Leaf trait modification in European beech trees in response to climatic and edaphic drought

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ABSTRACT

- Leaf morphological and physiological traits control the carbon and water relations of mature trees and are determinants of drought tolerance, but it is not well understood how they are modified in response to water deficits.
- We analysed five sun-canopy leaf traits (mean leaf size (LS), specific leaf area (SLA), Huber value (HV), water potential at turgor loss point ($\Psi_{tlp}$) and foliar carbon isotope signature ($\delta^{13}C$)) in European beech (Fagus sylvatica L.) across three precipitation gradients sampled in moist (2010), dry (2019) and very dry (2018) summers, and tested their response to short-term water deficits (climatic water balance (CWB) preceding sample collection) and long-term water availability (mean annual precipitation (MAP), plant-available soil water capacity (AWC) and neighbourhood competition).
- Across the 34 sites, LS varied seven-fold (3.9−27.0 cm²), SLA four-fold (77.1−306.9 cm²·g⁻¹) and HV two-fold (1.0−306.9 cm²·g⁻¹). In the 2018 dataset, LS showed a negative and HV a positive relationship to MAP, which contradicts relations found in multi-species samples. Average $\Psi_{tlp}$ ranged from −1.90 to −2.62 MPa and decreased across the sites with decreasing CWB in the month prior to measurement, as well as with decreasing MAP and AWC in 2019. Studied leaf traits varied considerably between years, suggesting that mast fruiting and the severe 2018 drought caused the formation of smaller leaves.
- We conclude that sun-canopy leaf traits of European beech exhibit considerable plasticity in response to climatic and edaphic aridity, and that osmotic adjustment may be an important element in the drought response strategy of this anisohydric tree species.

INTRODUCTION

European beech (Fagus sylvatica L.) is Europe’s most important temperate tree species, which dominates the natural forest vegetation of Central Europe and represents an important timber species (Leuschner & Ellenberg, 2017). Both experimental evidence with saplings and observations in adult trees indicate that beech is moderately sensitive to drought (Geßler et al. 2004; Leuschner, 2020; Braun et al. 2021). Yet, the species follows an anisohydric water potential regulation strategy, encountering considerable drops in leaf water potential (Leuschner et al. 2021). Thus, European beech is dependent on sufficient water supply during summer and likely also high atmospheric humidity, while sensitivity to frost and summer heat limit the species to mesic cool-temperate regions (Walthert & Meier, 2017; Leuschner, 2020). These limitations suggest that this species may, in large parts of its distribution range, be threatened by future increases in the frequency of ‘hotter droughts’, i.e. a combination of drought and high temperatures (Buras et al. 2020). The high mortality of European beech across Central Europe in consequence of the extreme 2018/2019 drought event suggests, indeed, a high vulnerability to climate warming (Braun et al. 2020; Schuldt et al. 2020; Walthert et al. 2021). Apart from dieback, especially on shallow soils and exposed ridges, many beech forests were left with visible foliar loss and crown damage, with only 11% of Germany’s beech forests being assessed as vital in the federal forest health assessment (BMEL 2020; 2021). On the other hand, beech is capable of recovering from moderate drought if water deficits are not too extreme (van der Werf et al. 2007; Pflug et al. 2018; Dietrich et al. 2019).

Increasing evidence suggests that hydraulic failure is a main mechanism leading to tree death in the context of recent climate warming-related forest dieback (Choa et al. 2012, 2018; Anderegg et al. 2016; Adams et al. 2017; Brodribb et al. 2020). Consequently, several studies have investigated the acclimation and adaptation potential of the hydraulic system of mature beech trees, finding only limited adjustment of branch xylem embolism resistance to increasing climatic aridity when comparing different populations (Herbette et al. 2010; Wortemann et al. 2011; Schuldt et al. 2016; Weithmann et al., unpubl.). Further, variability in embolism resistance within populations was usually higher than between populations (Aranda et al. 2015; Hajek et al. 2016; Stojnić et al. 2018). Thus, it remains...
questionable whether acclimation or adaptation of embolism resistance is an important element in the drought adaptation of European beech, and leaf-level responses might provide relevant insights into the species’ performance under drought.

Adaptation responses of leaf morphological and functional traits to increasing aridity have mostly been investigated at the interspecific level, i.e. across species and at higher taxonomic levels, while much less is known about the acclimation and adaptation potential of leaf traits of mature trees to increasing water deficits at the intraspecific level. Adjustment to water deficits at the leaf morphological and physiological levels has been demonstrated in numerous interspecific studies, notably through a decrease in leaf size (LS) and specific leaf area (SLA), alteration of sapwood-to-leaf area ratio (Huber value, HV) and shifts in leaf tissue osmotic and elastic properties (Bussotti, 2008; Bartlett et al. 2016; Powell et al. 2017; McGregor et al. 2020). However, linkages detected between leaf traits across species on a global scale may not hold at the intraspecific level (Niinemets, 2015; Anderegg et al. 2018). This is demonstrated by the trait mean LS and SLA, which are typically lower in arid than in wet environments in global or continent-wide species samples (Wright et al. 2002, 2017), while the opposite was found at the intraspecific level in European beech: Meier & Leuschner (2008) and Salehi et al. (2020) observed increases in LS and SLA of sun-canopy leaves with decreasing mean annual precipitation along two precipitation gradients in Central European mature beech forests. These were explained by higher temperatures at the drier sites that were still moist during leaf unfolding in early summer. Similarly, a study on changes in HV with increasing climatic aridity found an increase in a global multi-species dataset (Mencuccini et al. 2019), but no such relationship appeared at the intraspecific level for beech populations along a precipitation gradient (Schuldt et al. 2016).

A reduction in stomatal conductance is not only a short-term response to atmospheric and soil water deficits but may also represent a longer lasting acclimation response of beech to reduced precipitation levels, reflected in elevated levels of leaf tissue δ¹³C values (Meier & Leuschner, 2008; but see Schuldt et al. 2016). It is possible that populations differ in the extent of stomatal closure, but evidence from mature trees seems to be lacking.

