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Stomatal and Leaf Morphology Response of European Beech (*Fagus sylvatica* L.) Provenances Transferred to Contrasting Climatic Conditions

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Abstract: Climate change-induced elevated temperatures and drought are considered to be serious threats to forest ecosystems worldwide, negatively affecting tree growth and viability. We studied nine European beech (*Fagus sylvatica* L.) provenances located in two provenance trial plots with contrasting climates in Central Europe. Stomata play a vital role in the water balance of plants by regulating gaseous exchanges between plants and the atmosphere. Therefore, to explain the possible adaptation and acclimation of provenances to climate conditions, stomatal (stomatal density, the length of guard cells, and the potential conductance index) and leaf morphological traits (leaf size, leaf dry weight and specific leaf area) were assessed. The phenotypic plasticity index was calculated from the variability of provenances' stomatal and leaf traits between the provenance plots. We assessed the impact of various climatic characteristics and derived indices (e.g., ecodistance) on intraspecific differences in stomatal and leaf traits. Provenances transferred to drier and warmer conditions acclimated through a decrease in stomatal density, the length of guard cells, potential conductance index, leaf size and leaf dry weight. The reduction in stomatal density and the potential conductance index was proportional to the degree of aridity difference between the climate of origin and conditions of the new site. Moreover, we found that the climate heterogeneity and latitude of the original provenance sites influence the phenotypic plasticity of provenances. Provenances from lower latitudes and less heterogeneous climates showed higher values of phenotypic plasticity. Furthermore, we observed a positive correlation between phenotypic plasticity and mortality in the arid plot but not in the more humid plot. Based on these impacts of the climate on stomatal and leaf traits of transferred provenances, we can improve the predictions of provenance reactions for future scenarios of global climate change.

Keywords: acclimation; adaptation; common garden; drought; ecodistance; mortality; phenotypic plasticity; stomatal frequency; stomatal size

1. Introduction

European beech forests may be seriously affected by climate change-induced drought due to their well-known vulnerability to water shortages [1,2]. Combinations of heat and drought stress may cause a decrease in the vitality and competitive ability of beech populations [3–5]. There have been reports of beech populations facing strong selective pressures [6], which are foreseen to become more intense due to upcoming alterations in rainfall patterns and temperatures with ongoing climate change [7,8]. To mitigate these negative effects on the future performance of beech forests in afforestation programs in Europe, there has been increased interest in research on the intraspecific variation in beech responses to environmental changes [9–11].

Large intraspecific differences in morphological and physiological traits among the beech provenances of distinct origin reflect possible strategies which are expected to modify their response to drought. Beech populations show divergent water use strategies reflected in the differences of photosynthetic performance, water-use efficiency, leaf water potential, xylem embolism resistance and leaf morphology [10–13]. The intraspecific variation in tolerance of water deficit follows a pattern shaped by both regional and local scale effects. Beech populations originating from the sites with low precipitation [14–16], lower altitude [17] or marginal distribution range [9,18,19] show higher drought resistance in comparison with the populations from more humid environments. The observed functional variation between beech populations reaffirms the importance of local adaptation to water deficit in the context of climate change [13,20].

Common garden experiments allow us to assess the relative importance of adaptation to the site of origin and acclimation to the new environment in the expression of phenotypic traits, as all provenances are exposed to the same conditions in provenance trial plots [21]. As a result of adaptation to local original conditions, the performance and vitality of populations show a correlation with ecological characteristics at the site of origin, even after their transfer to a new environment. The effect of environmental change on a provenance planted at a given location can be expressed as the difference between the ecological characteristics of the trial plot and the site of provenance origin, called the ecodistance [22]. Moreover, if we study the performance of populations in different provenance trial plots and the differences between plots are greater than within, we expect that the differences between phenotypes are driven more by acclimation to current environmental conditions than by local adaptation [23,24]. Stomatal and leaf morphological traits such as stomatal density, potential conductance index and specific leaf area affect stomatal conductance and transpiration (functional traits) which in return influence performance, growth and survival [13,19]. Therefore, stomatal and leaf morphological traits represent a viable means to identify populations suitable for a specific environment.

The populations that possess stomatal and leaf morphological traits adapted to drought and heat stress will have an evolutionary advantage under future scenarios [3]. Hence, a plant strategy to cope with differences in water regimes involves altering stomatal density and stomatal size [25–27]. Some studies have shown that smaller stomata close more quickly than larger stomata do, thus indicating that this could enhance plant adaptation to drought [28]. However, there remains the debated issue of how stomatal density varies within a particular environment. It has been reported that drought resistant plants show higher stomatal density [27,29,30], but the results of more recent studies performed in controlled environments suggest that lower stomatal density improves drought tolerance [31,32]. Other functional traits frequently utilized in ecological studies and linked to drought tolerance are specific leaf area and leaf size. Several studies have revealed that changes in environmental factors such as light, temperature or nutrients strongly influence leaf traits [33,34]. Species with smaller, thicker leaves mainly occur in more stressful habitats and exhibit lower specific leaf areas. This trait is related to the species water use strategy [34], and it is highly plastic [35], although the precise physiological regulation mechanism of specific leaf area is still uncertain [33,36].

Furthermore, the phenotypic plasticity, defined as the capacity for a genotype to alter its morphology and/or physiology under altered environmental conditions [37], can play a major role in the survival and sustainability of forest populations subjected to global change [38–40]. This is generally seen as favourable under stress conditions because it enables plants to react to fluctuations in the environment [41,42]. However, several studies have reported a potential trade-off between phenotypic plasticity and individual fitness [43], suggesting reduced performance with increasing plasticity [38,44,45]. Therefore, it is crucial to assess the ability for stomatal and leaf morphological traits to react plastically to their environment and to test the connection between their phenotypic plasticity and plant fitness and performance.

We investigated stomatal and leaf morphological traits, their phenotypic plasticity and a link to the climate of origin and current climate in nine European beech provenances located in two provenance trial plots with contrasting climates (warmer and drier/colder and more humid) in Central Europe. Based on the premise that environmental differences between provenance trial plots can alter the stomatal and leaf morphological traits of European beech, we hypothesized that (i) provenances that grow in drier and warmer provenance plots will exhibit lower values of measured traits than those growing in more humid and colder plots to increase their performance under suboptimal conditions. We further expected to find that (ii) the climate of the provenance's original site would affect the provenance's phenotype even 18 years after transfer to a different environment. In addition to the relationship between the climate of origin and phenotype itself, we also hypothesized that (iii) the provenance climate of origin should affect the phenotypic plasticity of provenances, where provenances from more heterogeneous environments show higher phenotypic plasticity. Finally, we hypothesized (iv) a negative relationship between phenotypic plasticity and tree mortality, and provenances with higher value of plasticity would acclimate better under different environments, thus mitigating the risk of mortality.

