J. Klápště, J. La Mantia, C. P. Constabel, Y. A. El-Kassaby, R. C. Hamelin, M. Zifkin & M. S. Azam (2014) Association genetics, geography and ecophysiology link stomatal patterning in P opulus trichocarpa with carbon gain and disease resistance trade-offs. *Molecular Ecology,* 23**,** 5771-5790.
Momirović, N. 2019. Environmental and genetic control of functional traits in a provenance trial of oak (Quercus robur). 80 Blätter, Diagramme, Karten. Vienna, 2019.
Mudrák, O., J. Doležal, A. Vítová & J. Lepš (2019) Variation in plant functional traits is best explained by the species identity: Stability of trait-based species ranking across meadow management regimes. *Functional Ecology,* 33**,** 746-755.
O'Leary, M. H. (1981) Carbon isotope fractionation in plants. *Phytochemistry,* 20**,** 553-567.
Oguro, H., K. Hinata & S. Tsunoda (1985) Comparative anatomy and morphology of leaves between C3 and C4 species in Panicum. *Annals of botany,* 55**,** 859-867.

Organisation for Economic Cooperation and Development. 2018. OECD Scheme for the Certification of Forest Reproductive Material moving in International Trade. Paris: OECD.
Park, R. & S. Epstein (1960) Carbon isotope fractionation during photosynthesis. *Geochim. et cosmochim. acta,* 21.
Parmesan, C. & G. Yohe (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* 421**,** 37.
Pearce, D. W., S. Millard, D. F. Bray & S. B. Rood (2006) Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree Physiology,* 26**,** 211-218.
Peguero-Pina, J. J., S. Sisó, D. Sancho-Knapik, A. Díaz-Espejo, J. Flexas, J. Galmés & E. Gil-Pelegrín (2015) Leaf morphological and physiological adaptations of a deciduous oak (Quercus faginea Lam.) to the Mediterranean climate: a comparison with a closely related temperate species (Quercus robur L.). *Tree physiology,* 36**,** 287-299.
Pinti, D. 2011. Delta, Isotopic. In *Encyclopedia of Astrobiology,* eds. M. Gargaud, R. Amils, J. C. Quintanilla, H. J. Cleaves, W. M. Irvine, D. L. Pinti & M. Viso, 418-418. Berlin, Heidelberg: Springer Berlin Heidelberg.
Plymale, E. L. & R. B. Wylie (1944) The major veins of mesomorphic leaves. *American Journal of Botany***,** 99-106.
Ponton, S., J.-L. Dupouey, N. Bréda & E. Dreyer (2002) Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype× environment interactions. *Tree Physiology,* 22**,** 413-422.
Ponton, S., J. L. Dupouey, N. Breda, F. Feuillat, C. Bodenes & E. Dreyer (2001) Carbon isotope discrimination and wood anatomy variations in mixed stands of Quercus robur and Quercus petraea. *Plant, Cell & Environment,* 24**,** 861-868.
Poorter, H., Ü. Niinemets, N. Ntagkas, A. Siebenkäs, M. Mäenpää, S. Matsubara & T. Pons (2019) A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist*.
Poorter, L. & F. Bongers (2006) Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology,* 87**,** 1733-1743.
Price, C. A., P. R. T. Munro & J. S. Weitz (2014) Estimates of leaf vein density are scale dependent. *Plant physiology,* 164**,** 173-180.
Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, W. K. Cornwell, J. M. Craine, D. E. Gurvich, C. Urcelay, E. J. Veneklaas, P. B. Reich, L. Poorter, I. J. Wright, P. Ray, L. Enrico, J. G. Pausas, A. C. de Vos, N. Buchmann, G. Funes, F. Quétier, J. G. Hodgson, K. Thompson, H. D. Morgan, H. ter Steege, L. Sack, B. Blonder, P. Poschlod, M. V. Vaieretti, G. Conti, A. C. Staver, S. Aquino & J. H. C. Cornelissen (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany,* 61**,** 167-234.
Ramírez-Valiente, J. A., D. Sánchez-Gómez, I. Aranda & F. Valladares (2010) Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiology,* 30**,** 618-627.
Raven, J. A. (2014) Speedy small stomata? *Journal of Experimental Botany,* 65**,** 1415-1424.
Reich, P. B., M. G. Tjoelker, J.-L. Machado & J. Oleksyn (2006) Universal scaling of respiratory metabolism, size and nitrogen in plants. *Nature,* 439**,** 457.
Rellstab, C., S. Zoller, L. Walthert, I. Lesur, A. R. Pluess, R. Graf, C. Bodénès, C. Sperisen, A. Kremer & F. Gugerli (2016) Signatures of local adaptation in candidate genes of oaks (Quercus spp.) with respect to present and future climatic conditions. *Molecular Ecology,* 25**,** 5907-5924.
Robinson, D. (2001) δ15N as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution,* 16**,** 153-162.
Roth-Nebelsick, A., D. Uhl, V. Mosbrugger & H. Kerp (2001) Evolution and function of leaf venation architecture: a review. *Annals of Botany,* 87**,** 553-566.