Widespread leaf shedding after extreme droughts in beech is thought to result from xylem embolism, as none of the trees with leaf loss and crown damage studied by Walthert et al. (2021) showed recovery in the subsequent year. This was also observed by Wohlgemuth et al. (2020), suggesting that leaf shedding must be considered as a symptom of vulnerability rather than protection against excessive water loss. Similarly, a lower HV is usually linked to significantly higher embolism rates in mature beech trees (Schuldt et al. 2020). This is in line with results of long-term canopy monitoring, which showed that beech usually responds to hot summers with elevated defoliation in the following year (Seidling, 2007).

The water potential at turgor loss point (Ψₜlp) was identified in a global dataset as the single most influential leaf functional trait determining the drought tolerance of plants, with Ψₜlp generally decreasing from mesic to xeric habitats (Bartlett et al. 2012b). Ψₜlp may also vary at the intraspecific level among populations along gradients of climatic aridity, as observed e.g. in Eucalyptus obliqua (Pritzkow et al. 2020) and Castanopsis fargesii (Liang et al. 2019). In contrast, a comparison of growing-season Ψₜlp means among four mature European beech stands covering a reduction in mean annual precipitation (MAP) of 520 mm·year⁻¹ showed no Ψₜlp decrease with increasing climatic aridity (Schipka, 2002), while others observed osmotic adjustment during the summer period in this important Central European tree species (Backes & Leuschner, 2000; Leuschner et al. 2019, 2021). This matches the reduction in Ψₜlp after drought exposure found for sapling and adult beech trees by Tomasella et al. (2018, 2019), whereas others did not observe any osmotic adjustment in saplings (Knutzen et al. 2015; Lübbe et al. 2017). Overall, osmotic adjustment in European beech might be less pronounced than in other tree species and may only be important under dry conditions (Leuschner, 2020). It is possible that anisohydric trees such as European beech can conduct significant active osmotic adjustment only upon pronounced drops in leaf water potential (Hartmann et al. 2021; Leuschner et al. 2021).

Apart from precipitation, soil water storage capacity is an important determinant of plant water relations, the importance of which increases with drought and heat intensity during the course of climate warming. As expected, beech trees on deep soils with a higher capacity to access plant-available water (AWC) were less affected by the severe 2018/2019 drought (Walthert et al. 2021). While soil physical properties have been considered less often in tree dieback studies than climatic variables, it is obvious that including soil parameters in models dealing with the distribution, vitality or mortality of woody species always improve the predictions (Tai et al. 2017; Walthert & Meier, 2017; Renne et al. 2019). This highlights the importance of including local data on soil water storage capacity when investigating effects of water availability on tree performance. Another factor, which may influence water availability and thus tree vitality and mortality, is competition with neighbouring trees (Lübbe et al. 2017; Hajek et al. 2020), which has rarely been considered.

In the face of changing climate conditions, phenotypic variation, in combination with intraspecific genetic diversity, will improve the acclimation and adaptation potential of a plant species (Aubin et al. 2016). Despite the wide distribution range of F. sylvatica, this important tree species appears to be vulnerable to global change-type drought events, which demands a better understanding of the acclimation and adaptation potential of the species to drought and heat. In this study, we analysed the variation in LS, SLA and HV, the foliar δ¹³C signature and Ψₜlp in foliage from upper sun-canopy branches of 34 mature European beech forests on mostly sandy soil across three extended mean annual precipitation (MAP) gradients (866–497 mm·year⁻¹) in oceanic to sub-continental temperate climates. All sampled branches were collected during mid-summer in the upper sun canopy at similar distances to the tree tip. Sampling was conducted in three years with contrasting spring and early-summer moisture conditions, when leaf out of beech occurs, i.e. moist (2010, 4 stands, MAP reduction: 229 mm·year⁻¹), extremely dry (2018, 11 stands, MAP reduction: 353 mm·year⁻¹) and dry conditions (2019, 19 stands, MAP reduction: 301 mm·year⁻¹). Besides precipitation and climatic aridity, we additionally investigated the influence of local soil water storage capacity and competition intensity in the stand on the above five leaf traits to assess the influence of site water availability in a more comprehensive way. Sampling ten (or four) trees per stand and three to five branches per tree allowed us to partition the
observed leaf trait variation to within-tree, within-population and between-population components. We hypothesised, in accordance with the existing evidence on climatic effects on leaf morphological and hydraulic traits, that long-term site water availability (including climatic, edaphic and competition-related effects) will affect leaf traits across the gradients of the 2018 and 2019 sampling campaigns. Across the study years and with decreasing climatic water balance in the period preceding sample collection, we expected LS, SLA and $\Psi_{\text{tlp}}$ to decrease, while $\delta^{13}$C and HV were expected to increase.

**MATERIAL AND METHODS**

**Study sites and climate conditions**

The 34 monospecific mature beech (*Fagus sylvatica* L.) stands are located in the Pleistocene lowlands of northern Germany at elevations between 19 and 159 m a.s.l. (Fig. 1A). Across the gradient, the climate is cool-temperate with mean annual temperature (MAT, sites 1–30: 1958–2017, sites 31–34: 1950–2009) increasing from 8.3 to 9.6°C from West to East and mean annual precipitation (MAP) decreasing from 866 to 497 mm-year$^{-1}$, reflecting a gradient from an oceanic to a subcontinental cool-temperate climate (Fig. 1B, Table 1). The data for monthly precipitation, air temperature and potential evapotranspiration were extracted for the different sites for the period 1950–2019 from 1 km-gridded data retrieved from the Climate Data Centre (CDC) of the German Weather Service (DWD, Deutscher Wetterdienst, Offenbach, https://opendata.dwd.de/, accessed 2/4/2021) using the R package `rdwd` version 1.2.0 (Boessenkool, 2020). The climatic water balance of the spring months (March to May) of the three sampling years 2010, 2018 and 2019 (CWBS current year), the climatic water balance of the month preceding sample collection (CWBS$_{\text{pre}}$),