2. Materials and Methods

2.1. Locality Description and Plant Material

The material used for this experiment was collected from two European beech (*Fagus sylvatica* L.) provenance trial plots: Tále in the Slovak Republic (near Zvolen, 48°38′ N, 19°02′ E, 810 m a.s.l.) and Zbraslav in the Czech Republic (near Prague, 49°57′ N, 14°22′ E, 360 m a.s.l.). The Slovak provenance plot included loam soil with good nutrient availability and high water holding capacity, while the Czech provenance plot included sandy loam soil with poor nutrient availability and average water holding capacity [12]. Climate data for the original provenance sites were obtained from the WorldClim high-resolution climate database [46]. The climate characteristics of the Czech provenance plot were obtained from the Praha-Libuš meteorological station, and freely available data were provided by the Czech Hydrometeorological Institute (http://portal.chmi.cz). Climate data for the Slovak provenance plot were obtained from the nearby Kremnické Bane meteorological station monitored by the Slovak Hydrometeorological Institute. We calculated additional indices from the above climate data:

Ellenberg quotient (EQ) [47]

$$EQ = 1000 \times \frac{T_h}{Prec} \tag{1}$$

T_h—mean temperature of the hottest month (here July), $Prec$—annual sum of precipitation.

Forest Aridity Index (FAI) [48]

$$FAI = 100 \times \frac{T_{7-8}}{Prec_{5-7} + Prec_{7-8}} \tag{2}$$

T_{7-8}—mean temperature of July and August, $Prec_{5-7}$—precipitation sum for May to July, $Prec_{7-8}$—precipitation sum for July to August.

Isothermality (Iso_T)

$$Iso_T = \frac{T \times (T_h - T_c)}{T_{MAX} - T_{MIN}} \times 100 \tag{3}$$

T—annual mean temperature, T_h—mean temperature of warmest month, T_c—mean temperature of coldest month, T_{MAX}—max temperature of warmest month, T_{MIN}—min temperature of coldest month.

Precipitation seasonality ($Seas_{Prec}$)

$$Seas_{Prec} = \frac{\sigma_{Prec}}{Prec} \tag{4}$$

σ_{Prec}—annual standard deviation of precipitation, *Prec*—annual sum of precipitation.

All geographical and climatic data are presented in Table 1. Optimal hydric conditions of European beech stands are represented by *EQ* values of below 20. European beech starts to lose its competitive performance in environments with *EQ*s of above 20 and is replaced by more xerotic tree species in places with *EQ*s of above 30 [49,50]. Moreover, locations with optimal rainfall patterns during the vegetation season for European beech are defined by an *FAI* of under 4.75 [48,51]. The *EQ* for the Czech provenance plot is 33.5, and the *FAI* is 5.1, which characterizes the plot as a location with marginal environmental conditions for European beech occurrence. The Slovak provenance plot with an *EQ* of 19.1 and *FAI* of 2.5 represents the optimal hydric environment according to the classifications mentioned above. Both provenance plots were established in 1998 as a part of the European provenance plot network, whereas proven ances were planted as two-year-old seedlings [52]. Both plots were planted with 2 × 1 m spacing under a randomized block design with three blocks and fifty seedlings per block. Nine provenances were chosen for the analysis to capture the whole altitude range of the distribution of European beech (Figure 1). The distribution of provenance original sites regarding climate characteristics is visualized in Figure A1 of the Appendix A. We were not able to sample some of the provenances in multiple blocks due to high spatial mortality. The third block of the Czech provenance plot completely died off, and some of the provenances remained only in one block. Similarly, the third block of the Slovak provenance plot suffered high mortality, and we avoided this to minimize sample heterogeneity due to unknown factors.

Table 1. Geographic and climate characteristics of provenance plots and their original locations.

Provenance	***Long***	***Lat***	***Alt***	***T***	T_{59}	***Prec***	$Prec_{59}$	***EQ***	***FAI***	Iso_T	$Seas_{prec}$
FR04	2.58	44.15	850	10.8	16.8	804	344	23.8	5.4	3.8	14
LUX12	6.2	49.67	400	8.6	14.9	866	365	19.7	4.8	3.1	11
UK17	−3.42	57.67	10	8.2	12.7	671	303	21.8	5.3	3.6	19
SWE23	13.2	55.57	40	7.9	14.3	640	286	25.9	6.2	2.6	21
GER26	10.67	53.65	55	8.3	15	678	319	25.5	5.1	3.0	17
AU35	14.1	47.72	1250	2.4	9.2	1495	779	7.6	1.3	3.2	26
AU36	14.85	47.53	1100	2.9	9.9	1168	648	10.4	1.6	3.1	32
PL43	22.82	49.25	900	6.3	14.1	762	433	21.5	2.9	2.7	35
PL67	18.17	54.33	250	5.8	13.2	633	336	24.6	4.3	2.4	30
CZ Zbraslav	14.37	49.95	360	8.25	15.6	532	330	33.5	5.1	na	na
SK Tále	19.03	48.63	850	6.58	14.1	842	441	19.1	2.5	na	na

Long—longitude, *Lat*—latitude, *Alt*—altitude, *T*—annual average temperature, T_{59}—average temperature during the vegetation season, *Prec*—sum of annual precipitation, $Prec_{59}$—sum of precipitation during the vegetation season, *EQ*—Ellenberg quotient, *FAI*—Forest Aridity Index, Iso_T—isothermality, $Seas_{prec}$—precipitation seasonality, na—not available.

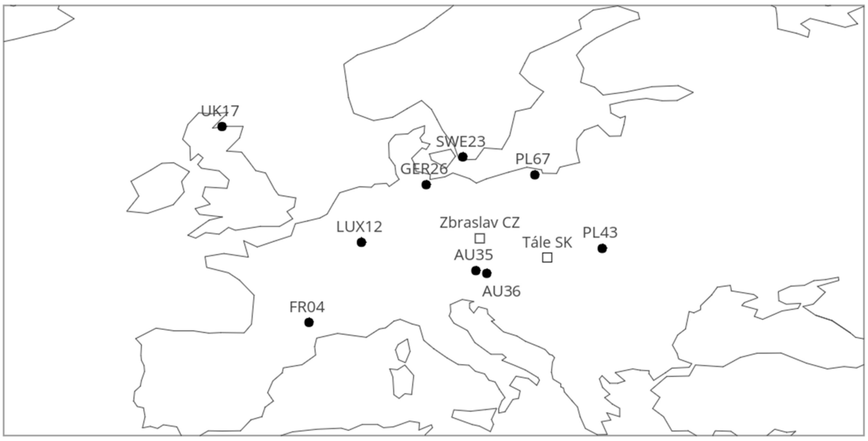

Figure 1. Localities of the tested provenances (dots) and provenance plots (squares).