Rounick, J. S. & M. J. Winterbourn (1986) Stable carbon isotopes and carbon flow in ecosystems. *BioScience,* 36**,** 171-177.
Roussel, M., D. Le Thiec, P. Montpied, N. Ningre, J.-M. Guehl & O. Brendel (2009) Diversity of water use efficiency among Quercus robur genotypes: contribution of related leaf traits. *Annals of Forest Science,* 66**,** 1-10.
RStudio Team (2018) RStudio: Integrated Development for R. *RStudio, Inc., Boston, MA URL http://www.rstudio.com*.
Sack, L. & N. M. Holbrook (2006) Leaf hydraulics. *Annu. Rev. Plant Biol.,* 57**,** 361-381.
Sack, L. & C. Scoffoni (2013) Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist,* 198**,** 983-1000.
Savolainen, O., T. Pyhäjärvi & T. Knürr (2007) Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Evol. Syst.,* 38**,** 595-619.
Shukla, P. R., J. Skea, R. Slade, R. van Diemen, E. Haughey, J. Malley, M. Pathak & J. Portugal Pereira. 2019. Technical Summary. In *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems,* eds. P. R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.-O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi & J. Malley. In press.
Simonin, K. A. & A. B. Roddy (2018) Genome downsizing, physiological novelty, and the global dominance of flowering plants. *PLoS biology,* 16**,** e2003706.
Sork, V. L., K. A. Stowe & C. Hochwender (1993) Evidence for Local Adaptation in Closely Adjacent Subpopulations of Northern Red Oak (Quercus rubra L.) Expressed as Resistance to Leaf Herbivores. *The American Naturalist,* 142**,** 928-936.
Streiff, R., A. Ducousso, C. Lexer, H. Steinkellner, J. Gloessl & A. Kremer (1999) Pollen dispersal inferred from paternity analysis in a mixed oak stand of Quercus robur L. and Q. petraea(Matt.) Liebl. *Molecular Ecology,* 8**,** 831-841.
Tanksley, S. D. (1993) Mapping polygenes. *Annual review of genetics,* 27**,** 205-233.
Tieszen, L. L. & T. W. Boutton. 1989. Stable Carbon Isotopes in Terrestrial Ecosystem Research. In *Stable Isotopes in Ecological Research,* eds. P. W. Rundel, J. R. Ehleringer & K. A. Nagy, 167-195. New York, NY: Springer New York.
Torres-Ruiz, J. M., A. Kremer, M. R. Carins Murphy, T. Brodribb, L. J. Lamarque, L. Truffaut, F. Bonne, A. Ducousso & S. Delzon (2019) Genetic differentiation in functional traits among European sessile oak populations. *Tree physiology,* 39**,** 1736-1749.
Uhl, D. & V. Mosbrugger (1999) Leaf venation density as a climate and environmental proxy: a critical review and new data. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 149**,** 15-26.
Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen & M. Diemer (2004) The worldwide leaf economics spectrum. *Nature,* 428**,** 821.
Wu, T., Y. Dong, M. Yu, G. G. Wang & D.-H. Zeng (2012) Leaf nitrogen and phosphorus stoichiometry of Quercus species across China. *Forest ecology and management,* 284**,** 116-123.
Wu, T., G. G. Wang, Q. Wu, X. Cheng, M. Yu, W. Wang & X. Yu (2014) Patterns of leaf nitrogen and phosphorus stoichiometry among Quercus acutissima provenances across China. *Ecological Complexity,* 17**,** 32-39.
Xu, Z. & G. Zhou (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of experimental botany,* 59**,** 3317-3325.
Zhang, L., J. Yang, Y. Huang, Z. Jia & Y. Fang (2018) Leaf venation variation and phenotypic plasticity in response to environmental heterogeneity in Parrotia