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**Fig. 1.** A: Map of the northern part of Germany, with federal states and location of the 34 investigated beech stands sampled in three different years. Colours and shape of the stand symbols show the sampling campaign (2010, 2018 or 2019) and mean annual precipitation (MAP, 1958–2017, data provided by DWD) is indicated in colours. For site codes and more information on stands, see Table 1 and Table S1. B: 60-year mean annual precipitation (MAP, left), mean annual temperature (MAT, middle) and climatic water balance of the current year (CWBS, right) of the sites where sample collection took place in 2010 (n = 4), 2018 (n = 11) or 2019 (n = 19). 60-year mean values refer to the period 1950–2009 for the sites sampled in 2010 and to the period 1958–2017 for the 2018 and 2019 sites.
and the full-year climatic water balance for the three sampling years (CWBU) were calculated for each site as the difference between precipitation and potential evapotranspiration of the respective months (see Table S2 for climate data of two selected study sites, one in the west and one in the east of the study region).

### Edaphic conditions

The sandy soils of the 34 sites developed from glacial deposits of the last (Weichselian) or penultimate (Saalian) Ice Age and are predominantly nutrient-poor, with profile depths exceeding 120 cm. None of the soils were influenced by groundwater. For sites 1–30, details on soil chemical and physical properties are given below, for sites 31–34 (Calvörde, Göhrde, Sellhorn and Unterlüss with sandy soil texture) see information in Müller-Haubold et al. (2013). For analysing soil chemical properties, soil samples were taken in soil pits dug at two randomly placed locations per site, and for estimation of the plant-available soil water capacity (AWC, mm), samples from one soil pit each were taken from three different depth layers (0–10 cm, 10–30 cm, 30–60 cm). Furthermore, the soil stone content (particles >2 mm) was determined at one pit per site by sieving a soil volume of 45,000 cm³. In the laboratory, pH in water was determined in the fresh soil, while soil nitrogen content and soil organic carbon and C/N ratio were determined through gas chromatographic analysis of the dried soil (70°C, 48 h) (see Table S1). To estimate AWC, particle size distribution, i.e. the relative proportions of sand (63–2000 μm), silt (2.0–63 μm) and clay-sized (<2.0 μm) particles, and soil bulk density were determined. The sand fraction was measured by sieving, and the silt and clay fractions by differential sedimentation in a PARI Soil Particle Analysis System.
Analyser (METER Group, Munich, Germany). To estimate soil hydraulic properties (the van Genuchten (1980) parameters), we then employed pedotransfer functions according to Schaap et al. (2001), using the module ROSETTA 1.1 implemented in the software RETC (version 6.02; van Genuchten et al. 1991). From the modelled water retention curves, volumetric water content at permanent wilting point (at a water potential of −1.5 MPa; pF 4.2) and water content at field capacity (at a water potential of −60 hPa; pF 1.8) were extracted and AWC calculated as the difference between these two water contents for the three depth layers to 60 cm and extrapolated to a standard depth of 100 cm, assuming a homogenous soil particle composition in the 30–100 cm layer.

Tree size and competition intensity in the canopy

Mean tree height of the study sites ranged from 21.1 to 34.4 m, and diameter at breast height (DBH) from 36 to 58 cm (Table 1). Because we assumed that soil water availability to the tree individual may depend on the intensity of competition of the target tree with their direct neighbours, we calculated the Hegyi competition index (CI; Hegyi, 1974) for the sites 1–30 for each sampled tree i (10 per site) from the distance and height of the three nearest neighbouring trees j as:

\[ CI_i = \frac{\sum_{j=1}^{n} d_j / D_{ij}}{n}, \]

where \( d_i \) is the DBH of the sampled tree i (cm), \( d_j \) the diameter at breast height of the competitor j (cm), \( D_{ij} \) the distance between target tree and competitor (m), and \( n \) is the number of directly neighbouring trees considered (\( n = 3 \)).

Leaf sampling and analysis of foliar traits

Sample collection took place between June and August in 2010 (sites 31–34) and in 2018 and 2019 (sites 1–30). All three sampling campaigns covered sites with a broad range of MAP levels, each representing a precipitation gradient, but the moisture conditions during leaf formation in spring/early summer differed between the 2010 (moist), 2018 (extremely dry) and 2019 (dry) datasets (Table S2). At all sites, branches were cut from the uppermost sun-exposed crowns by professional tree climbers. Fully expanded intact leaves were sampled from first-order side branches of the collected main branch at a uniform distance of 1–2 m below the tip. Per site, we sampled leaves of four to five branch segments each of four trees at sites 31–34 and leaves of three branches of ten trees each at the sites 1–30. For all leaf samples, mean leaf size (LS, cm²), specific leaf area (SLA, cm² g⁻¹), sapwood-to-leaf area ratio (Huber value, HV, cm² m⁻²) and the carbon isotope signature of leaf dry mass (δ¹³C, ‰; not available for samples of sites 31–34) were determined. Additionally, three small distal twigs per tree with fully intact leaves were cut to determine turgor loss point (ψₜlp; see Table 2) for a list of all variables)

From the samples from sites 1–30, all leaves distal to the basipetal end of the sample (50–100 cm from the apex, branch diameter between 5.5 and 14.6 mm) were detached and stored at about 6°C. Per branch sample, a subsample of the leaves was scanned for leaf area determination (two scans per branch with an average of 58 leaves) using a flat-bed scanner and the software WinFOLIA Reg 2014a (Régent Instruments, Quebec City, Canada). At sites 31–34, leaves of four to five branch segments per tree of 5–6 cm in length with diameters of 6.0–8.0 mm were scanned.