2.2. Stomatal and Leaf Morphological Traits

The samples were taken during June 2016 from full sun-exposed leaves located in the upper third of the crown to minimize irradiation and canopy position effects on stomatal and leaf morphology development [53,54]. We sampled six individuals per provenance per plot and made two imprints per individual. The imprints were made by the application of transparent nail polish to the abaxial side of the leaves [55]. The layer of polish was then transferred to a microscope slide with transparent tape. To avoid possible variations in stomatal distribution within the leaves, we took imprints between the second and third veins from the base of the leaves [17]. Six images were taken from each imprint using a Motic BA210 microscope with an integrated camera (Motic Electric, Linz, Austria). Three of these images were captured at 40 × 10 magnification, and three photos were captured at 10 × 10 magnification. The images at 10 × 10 magnification were used to assess stomatal density (*SD*). The number of stomata was calculated within a 750 × 750 µm square per image with a random position using ImageJ 1.51k software (National Institute of Health, Bethesda, MD, USA). The assessed value of the number of stomata per square was converted to the number per square millimetre. The length of guard cells (L_A) was measured for ten stomata in a 40 × 10 magnification image using ImageJ software. The *SD* and L_A values were then averaged per individual for further analysis. Additional leaves from the same branch were scanned with a HP Scanjet G4010 scanner (Hewlett Packard, California, USA), and the leaf size (S_{leaf}) was subsequently measured by ImageJ software. The scanned leaves were stored in silica gel, after which the leaves were dried at 75 °C to a constant weight (approximately 48 h). Afterwards, the dry weight (m_{leaf}) was assessed. From the measured parameters, we calculated the following traits:

The potential conductance index *(PCI)*, an integrative variable of stomatal density and the length of guard cells, which can be used as a proxy for the theoretical maximal stomatal water vapour conductance [56,57]:

$$PCI = L_A^2 \times SD \times 10^{-4} \tag{5}$$

L_A—length of guard cells, *SD*—stomatal density.

Specific leaf area (*SLA*), a parameter that corresponds to the thickness and density of leaf lamina [58,59]:

$$SLA = \frac{S_{leaf}}{m_{leaf}} \tag{6}$$

S_{leaf}—leaf size and m_{leaf}—dry weight of leaves.

2.3. Quantification of Phenotypic Plasticity

The plasticity index based on maximum and minimum means (PI_v) was calculated for each trait and provenance, respectively [37].

$$PI_v = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_{max}} \tag{7}$$

$\overline{x}_{max}$—maximum mean (mean of the group showing the maximal value relative to that of other groups); $\overline{x}_{min}$—minimum mean (mean of the group showing the minimal value relative to that of other groups). In our case, the groups refer to identical provenances from two different provenance plots.

2.4. Statistical Analysis

Statistical analysis was performed using R statistical software (Version 4.0.3, R Core Team, Vienna, Austria). Original climate characteristics of provenances were analysed by principal component analysis to visualize likeness or disparity between the original sites of the provenances. The normal distribution of the obtained data was first tested by the Shapiro–Wilk test. A two-way analysis of variance was used where provenance and plots were set as factors with fixed effects. Moreover, differences between provenances were tested separately for each plot by Fisher's LSD post hoc test. We used ecodistance to capture shifts between the climate of origin and the climate of the provenance plot. The ecodistance was defined as the difference between the investigated ecologically relevant variables at the test site and at the population origin [22,60]. Furthermore, mixed models in the R "nlme" package [61] were used to explore relationships between individual stomatal and leaf morphological traits (dependent variables) and various explanatory variables, such as *EQ*, *FAI*, latitude and longitude. To account for between-plot variability, we included plot as a random effect variable, and the between-plot variance in the intercept estimation was quantified. We were not able to include a block design in the mixed models, as there were missing data due to spatial mortality within the plots. We used maximum likelihood to estimate the parameters of the model. To estimate the importance of individual explanatory variables, we calculated the normalized model likelihoods (Akaike weights). First, we fitted all possible model variants, including the null model (including only the intercept) and full model (including all explanatory variables). Then, Akaike weights were calculated for each model based on the corrected Akaike information criterion using the "Weights" function in the "MuMIn" R package [62]. The models with the highest weights were further selected and interpreted. In addition to that, we calculated marginal R^2 ($R^2{}_m$) and conditional R^2 ($R^2{}_c$) for better comparison of fixed and random factors in model [63]. Statistical significance of differences between the plasticity of the traits was assessed by analysis of variance and Tukey's post-hoc test. The relationships between original climate, phenotypic plasticity and tree mortality were tested by linear regression.

3. Results

3.1. Stomatal and Leaf Morphological Traits

The effects of the tested factors, provenance, plot and provenance-by-plot interactions were statistically significant for all traits except in the case of specific leaf area (*SLA*) (Table 2, Table A1 in Appendix A). Provenances growing in a warmer and drier site in the Czech Republic showed lower guard cell length (L_A, Figure 2A), stomatal density (*SD*, Figure 2B), potential conductance index (*PCI*, Figure 2C), leaf dry weight (m_{leaf}, Figure 2D) and leaf size (S_{leaf}, Figure 2E) values than the provenances in the colder and more humid Slovak plot. Provenances in the Czech provenance plot showed an average 57% reduction in the *PCI* for leaves that were 12% smaller than those in the Slovak provenance plot. Based on the average m_{leaf}, the provenances in the drier Czech plot accumulated 39% less biomass per leaf than the provenances in the more humid Slovak plot. Values of *SLA* were higher on average for the drier provenance plot, but changes were inconsistent and insignificant between provenances (Figure 2F).

Table 2. Results of two-way ANOVAs for each stomatal and leaf morphological trait.

Factor	Df	Trait	L_A	*SD*	*PCI*	m_{leaf}	S_{leaf}	*SLA*
Provenance	1	F	57.95	17.65	11.59	3.79	10.83	0.69
		p	***	***	***	**	***	0.64
Plot	8	F	1771.5	457.4	1221.25	31.35	21.58	9.48
		p	***	***	***	***	***	**
Provenance × Plot	8	F	18.09	4.642	7.66	2.72	3.92	1.64
		p	***	***	***	*	***	0.13

L_A—length of guard cells, *SD*—stomatal density, *PCI*—potential conductance index, S_{leaf}—leaf size, m_{leaf}—dry weight per leaf, *SLA*—specific leaf area, significance levels *** <0.001 ** <0.01 * <0.05.