subaequalis (HT Chang) RM Hao et HT Wei, an endemic and endangered tree species from China. *Forests,* 9**,** 247.
Zhang, S.-B., Z.-J. Guan, M. Sun, J.-J. Zhang, K.-F. Cao & H. Hu (2012) Evolutionary association of stomatal traits with leaf vein density in Paphiopedilum, Orchidaceae. *PloS one,* 7**,** e40080.
Zhu, Y., H. Kang, Q. Xie, Z. Wang, S. Yin & C. Liu (2012) Pattern of leaf vein density and climate relationship of Quercus variabilis populations remains unchanged with environmental changes. *Trees,* 26**,** 597-607.
Zwieniecki, M. A., C. K. Boyce & N. M. Holbrook (2004) Hydraulic limitations imposed by crown placement determine final size and shape of Quercus rubra L. leaves. *Plant, Cell & Environment,* 27**,** 357-365.

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Appendices

Protocol for isotope ratio mass spectronomy

Plant material | Einwaage: 3.0mg | tin cups/ small

Tray Name: _____

Operator: _____

	Blank blank			Standards	Weight
1	A	1	0,4	Acetanilide	
2	A	2		Blank cup	
3	A	3	0,4	Acetanilide	
4	A	4	0,4	Acetanilide	
5	A	5	0,4	IVA Protein	
6	A	6	0,4	IVA Protein	
7	A	7	0,4	IVA Urea	
8	A	8	0,4	IVA Urea	
9	A	9	3,0	IVA Sorgum	
10	A	10	3,0	IVA Sorgum	
11	A	11	3,0	IVA wheat	
12	A	12	3,0	IVA wheat	

				Sample	Weight
13	B	1			
14	B	2			
15	B	3			
16	B	4			
17	B	5			
18	B	6			
19	B	7			
20	B	8			
21	B	9			
22	B	10			
23	B	11			
24	B	12			

				Sample	Weight
28	C	1			
29	C	2			
30	C	3			
31	C	4			
32	C	5			
33	C	6			
34	C	7			
35	C	8			
36	C	9			
37	C	10			
38	C	11			
39	C	12			

				Sample	Weight
43	D		1		
44	D		2		
45	D		3		
46	D		4		
47	D		5		
48	D		6		
49	D		7		
50	D		8		
51	D		9		
52	D		10		
53	D		11		
54	D		12		

				Sample	Weight
58	E		1		
59	E		2		
60	E		3		
61	E		4		
62	E		5		
63	E		6		
64	E		7		
65	E		8		
66	E		9		
67	E		10		
68	E		11		
69	E		12		

				Sample	Weight
73	F		1		
74	F		2		
75	F		3		
76	F		4		
77	F		5		
78	F		6		
79	F		7		
80	F		8		
81	F		9		
82	F		10		
83	F		11		
84	F		12		

				Standards	Weight
85	G	1			
86	G	2			
87	G	3			
88	G	4			
89	G	5		Blank cup	
90	G	6	0,3	IVA wheat	
91	G	7	0,3	IVA wheat	
92	G	8	0,4	Acetanilide	
93	G	9	0,4	Acetanilide	
	G	10			
	G	11			
	G	12			

				Standards	Weight
25	H	1		Blank cup	
26	H	2	3,0	IVA wheat	
27	H	3	3,0	IVA wheat	
40	H	4		Blank cup	
41	H	5	3,0	IVA wheat	
42	H	6	3,0	IVA wheat	
55	H	7		Blank cup	
56	H	8	3,0	IVA wheat	
57	H	9	3,0	IVA wheat	
70	H	10		Blank cup	
71	H	11	3,0	IVA wheat	
72	H	12	3,0	IVA wheat	

Notizen