LS was calculated by dividing the total area of the scanned leaves by the number of leaves, and SLA by dividing the total area of scanned leaves by their dry weight (oven-dried at 70°C for 48 h). The total leaf area per branch was approximated by multiplying the total leaf dry mass per branch with SLA. For determination of HV, the xylem cross-sectional area without pith and bark (Aₓylem), which was estimated from the branch cross-sectional area (Aₓcross) as \( Aₓylem = Aₓcross - 3.715 + 0.770 \times Aₓcross \) (Schuldt et al. 2016), was divided by the total leaf area per branch. Thereafter, the dried leaf samples were ground and leaf N content (see Table S3) and carbon isotope signature of the dry mass (δ¹³C) were analysed by mass-ratio spectroscopy (Delta Plus; Thermo Finnigan, Bremen, Germany) at the Centre for Stable Isotope Research and Analysis (KOSI) of the University of Göttingen.

The twigs collected for determination of ψₜlp at sites 1–30 were recut under water and transported to the laboratory in a cooling box at 6°C under high air humidity with the cut surface placed under water. ψₜlp was determined within 72 h after sampling using the osmometer method, where ψₜlp is estimated based on the leaf osmotic potential at full hydration (ψₑ; Bartlett et al. 2012a). The leaf osmotic potential was measured in a vapor pressure osmometer (VAPRO 5600, Wescor, Logan, UT, USA) on leaf discs of fully rehydrated leaves after a 2 min submersion in liquid nitrogen, following the protocol of Bartlett et al. (2012a). ψₜlp was estimated as \( ψₜlp = 0.832 \times ψₑ - 0.631 \) (Bartlett et al. 2012a). Although the osmometric method has since been shown to deliver valid results for several growth forms including grasses, herbaceous and woody plants (Arndt

### Table 2. List of variables included in the study, with abbreviations and units.

| Abbreviation | Unit | Definition |
|-------------|------|------------|
| LS          | cm²  | Mean leaf size |
| SLA         | cm² g⁻¹ | Specific leaf area |
| HV          | cm² g⁻¹ m⁻² | Sapwood-to-leaf area ratio (Huber value) |
| δ¹³C        | ‰   | Carbon isotope signature |
| ψₜlp        | MPa | Water potential at turgor loss point |
| MAP         | mm year⁻¹ | Mean annual precipitation |
| MAT         | °C   | Mean annual temperature |
| CWB         | mm year⁻¹ | Climatic water balance (precipitation – pot. evapotranspiration) |
| CWBS        | mm   | Cumulative climatic water balance of March, April and May |
| CWBSpre     | mm   | CWB of the month preceding sample collection |
| AWC         | mm   | Plant-available water capacity of the soil (referring to 1-m depth) |
| CI          |      | Hegyi competition index |
| Height      | m    | Tree height |
et al. 2015; Griffin-Nolan et al. 2019; Majekova et al. 2019), it should be kept in mind that differences in the apoplastic fraction and leaf modulus of elasticity across populations and environmental conditions might affect the estimated $Ψ_{tlp}$ values.

For sites 31–34, $Ψ_{tlp}$ and $Ψ_0$ were estimated from pressure-volume curves established with the over-pressurization technique in a Scholander pressure chamber (Koide et al. 2000). In July 2010, shoots with four to six leaves were collected in the lower sun canopy, cut under water, transported to the laboratory in a humidified plastic bag and rehydrated overnight at room temperature. Leaves were then weighed to obtain initial mass, inserted into the pressure chamber to determine the initial water potential, and subsequently subjected to a series of increasing pressures ranging from 0.4 to 3.1 MPa. The extruded water was collected in Eppendorf cups containing cellulose pads and weighed immediately. After each step, the leaves were oven-dried to determine dry mass and initial relative water content. $Ψ_{tlp}$ was determined from the intersection between the nonlinear and linear branch of the plot of inverse water potential against cumulative expressed water fraction.

In addition, data of three trees (three samples per tree) from a stand in Unterlüss (close to sites 24 and 34) were used to test for significant differences in LS, SLA and HV of upper canopy branches collected in 2018 and 2019.

Statistical analyses

All statistical analyses were carried out with the software R, version 4.0.3 (R Core Team, 2020) using the tidyverse package, version 1.3.0 (Wickham et al. 2019).

In our dataset, the effect of the sampling year (2010, 2018, 2019) on the leaf traits cannot reliably be separated from site and climate effects, as testing for this effect was not planned in the original design stage of the study, and sampling could not be repeated on the same sites in consecutive years. We therefore refrain from formally testing for year differences, and only present visual comparisons of leaf traits at the tree level (average of three samples per tree) sampled at different sites in the summers 2010, 2018 and 2019. Only at one site (Unterlüss), were data from repeated sampling in 2018 and 2019 on the same trees available, with differences in LS, SLA and HV between 2018 and 2019 being tested with an unpaired two-sample Wilcoxon test.

Linear regression analyses were conducted to test for the effect of sampling year’s climatic water balance in spring (CWBS, March to May) on the site means of LS, SLA, HV and δ¹³C. In case of $Ψ_{tlp}$ with higher seasonal variation than in the other traits, the climatic water balance of the month prior to sampling (CWBS_m), instead of CWBS, was analysed.

To test for the effect of long-term water availability (combined effects of MAP [1958–2017], AWC and CI) on leaf traits, linear mixed effects models were fitted with the R package lme4, version 1.1.26 (Bates et al. 2015) for leaves collected in 2018 and 2019 and for each trait separately. LS, SLA and HV were natural log-transformed and the five leaf traits were modelled as a function of MAP, AWC and CI (centred, scaled and, in case of CI, log-transformed) as fixed effects, and with random intercepts for tree nested in site. Due to convergence issues, the site-wise random intercept had to be omitted in the model for HV in 2018. The variance in the traits was then decomposed into variance components for the fixed effects, the random effects (between-sites and between-trees) and the residual variance (within-tree). The marginal and conditional R² (Nakagawa et al. 2017) were computed based on R package MuHti version 1.43.17 (Bartoń, 2020).