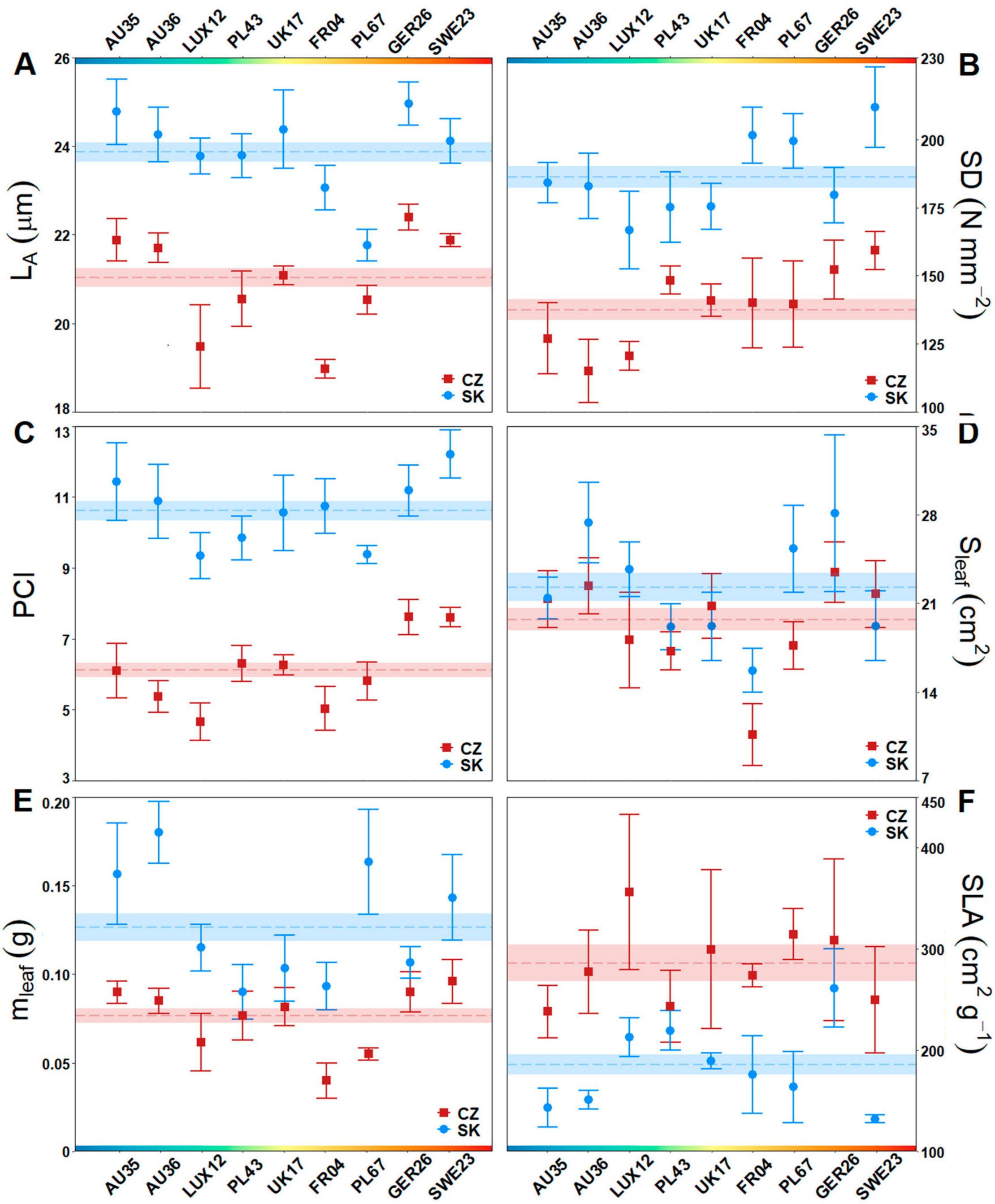

Figure 2. Mean values and standard errors of stomatal and leaf morphological traits per provenance: length of guard cell (L_A, (**A**)), stomatal density (*SD*, (**B**)), potential conductance index (*PCI*, (**C**)), leaf size (S_{leaf}, (**D**)), leaf dry weight (m_{leaf}, (**E**)) and specific leaf area (*SLA*, (**F**)). The red colour represents provenances growing in the Czech provenance plot, and the blue colour represents provenances growing in the Slovak provenance plot. The dashed horizontal line represents the average per plot with the surrounding standard error interval band. Provenances are arranged based on the Ellenberg quotient of the original site (blue to red x axis band).

3.2. Impact of Climate Ecodistance on Stomatal and Leaf Morphological Traits

The multifactorial approach based on mixed models showed that the models with singular explanatory variables performed better than those with multiple factors; thus, a further analysis employed simple linear regression models (Tables A2 and A3). The degree of aridity and temperature differences between provenances' origins and new plots (ecodistance) had a significant effect on provenances' stomatal development (Figure 3). The ecodistance of the Ellenberg quotient (EQ_{ED}) and forest aridity index (FAI_{ED}) had a significant negative influence on *SD* (Figure 3A,B) and the *PCI* (Figure 3C,D). Provenances transferred to a drier environment relative to their original site showed a proportionally lower density of stomata with lower potential conductance. The ecodistance of average temperature (T_{ED}) and average temperature during the vegetation season (T_{59ED}) also had a significant negative influence on *SD*, and provenances transferred to a climate warmer than that of their original site showed a proportional decrease in *SD* (Figure 3E,F). Other climate ecodistance indices showed no significant correlations with stomatal and leaf morphological traits.

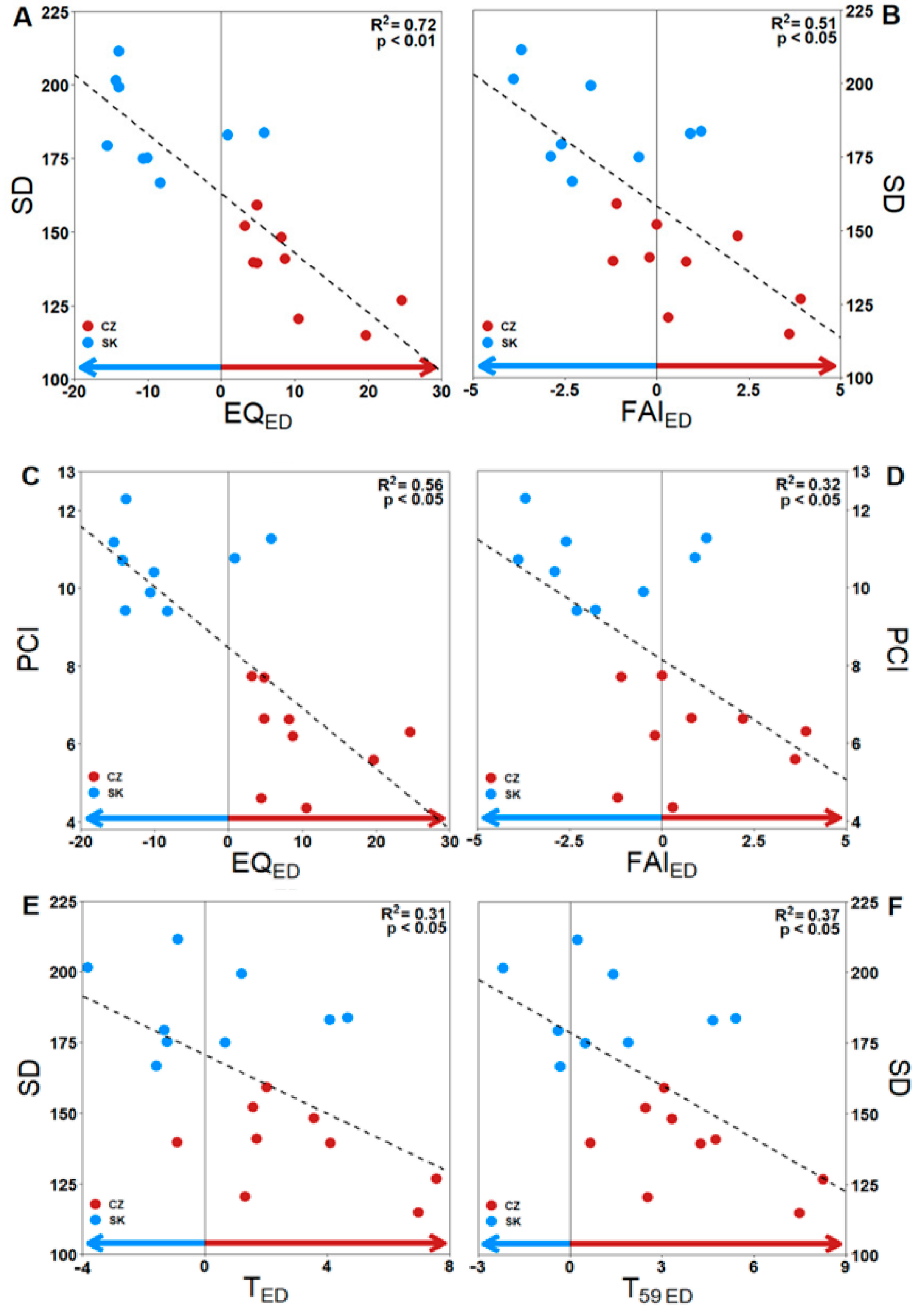

Figure 3. Linear regression results showing the relationship between ecodistance (EQ_{ED}, FAI_{ED}, T_{ED}, $T_{59\,ED}$) and stomatal morphological traits, stomatal density (*SD*, (**A**,**B**,**E**,**F**)) and the potential conductance index (*PCI*, (**C**,**D**)). Positive values on the horizontal axis represent transfer to a drier/hotter environment (red arrow), and negative values represent transfer to a more humid/colder environment (blue arrow).

3.3. Phenotypic Plasticity of Stomatal and Leaf Morphological Traits

To quantify the acclimation response, we calculated the phenotypic plasticity index for each trait and provenance. Plasticity indices of the potential conductance index (PCI_{PI}), leaf dry weight ($m_{leaf\,PI}$) and specific leaf area (SLA_{PI}) showed the highest values of plasticity among the provenances. In contrast, the plasticity index of the length of guard cells ($L_{A\,PI}$) was the lowest among the provenances (Figure 4). The climate of the original provenance locations affected the plasticity response of acclimation after transfer to a new environment. Provenances from locations with more heterogeneous environmental temperatures showed lower PCI_{PI} and $L_{A\,PI}$ plasticity. We found a positive relationship between the isothermality (Iso_T) of the original location and provenances $\mathrm{PCI_{PI}}$ and $L_{A\,PI}$ (Figure 5A,C). Moreover, provenances from environments with more heterogeneous precipitation distributions showed lower plasticity of $\mathrm{L_A}$. The seasonality of precipitation ($Seas_{Prec}$) at the original locations of the provenances negatively influenced the $L_{A\,PI}$ of the provenances (Figure 5D). Furthermore, the original latitude (*Lat*) affected the PCI_{PI}, as provenances from lower latitudes showed a higher PCI_{PI} (Figure 5B). We found a significant negative relationship between provenance mortality and the plasticity of stomatal and leaf morphological traits (PCI_{PI} and SLA_{PI}) in the drier and hotter Czech provenance plot (Figure 6) but no significant relationship between mortality and plasticity in the more humid Slovak provenance plot.

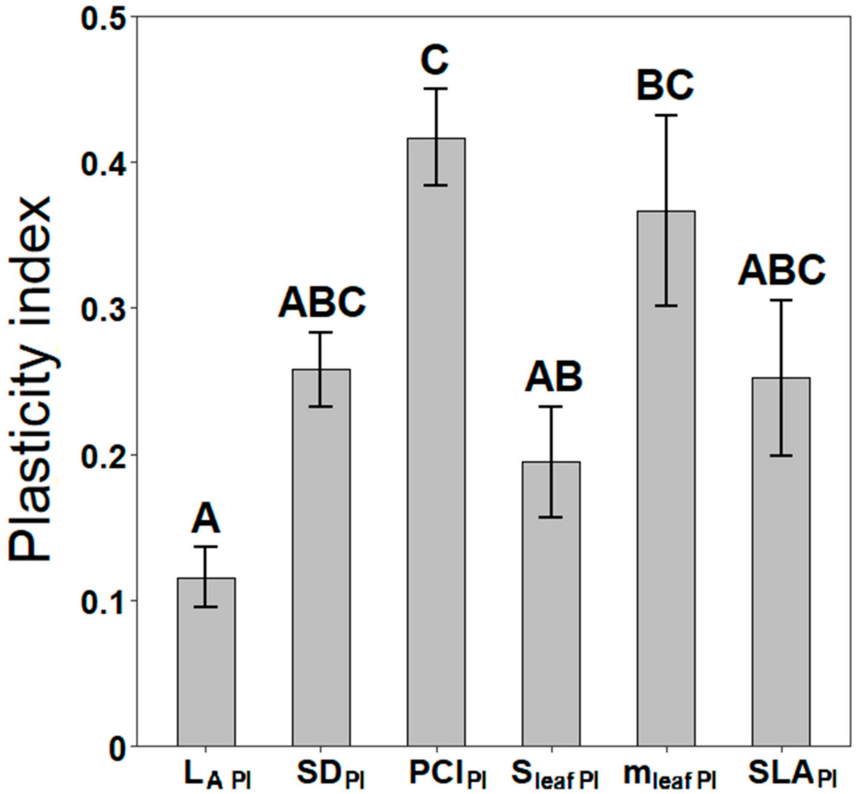

Figure 4. Phenotypic plasticity indices of stomatal density (SD_{PI}), guard cell length ($L_{A\,PI}$), the potential conductance index (PCI_{PI}), leaf size ($S_{leaf\,PI}$), leaf dry weight ($m_{leaf\,PI}$) and specific leaf area (SLA_{PI}) for each provenance. Plasticity indices obtain values of 0 to 1, where 0 denotes no plasticity and 1 denotes theoretical maximal plasticity. Confidence intervals represent standard error and the capital letters correspond to results of post-hoc analysis.