RESULTS

Climate conditions and soil water availability across the sites

The range of climate conditions covered by the three datasets (2010, 2018, 2019) was not identical, but relatively similar. Sixty-year mean annual precipitation (MAP) was slightly higher at the sites sampled in 2019 (566–886 mm-year⁻¹, 1958–2017, n = 19) than at the sites of 2010 (509–738 mm-year⁻¹; 1950–2009, n = 4) and 2018 (497–850 mm-year⁻¹, 1958–2017, n = 11; Fig 1), while 60-year mean annual temperature (MAT) was slightly lower in the 2010 dataset (8.3–8.9°C) than in the 2018 (8.4–9.6°C) and 2019 datasets (8.5–9.2°C). As the mid- and late-summer of 2018 was extremely dry, the climatic water balance (CWBS) of the full year at the sites sampled in 2018 was the lowest (between −564 and −138 mm-year⁻¹) compared to −287–156 mm-year⁻¹ in the 2019 sample and 133–297 mm-year⁻¹ in the 2010 sample. In the 60-year record of climate data of the study sites, early growing season (MSP; April to June) and growing season precipitation (MGSP; April to October) were tightly correlated with full-year MAP, while early growing season temperature (MST; April to June) and growing season temperature (MGST) showed a weaker relationship with full-year MAT (Fig S1). CWBS deviated from the 60-year MAP most in 2019, intermediate in 2018 and least in 2010. The relationship between MAP and CWBS was strongest for the 2018 dataset, the year with the most negative climatic water balance (Fig 1B).

Leaf traits in the 2010, 2018 and 2019 datasets

Across the 34 sites, mean leaf size (LS) of sun-canopy foliage varied nearly seven-fold between 3.9 ± 0.2 and 27.0 ± 1.8 cm², specific leaf area (SLA) four-fold between 77.1 ± 1.0 and 306.9 ± 18.2 cm²g⁻¹, and Huber value (HV) six-fold between 1.0 ± 0.1 and 6.65 ± 1.3 cm²m⁻² (averaged over a tree, n = 3–5 samples per tree; Fig 2A). Average LS in the 2018 dataset (17.3 ± 0.3, n = 110 trees) was clearly higher than in the 2010 data (13.8 ± 0.4, n = 16 trees) and the 2019 data (12.5 ± 0.3, n = 190 trees). Compared to LS, SLA varied much less between the three datasets, with the 2019 data having a slightly higher mean (144.4 ± 3.0 cm²g⁻¹) than the 2010 and the 2018 data (111.9 ± 3.5 and 128.9 ± 2.0 cm²g⁻¹, respectively). Yet, the range of average SLA values per tree spanned 229.8 cm²g⁻¹ in the 2019 dataset and was remarkably large. HV showed the inverse pattern of LS, with mean HV being higher in the 2019 data (2.7 ± 0.1) than in the 2018 and 2010 datasets (2.2 ± 0.1 and 2.3 ± 0.1, respectively).

The average leaf mass δ¹³C value was higher in the 2018 dataset (−27.8 ± 0.1%) than in the 2019 data (−28.8 ± 0.1%), indicating a generally lower stomatal conductance in the former dataset (Fig 2B). $Ψ_{tlp}$ was lower in the 2018 dataset (−2.48 ± 0.01 MPa) compared to the 2019 data (−2.30 ± 0.02 MPa) and the 2010 data (−2.19 ± 0.02 MPa). The inter-
relationships between the studied leaf traits differed between the three datasets (Fig. S2).

Comparison of leaf traits in the same trees between 2018 and 2019 revealed significant differences in LS, SLA and HV, with a 50% reduction in LS (from 14.5 ± 0.9 cm² to 7.1 ± 0.5 cm²) and doubling in HV (from 1.8 ± 0.5 cm² m⁻² to 3.6 ± 1.1 cm² m⁻²), while SLA decreased by 15% from 150 ± 16 cm² g⁻¹ to 128 ± 8 cm² g⁻¹ (stand Unterluss, close to sites 24 and 34; Fig. 3).

Effects of long-term water availability
The effects of mean annual precipitation (MAP, 1958–2017), plant-available soil water capacity (AWC) and Hegyi competition index (CI) on leaf traits were tested with linear mixed effects (LME) models for the 2018 and 2019 datasets (Table 3). LS was significantly affected only by MAP in 2018 (negative effect), while no significant influence was detectable in the

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Fig. 2. (A) Mean leaf morphological and (B) physiological traits at the tree level. Average leaf size (LS), specific leaf area (SLA), Huber value (HV), carbon isotope signature ($\delta^{13}C$) and water potential at turgor loss point ($\Psi_{tlp}$) for the three sampling campaigns (2010: n_{tree} = 16, 2018: n_{tree} = 110, 2019: n_{tree} = 190). Box-whisker plots depict median and interquartile ranges (Q1–Q3); whiskers extend to max. 1.5 times the interquartile range.

Fig. 3. Mean leaf size, specific leaf area and Huber value of nine samples from three trees collected in Unterluss (close to stands 24 and 34) in 2018 and again in 2019. Asterisks indicate significant differences between years (**P < 0.01; ***P < 0.001). Box-whisker plots depict median and interquartile ranges (Q1–Q3); whiskers extend to max. 1.5 times the interquartile range.
### Table 3. Results from linear mixed-effects models examining the influence of 60-year mean annual precipitation (MAP), plant-available water capacity (AWC) and Hegyi competition index (CI, log-transformed) as fixed variables and random intercept for tree nested in site on mean leaf size (LS), specific leaf area (SLA), Huber value (HV), carbon isotope signature ($\delta^{13}$C) and pressure at turgor loss point ($\Psi_{tlp}$) sampled in 2018 or 2019.