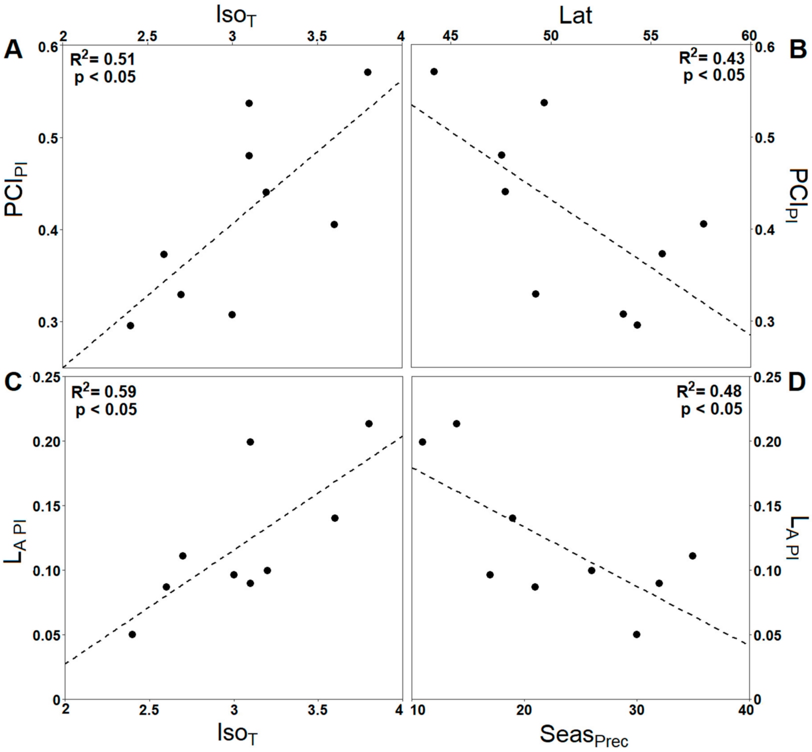

Figure 5. Linear regression results showing the relationship between isothermality (Iso_T, (**A**,**C**)), latitude (*Lat*, (**B**)) and precipitation seasonality ($Seas_{Prec}$, (**D**)) of the provenance's original site and plasticity indices ($L_{A\ PI}$, PCI_{PI}).

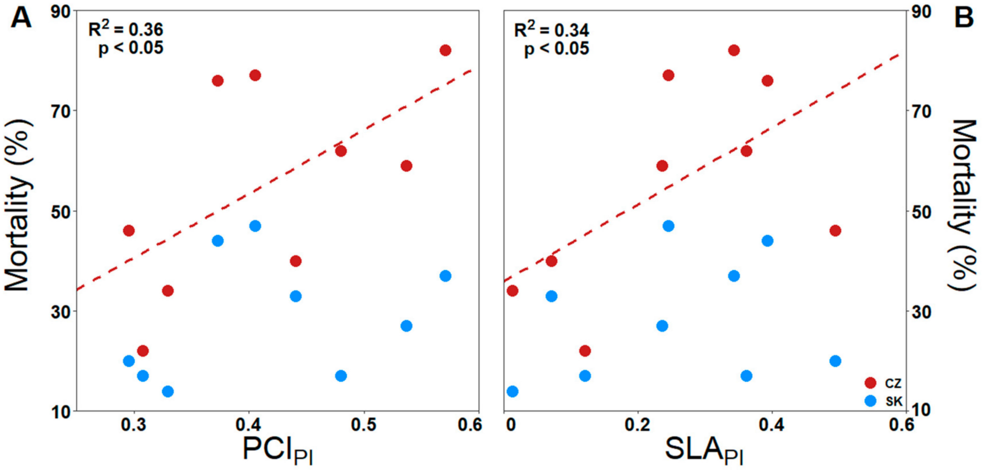

Figure 6. Visualization of the relationship between plasticity indices (PCI_{PI}, (**A**); SLA_{PI}, (**B**)) and the observed mortality of provenances in the Czech (red) and Slovak (blue) provenance plot. The linear regression was significant only for drier Czech provenance plot.

4. Discussion

4.1. Functional Aspects of the Adaptive Response

Our study demonstrates that European beech provenances adjusted their stomatal and leaf morphological traits in response to being transferred to a new environment. Provenances exhibited significantly lower *SD*, L_A, *PCI*, S_{leaf} and m_{leaf} levels in the drier Czech provenance plot than in the more humid Slovak provenance plot. There were no significant differences in *SLA* when we considered provenance–plot interactions. Lack of significant differences regarding *SLA* might be caused by low sample size and high variability of the trait. Nevertheless, we observed that provenances in more xeric plot showed higher overall *SLA*. This is in opposition to general consensus [13,36,64,65], but has already been observed in some studies [66,67]. The adjustment of stomatal and leaf development can be seen as an adaptive response to either suboptimal climatic conditions of the Czech plot or to favourable climatic conditions of the Slovak plot. It has been reported that plants might improve their drought tolerance and water use efficiency (*WUE*) by reducing *SD* [31,32,68] and L_A [28,69]. Both herbaceous plants and trees react to episodic drought and long-term xericity of the environment by decreasing *SD* [25,70] and developing smaller stomata with lower L_A [26,56,71–73]. Combined stomatal morphology (L_A) and the distribution of stomata on leaves (*SD*), represented as the *PCI*, might be seen as a proxy for structural constraints of maximal stomatal conductance. A reduction in the *PCI* under xeric conditions should then ultimately reduce stomatal conductance [74] and water loss, which can lead to improved *WUE* [73,75]. Acclimation through the development of smaller leaves (S_{leaf}) under xeric conditions leads to less water loss through transpiration [76] and higher WUE [77,78]. It should be mentioned that *WUE* is also influenced by photosynthetic capacity and not just stomata related traits [13]. Plants exposed to water deficit show a reduction in m_{leaf} [64,79]. The combination of lower S_{leaf} with m_{leaf} in drier site might be explained by trees' strategy to invest more in root biomass with the cost of lower leaf biomass and leaf size [80]. The above-mentioned traits, therefore, represent plants' adaptive mechanisms in mitigating drought stress [81]. As these stomatal and leaf morphological traits have a significant impact on plant performance under water stress, their adjustment is vital for plants to successfully acclimate under changing conditions due to either anthropogenic transport to new environments or accelerating global climate change.

On the other hand, an increase in *SD* and $\mathrm{L_A}$ can enhance photosynthetic capacity [82,83], which could enhance tree performance under optimal climatic conditions where the strongest selective pressure is competition [84]. Leaves with higher *SD* and larger stomata (L_A) show an increase in maximal stomatal conductance [85–87], which leads to higher biomass accumulation and growth [88]. A higher *PCI* increases the maximal limit of stomatal conductance, which might improve photosynthetic capacity [89]. Moreover, the *PCI* has also been found to be related to leaf hydraulic conductance [57], which has been correlated with photosynthesis rates across plant species [89,90]. Higher S_{leaf} values under favourable conditions lead to higher photosynthesis rates [91,92], which positively affect leaf biomass production with higher m_{leaf} values [93]. According to our results, provenances in the drier and hotter Czech plot might acclimatise to their environment by undertaking a water conservation strategy with decreasing *SD*, L_A, *PCI*, S_{leaf} and m_{leaf} values. Conversely, provenances growing on the more humid Slovak plot could utilize the development of larger leaves (S_{leaf}, m_{leaf}) with higher *SD*, L_A and *PCI* values to maximize photosynthetic activity and growth in a competitive environment. Despite the clear theoretical basis for why the provenances showed significantly different values of the tested traits, we did not find any significant relationship between the tested traits and mortality. To address our first hypothesis (i), we found the morphological response as expected, but there is no evidence that the alternation of stomatal and leaf morphological traits had a significant positive effect on provenance performance. To capture the drought resistance profile of provenances for practical application, we suggest analysing additional physiological and functional traits, such as *WUE*, cuticular conductance, xylem embolism resistance and the turgor loss point [10,13,94]. Stomatal and leaf morphological traits alone are not satisfactory to define which provenances would be favourable for hotter and more xeric conditions in the near future.

4.2. Climate Ecodistance as an Effective Tool for Provenance Research

We found that the *SD* and *PCI* of provenances depend on the aridity ecodistance (EQ_{ED} and FAI_{ED}) and temperature ecodistance (T_{ED} *and* T_{59ED}). The ecodistance represents the climatic shift between the original provenance site and the provenance plot to which it is transferred. The connection between both the climate of origin and the current climate and stomatal phenotype might suggest strong coordination between genetic and environmental impacts on stomatal development. A previous provenance study showed strong significant relationships between temperature and ecodistance and the phenology, morphology and dendrometric traits of European beech provenances [22]. Aridity ecodistance (*EQ*) has been found to be a significant explanatory variable for the vitality [95] and growth [96] of European beech provenances. A study of four temperate tree species (*Fagus sylvatica*, *Picea abies* (L.) Karst., *Pinus sylvestris* L. and *Quercus petraea* (Matt.) Liebl.) has also shown a significant relationship between aridity ecodistance (the annual aridity index) and the growth of provenances after transfer to a new environment [97]. Our second hypothesis (ii) is confirmed by a significant relationship between *SD*, the *PCI* and ecodistance, which incorporates the climate of origin as well as the current climate of new plots. The ecodistance has not attracted much popularity since its first formulation [22], but it seems that this simple mathematical formulation of climate transfer could capture the physiological, morphological and growth reactions of tree provenances to their new environments. More studies have focused on additional tree species, and other aspects of tree phenotyping are needed to test whether ecodistance is robust enough to be useful and reliable for forestry applications. The ecodistance could then be used not only to explain the effect of climatic shifts caused by the spatial transfer of provenances but also to predict provenance reactions to temporal changes caused by accelerating anthropogenic global climate change.

4.3. Phenotypic Plasticity

Populations with higher phenotypic plasticity can adapt to higher environmental variability and thus can minimize the risk of mortality [65,98,99]. Our results suggest that the *PCI*, m_{leaf} and *SLA* are the most plastic, while L_A is the least plastic trait among provenances. The low plasticity of $\mathrm{L_A}$ might suggest higher genetic control of stomatal size relative to the other tested traits. Similar results of low plasticity for L_A and high plasticity for the *PCI* and leaf morphology were also observed in beech provenance studies [71,95]. Furthermore, we found a significant relationship between climate heterogeneity of the original site (Iso_T and $Seas_{Prec}$) and the plasticity of L_A and *PCI* ($L_{A\ PI}$ and PCI_{PI}). We expected populations that had evolved under more heterogeneous environments to favour higher phenotypic plasticity to quickly adjust their phenotype if needed [11,37,99,100]. In contrast to what we expected, the provenances from the most heterogeneous environment showed the lowest $L_{A\ PI}$ and $\mathrm{PCI_{PI}}$ values, so hypothesis (iii) cannot be confirmed. We also found a negative significant relationship between latitude and PCI_{PI}, which might be attributable to a reduction in genetic diversity from lower to higher latitudes after the recolonization of habitats after the last glacial maximum [101–103]. A harsher northern environment or higher competition at the distribution edge may create more selection pressure than elsewhere in the distribution range [44]. Therefore, populations that evolved under strong selection pressure might favour more efficient strong genetic control over high phenotypic plasticity within the population [104]. This could lead to trait canalization which might be translated to lower phenotypic plasticity [105–107].

We found a significant positive relationship between PCI_{PI}, SLA_{PI} and mortality values, but only in the drier and hotter Czech provenance plots. However, in this study with phenotyping data from only the remaining trees, we cannot conclude that the high plasticity of these two traits causes high provenance mortality as the plasticity was calculated from surviving individuals and thus might be biased. Higher mortality, which created more open canopies and less competition among the remaining trees, might have caused the higher values of phenotypic plasticity, as trees reacted to newly available canopy space. High phenotypic plasticity is generally seen as a favourable property of plants, trees or populations under global climate change [37,108,109]. We cannot confirm our hypothesis that (iv) there is a negative relationship between phenotypic plasticity and tree mortality. Despite this, we find it important to discuss phenotypic plasticity. Both recent and earlier studies have shown that the high phenotypic plasticity of plants should not be universally seen as a positive attribute [43,45,110,111]. The plasticity cost might not be pronounced under normal conditions, but when plants are exposed to a highly stressful environment, the plasticity cost might outweigh the fitness gain [112,113]. The results of several studies suggest reduced performance with increasing phenotypic plasticity under stress [38,44]. Thus, phenotypic plasticity, measured by common metrics [37], should not be automatically interpreted as beneficial for plants under global climate change. The high phenotypic plasticity of populations exposed to severe environmental stress might be associated with increased mortality and reduced fitness.

5. Conclusions

European beech provenances have shown a high degree of both adaptation and acclimation after transfer to a new environment. The observed differences in stomatal morphological traits were linked to the long-term aridity and air temperature of both the original site and the current provenance plot. The heterogeneity of the original site's climate and latitude affected the phenotypic plasticity of stomatal traits. Higher phenotypic plasticity was associated with higher mortality under suboptimal conditions but not under favourable hydric conditions. Additional functional and physiological traits should be considered to evaluate the resistance or performance of European beech provenances, as we have not found any direct link between mortality and the tested stomatal and leaf morphological traits. Ecodistance can be considered as an easy to use and robust tool for analysing adaptive responses of tree provenances under global climate change. Studies of phenotypic plasticity should not interpret the positive effects of high plasticity without taking into consideration the performance, fitness or vitality of plants.