| Variable | Year | Fixed parts | Random parts | Obs. |
|----------|------|-------------|--------------|------|
|          |      | Intercept   | MAP          | AWC  | CI (log) | tree:site | site       | residual   |      |
| LS (log) | 2018 | 2.82        | −0.10        | −0.06 | 0.01    | 0.17      | 0.11       | 0.10       | 330   |
|          | 2019 | 2.47        | −0.01        | 0.06  | −0.01   | 0.26      | 0.23       | 0.13       | 564   |
| SLA (log)| 2018 | 4.84        | −0.02        | 0.02  | 0.03    | 0.09      | 0.08       | 0.13       | 330   |
|          | 2019 | 4.93        | 0.00         | 0.13  | 0.00    | 0.12      | 0.19       | 0.11       | 564   |
| HV (log) | 2018 | 0.70        | −0.01        | −0.08 | 0.02    | 0.20      | 0.28       | 0.33       | 330   |
|          | 2019 | 0.88        | 0.01         | 0.08  | 0.02    | 0.25      | 0.30       | 0.32       | 564   |
| $\delta^{13}$C | 2018 | −27.82      | −0.20        | −0.09 | −0.18   | 0.68      | 0.49       | 0.33       | 330   |
|          | 2019 | −28.78      | −0.27        | 0.01  | −0.05   | 0.72      | 0.68       | 0.39       | 564   |
| $\Psi_{tlp}$ | 2018 | −2.48       | 0.00         | 0.01  | 0.02    | 0.09      | 0.09       | 0.10       | 330   |
|          | 2019 | −2.30       | 0.09         | 0.08  | −0.01   | 0.11      | 0.14       | 0.12       | 564   |

Given are scaled estimates (est.), standard errors (SE), test statistics (t-val.), degrees of freedom (df) and P-values (fixed parts), the SD of the random intercepts and the residual SD (random parts) and the number of observations (Obs.) for each model.

2019 dataset. SLA was significantly positively influenced by CI in 2018, and by AWC in the 2019 data. MAP had a significant positive effect on HV in the 2018 dataset, while CI influenced HV negatively. In the 2019 dataset, HV was not significantly related to MAP or to AWC or CI. Foliar $\delta^{13}$C was significantly influenced by competition intensity only in the 2018 dataset,
with a positive effect of CI. \( \Psi_{\text{tlp}} \) significantly increased with MAP and AWC in the 2019 dataset, but the effects of MAP, AWC and CI were not significant in the 2018 dataset.

Relationship between leaf traits and short-term water availability

Linear regression analyses between the climatic water balance one month before bud burst and during leaf expansion (March to May of the sampling year, CWBS) and average LS, SLA, HV and \( \delta^{13}\text{C} \) of a site revealed significant decreases in LS and the \( \delta^{13}\text{C} \) signature, i.e. larger leaves, under higher climatic aridity (Fig. 4A and B), while SLA and HV were not significantly influenced. \( \Psi_{\text{tlp}} \) significantly decreased with diminishing CWBpre. The correlation between \( \delta^{13}\text{C} \) signature and leaf N content was very weak (Fig. S2), indicating that discrimination against \(^{13}\text{C} \) during photosynthesis was not dependent on foliar N content.

Variance components

Variance decomposition for the LME models in Table 3 revealed that site water availability, as expressed by the combined effects of MAP, AWC and CI, explained a rather small fraction of the variance of \( \Psi_{\text{tlp}} \) in the 2018 dataset (1%), while ranging from 5% (SLA) to 18% (LS) for the other traits (Fig. 5). This was different in the 2019 dataset, with a relatively high proportion of variance explained by MAP, AWC and CI in the case of \( \Psi_{\text{tlp}} \) (23%) and SLA (21%), while this proportion was lowest in the case of LS (3%). In both datasets, a substantial fraction of the between-stand variance was not explained by MAP, AWC or CI, ranging from 33% in \( \Psi_{\text{tlp}} \) to 45% in SLA in the 2019 data, and from 21% in LS and SLA to 32% in \( \Psi_{\text{tlp}} \) in the 2018 data. The proportion of within-population variance not explained by competition intensity was high, ranging from 24% in the case of SLA to 52% in \( \delta^{13}\text{C} \) in the 2018 dataset, and from 19% in SLA to 47% in LS in the 2019 data. Variance attributed to within-tree differences was high for SLA and HV in the 2018 dataset (50% and 59%), but much lower in the case of foliar \( \delta^{13}\text{C} \) in both datasets (12% and 13% in the 2018 and 2019 data, respectively).

**DISCUSSION**

Leaf traits in the three sampling campaigns 2010, 2018 and 2019

Our study of leaf trait variation in beech forests across three climatic aridity gradients covers a large part of the species’ habitat range in Central Europe and thus allows conclusions on the acclimation and adaptation potential of mature beech in response to climate warming. Mean leaf size (LS) in the upper sun canopy of the 34 stands (12.6–17.3 cm\(^2\)) was similar to LS determined in 2005 and 2006 across a comparable precipitation
gradient (520–970 mm·year⁻¹) in Central Germany, although on a different soil (Meier & Leuschner, 2008). It was clearly higher than LS of sun leaves collected in 2011 (8.22–11.48 cm²) in stands 31–34 (Schuldt et al. 2016), probably due to a strong masting event in 2011 (Müller-Haubold et al. 2013). However, fructification also occurred in 2019 in many beech forests of northern Germany (Scharnweber et al. 2020), probably induced by the warm and dry summer of 2018, as mass fructification in F. sylvatica was found to be stimulated by high temperatures and high solar radiation in June and July of the year preceding a mast year (Müller-Haubold et al. 2015). Hence, the nearly 40% higher mean LS in the 2018 dataset, as compared to the 2019 data, was probably, at least partly, caused by mast fruiting, which reduces carbohydrate allocation to leaves. As LS has been found to increase in beech with autumn temperature of the previous year (Zhu et al. 2021), the larger LS in the 2018 dataset could partly also be the result of the somewhat higher mean annual temperatures recorded at the sites sampled in 2018. However, repeated sampling of the same trees in 2018 and 2019 at the Unterliss site also revealed significantly smaller leaves in 2019 than in 2018, and cross-regional surveys after the severe 2018 drought for many Central European beech forests showed particularly small leaves in 2019 as an after-effect (Schuldt et al. 2020; Walthert et al. 2021; Zhu et al. 2021). From their analysis of a 25-year record of leaf morphological trait data from Swiss beech forests, Zhu et al. (2021) concluded that higher VPD results in a reduced specific leaf area (SLA) in 2019 might be caused by differences in soil water availability and VPD across the climate gradient, or by local differences in masting intensity, which influence both carbohydrate and nutrient availability.

Average Huber values (HV) varied inversely to LS across the three sampling years, with higher HV being negatively related to LS (Fig. S2). This suggests that inter-annual changes in HV are mainly driven by variation in LS and not by a variable leaf number per twig. The 100% increase in HV after the severe 2018 drought at one central site was likely driven by reduced water transport capacity of the branches due to drought legacy effects (Schuldt et al. 2020).

As expected, leaf carbon isotope signature (δ¹³C) was, on average, by about 1% higher in the drier and hotter summer of 2018 as compared to 2019, indicating reduced stomatal conductance and a higher intrinsic water use efficiency in the former summer (Farquhar et al. 1989). This was associated with an on average lower water potential at turgor loss point (Ψtlp) in 2018 than in 2019, and the highest Ψtlp in the moist summer of 2010, suggesting mean osmotic adjustment by about 0.2–0.3 MPa in response to inter-annual climatic differences. Seasonal osmotic adjustment in the leaves of adult beech trees has been observed in several studies in Central Europe, but its extent seems to depend on the drought intensity experienced during the growing season, as it was usually <0.4 MPa (Leuschner, 2020). In the foliage of drought-stressed beech saplings, amino acids accumulate together with mono- and disaccharides, and especially the trisaccharide raffinose, suggesting increased conversion of starch to non-structural carbohydrates under drought (Aranda et al. 2018). It is therefore possible that the less negative Ψlp values in the 2019 dataset compared to 2018 are a consequence of carbohydrate depletion due to mast fruiting in that year, as higher concentrations of osmotic substances come at the expense of elevated carbohydrate costs (Villagra et al. 2013; Martínez-Vilalta et al., 2016). The significant influence of climatic water balance of the month preceding sample collection (CWBₚₑₛ) on Ψlp over the three sampling years and the significant effect of mean annual precipitation (MAP) and plant-available water capacity (AWC) in 2019, confirm that Ψlp is largely under the influence of water availability, at least under dry conditions (cf. Sjöman et al. 2018; Aranda et al. 2021).
Effects of lasting water deficits on leaf traits

As expected, site water availability impacted on the studied leaf traits across the gradients in the 2018 and 2019 sampling campaigns. In our study, long-term site water availability was described in terms of mean annual precipitation (MAP), available water capacity (AWC) of the soil, and competition intensity (CI) between neighbouring trees. A striking result of this precipitation gradient study is the highly significant negative relationship of LS to MAP in the 2018 dataset, i.e. larger sun-canopy leaves at the drier sites, contradicting the general assumption that plants produce smaller leaves in more xeric environments (Parkhurst & Loucks, 1972). Our finding does also contrast with the results from an Italian gradient, where leaves of *F. sylvatica* were smaller at the southernmost sites that have a drier climate (Bussotti et al. 2005). However, our finding is in line with other precipitation gradient studies in beech forests in Germany and Switzerland (Meier & Leuschner, 2008; Schuldt et al. 2016; Salehi et al. 2020), which also found an LS increase with decreasing precipitation level. A likely explanation is that soil water availability is usually non-limiting in April and May even at the drier sites, which experience drought later in summer, so that higher temperatures stimulate the formation of large leaves in spring (Leuschner, 2020). As soil water reserves were not sufficiently refilled in winter 2018/19 after the very dry 2018 summer (Scharnweber et al. 2020), leaf expansion in 2019 could not profit from ample moisture availability in spring, which could explain the missing correlation between LS and MAP in the 2019 dataset. These results support the assumed mismatch between evaporative demand and transpiring leaf area, confronting beech with drought stress and often leading to pre-senescence leaf shedding, as the species is unable to produce a second, better-adapted leaf generation (Leuschner, 2020; Schuldt et al. 2020; Wohlgemuth et al. 2020; Walthert et al. 2021).

The Huber value (HV), i.e. the sapwood-to-leaf area ratio, of sun-canopy branches decreased with a MAP decrease in the 2018 dataset, which is opposite to earlier findings in intraspecific studies with trees, as e.g. in Scots pine (Mencuccini & Grace, 1995; Martínez-Vilalta et al. 2009; Sterck et al. 2012). This indicates that adult beech trees do not improve the water supply to their sun foliage under reduced precipitation through a reduction in the amount of leaf area supplied per unit hydraulic conductance, as would have been expected (Choat et al. 2018). This seems to be related to the finding that LS (Meier & Leuschner, 2008; Salehi et al. 2020) and also stand leaf area index (Meier & Leuschner, 2008) increase in beech stands with decreasing MAP, while the branch and stem hydraulic system responds to increased climatic aridity with a reduction in mean vessel diameter, and thus reduced specific conductance (Schuldt et al. 2016; Zimmermann et al. 2021; Weithmann et al., unpubl.). It appears that foliar and xylem responses to increasing climatic aridity are poorly coordinated in mature beech trees, which must expose the foliage to increased drought stress in summers with water deficits.

Our results further demonstrate effects of the local soil moisture regime (AWC) on certain leaf morphological and physiological traits. While LS was unaffected, SLA decreased in the 2019 dataset with a reduction in soil water storage capacity, suggesting that water deficits constrain leaf development in beech primarily through the soil water pool and less through the amount of rainfall. In correspondence, $\Psi_{\text{slp}}$ decreased with a reduction in AWC in the 2019 dataset, indicating that osmotic adjustment at the leaf level was driven by both rainfall deficit and a reduced soil water storage capacity. Walthert et al. (2021) and Obladen et al. (2021) observed more pronounced drought stress symptoms after the 2018/19 drought in Central European beech stands on soils with limited water storage capacity, which underpins the importance of soil physical properties for drought intensity.