Author Contributions: D.K., J.F. and A.K. conceived the experimental design and coordinated the experiment; P.P., A.P., A.K., J.F. and D.K. sampled the plant material; A.P. and P.P. processed the samples and performed the measurements; M.B., P.P. and P.F. performed the statistical analyses; P.P. and A.P. wrote the first version of the manuscript, which was discussed and approved by all of the co-authors. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Results of Fisher LSD post-hoc test.

SK	L_A	SD	PCI	S_{leaf}	m_{leaf}	SLA
FR04	BC	AC	AB	B	A	AB
LUX12	AB	B	A	AB	ABC	BC
UK17	AB	AB	AB	AB	AB	ABC
SWE23	AB	C	B	AB	ABCD	A
GER26	A	ABC	AB	A	ABC	C
AU35	A	ABC	AB	AB	BCD	A
AU36	AB	ABC	AB	A	D	AB
PL43	AB	AB	A	AB	A	BC
PL67	C	AC	A	A	CD	AB
CZ	L_A	SD	PCI	S_{leaf}	m_{leaf}	SLA
FR04	C	ABCD	AB	B	D	A
LUX12	C	AB	B	AB	ACD	A
UK17	AB	ABCD	ACD	A	ABC	A
SWE23	AB	D	CD	A	B	A
GER26	B	CD	D	A	AB	A
AU35	AB	ABC	AC	A	AB	A
AU36	AB	A	AB	A	ABC	A
PL43	AC	BCD	ACD	AB	ABCD	A
PL67	AC	ABCD	AB	AB	CD	A

SD—stomatal density, L_A—length of guard cells, PCI—potential conductance index, SLA—specific leaf area, S_{leaf}—leaf size, m_{leaf}—dry weight per leaf.

Table A2. AICc based variable weights of mixed models. Green to red spectrum represents the explanatory power of individual parameters and their combinations. Full model consists of all three parameters: aridity (expressed as the *EQ* and *FAI*), longitude and latitude.

Trait	EQ_{ED}	$Long_{ED}$	Lat_{ED}	EQ_{ED} + $Long_{ED}$	EQ_{ED} + Lat_{ED}	$Long_{ED}$ + Lat_{ED}	Full Model
SD	0.704	0.014	0.007	0.125	0.131	0.002	0.016
L_A	0.222	0.31	0.168	0.202	0.031	0.047	0.021
PCI	0.211	0.461	0.151	0.065	0.032	0.073	0.007
SLA	0.312	0.239	0.292	0.044	0.06	0.046	0.006
S_{leaf}	0.16	0.472	0.152	0.069	0.031	0.102	0.014
	FAI_{ED}	$Long_{ED}$	Lat_{ED}	FAI_{ED} + $Long_{ED}$	FAI_{ED} + Lat_{ED}	$Long_{ED}$ + Lat_{ED}	Full model
SD	0.263	0.056	0.029	0.046	0.532	0.009	0.065
L_A	0.286	0.284	0.154	0.163	0.049	0.043	0.021
PCI	0.177	0.482	0.158	0.068	0.031	0.076	0.008
SLA	0.316	0.241	0.296	0.047	0.048	0.047	0.005
S_{leaf}	0.149	0.345	0.111	0.273	0.021	0.075	0.027

SD—stomatal density, L_A—length of guard cells, *PCI*—potential conductance index, *SLA*—specific leaf area, S_{leaf}—leaf size, EQ_{ED}—Ellenberg quotient ecodistance, FAI_{ED}—Forest Aridity Index ecodistance, $Long_{ED}$—longitude ecodistance, Lat_{ED}—latitude ecodistance.

Table A3. Values of the marginal and conditional R^2 of mixed models presented in Table A2.

	SD		L_A		*PCI*		*SLA*		S_{leaf}	
	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c
EQ_{ED}	0.38	0.76	0.03	0.87	0.01	0.89	0.04	0.86	0.02	0.38
$Long_{ED}$	0.01	0.84	0.02	0.78	0.01	0.91	0.01	0.75	0.12	0.18
Lat_{ED}	0.00	0.84	0.00	0.78	0.00	0.91	0.01	0.77	0.03	0.22
EQ_{ED} + $Long_{ED}$	0.38	0.75	0.07	0.92	0.01	0.91	0.04	0.86	0.19	0.75
EQ_{ED} + Lat_{ED}	0.37	0.77	0.03	0.86	0.01	0.88	0.04	0.86	0.05	0.40
$Long_{ED}$ + Lat_{ED}	0.02	0.84	0.02	0.78	0.01	0.91	0.02	0.77	0.15	0.29
Full model	0.37	0.76	0.07	0.91	0.01	0.91	0.04	0.86	0.21	0.77
	SD		L_A		*PCI*		*SLA*		S_{leaf}	
	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c	R^2_m	R^2_c
FAI_{ED}	0.06	0.82	0.02	0.83	0.00	0.90	0.02	0.80	0.09	0.43
$Long_{ED}$	0.01	0.84	0.02	0.78	0.01	0.91	0.01	0.75	0.12	0.18
Lat_{ED}	0.00	0.84	0.00	0.78	0.00	0.91	0.01	0.77	0.03	0.22
FAI_{ED} + $Long_{ED}$	0.06	0.82	0.04	0.85	0.01	0.91	0.02	0.79	0.23	0.58
FAI_{ED} + Lat_{ED}	0.11	0.86	0.02	0.83	0.00	0.90	0.02	0.79	0.09	0.40
$Long_{ED}$ + Lat_{ED}	0.02	0.84	0.02	0.78	0.01	0.91	0.02	0.77	0.15	0.29
Full model	0.11	0.86	0.05	0.85	0.01	0.90	0.02	0.78	0.23	0.56

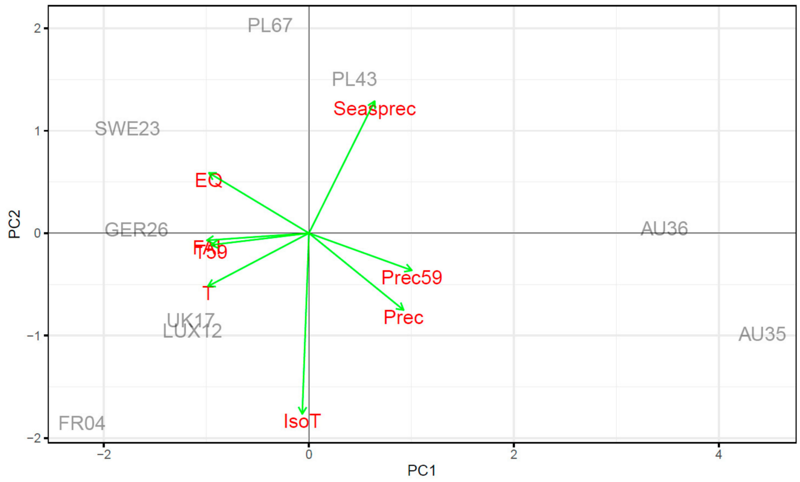

Figure A1. The principal component analysis biplot of provenance's original climate.

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