Influence of short-term water deficits on leaf traits

Regression of LS, SLA, HV and $\delta^{13}$C on the climatic water balance in the three months before and during leaf expansion (March to May, CWBS) across the 34 sites revealed significant positive effects of instantaneous water deficits on LS and $\delta^{13}$C, while SLA and HV were unaffected. The observed promotion of leaf expansion by higher climatic aridity in spring is in line with the discussed negative MAP effect on LS, probably driven by the stimulation of leaf expansion by higher temperatures. The observed negative CWBS–LS relationship contradicts our hypothesis that leaf size would decrease with decreasing CWBS. The $\delta^{13}$C decrease with increasing CWBS, on the other hand, is in agreement with our assumption, and suggests that leaf expansion at the sites with higher climatic aridity is closely linked to a reduction in stomatal conductance, relative to sites with lower aridity. Stomatal regulation in beech has been found to be relatively sensitive to atmospheric saturation deficits (Oren et al. 1999; Aranda et al. 2000; Leuschner et al. 2021), which enables beech to reduce stand transpiration to moderate levels under high evaporative demand, even when high leaf area indices (LAI) >7 developed at the drier sites (Meier & Leuschner, 2008).

For $\Psi_{\text{slp}}$, a significant negative effect of CWBpre was demonstrated, supporting our hypothesis and matching the MAP and AWC effects. Our observations confirm the capacity of mature beech trees for active osmotic adjustment to maintain their cell turgor under drought stress conditions. Among the studied leaf properties and other hydraulic traits investigated earlier (Weithmann et al., unpubl.), the water potential at turgor loss point appears as the trait most closely related to local climatic aridity and thus atmospheric drought stress exposure of the studied beech forests in northern Germany. This supports the conclusion of previous studies on the high indicative value of $\Psi_{\text{slp}}$ for assessing drought exposure of plants (e.g. Turner, 1986; Abrams, 1988; Bartlett et al. 2012b).

Leaf trait variance partitioning and the role of within-population variability

In correspondence with earlier studies, the portion of leaf trait variance attributed to unexplained within-population as well as within-tree differences was high in the studied leaf traits (Leonardi et al. 2006; Bresson et al. 2011; Hajek et al. 2016; Schuldt et al. 2016). Within-crown variability in leaf morphology and physiology, as caused by gradients in light intensity, atmospheric water vapour deficit and leaf water status, is large, especially in late-successional tree species such as European beech (Reiter et al. 2005; Niinemets, 2007; Oldham et al. 2010; Hagemeyer & Leuschner, 2019; Leuschner & Hagemeyer, 2020). Therefore, intraspecific comparison of leaf traits is only feasible...
for samples taken at equivalent crown positions, as realized in our study by collecting leaves from sun-exposed canopy branches uniformly at 1–2 m distance to the tree tip.

Considerable phenotypic plasticity and high genetic variance within populations are the basis of considerable leaf trait variation among neighbouring trees in a stand (Carsjens et al. 2014; Aranda et al. 2017; Frank et al. 2017). This is demonstrated by a proportion of >50% of within-tree or within-population variance (not explained by CI) in the total variance for most of the studied leaf traits in our study. The high intraspecific variance, be it phenotypic or genetic, represents the basis for successful adaptation of beech populations to drier environments (Aranda et al. 2018). We assume that the high small-scale variation in soil water deficits that developed as a consequence of the severe 2018 drought caused the observed higher proportion of unexplained site differences in the 2019 dataset. These water deficits were not represented in our AWC data that only considers water storage capacity but not actual water reserves. Moreover, including the atmospheric saturation deficit as an explanatory variable has the potential to increase the explained variance, as demonstrated by Zhu et al. (2021), who found a VPD effect on SLA in beech.

CONCLUSIONS

Even though our analysis includes only a limited dataset on inter-annual leaf trait variation in a given stand, the broad variation in climatic and soil physical conditions covered by the three climatic aridity gradients and in the three years’ weather conditions allow some conclusions on leaf trait plasticity and their adaptive modification in response to increasing climatic aridity. Despite sampling at equal positions in the crown, the spatial and inter-annual variation in sunny canopies mean leaf size (LS) and Huber value (HV), i.e. sapwood-to-leaf area ratio, is substantial across the study region, while specific leaf area (SLA) and the water potential at turgor loss point ($\Psi_{lp}$) were less variable. Reduced precipitation in more continental climates stimulates the production of larger (and thinner) sun leaves in beech, as long as water availability in spring is favourable. This prompts the trees to reduce stomatal conductance later in summer to reduce excess water loss and to achieve active osmotic adjustment to maintain cell turgor during periods of water deficits. In contrast to findings in other tree species, beech sapwood-to-leaf area ratio did not increase with decreasing precipitation, which will increase the drought stress exposure of beech sun foliage at drier sites and will constitute a bottleneck in the drought response strategy of this species.

In contrast to the effect of reduced MAP, inter-annual differences indicate that the severe 2018 drought event triggered the formation of smaller leaves with reduced SLA, increased HV and higher water use efficiency. Thus, European beech exhibits considerable plasticity in sun-canopy leaf traits, which may well exceed the variation in hydraulic traits, as demonstrated by climate gradient studies, but the degree of plasticity still await comparison with other tree species. As data from the mid-crown and shade crown are missing, it is unclear whether these modifications are restricted to the top crown or also occur in the lower crown. Continuous monitoring of inter-annual variation in leaf and hydraulic traits in response to climate fluctuations is needed for a better understanding of the drivers and functional consequences of foliage dynamics in trees.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Variability in leaf traits of European beech

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