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Drought Hardening of European Beech (*Fagus sylvatica* L.) and Silver Fir (*Abies alba* Mill.) Seedlings in Mixed Cultivation

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Abstract: To alleviate the enhanced frequency, duration, and intensity of drought as a consequence of global warming, admixing drought-sensitive European beech (*Fagus sylvatica* L.) with deep rooting silver fir (*Abies alba* Mill.) has been proposed. However, information on the performance of the admixtures of seedlings of these tree species at limited water availability has so far not been reported. In the present study, we investigated the significance of water deprivation in mixtures of beech and fir seedlings on the foliar relative water content (RWC), $\delta^{13}C$ signature, total C and N contents, and C:N ratios of both species in a drought-rewetting cycle. Surprisingly, moderate drought triggered increased RWC in beech leaves and current year fir needles indicating drought hardening. The enhanced foliar RWC was preserved after rewatering in beech leaves, but not in current year fir needles. Drought did not significantly affect $\delta^{13}C$ abundance in beech leaves, but enhanced the $\delta^{13}C$ abundance (less negative values) in current and one-year old fir needles, indicating stomatal control in fir needles but not in beech leaves upon moderate drought. Total C contents of beech leaves were significantly increased upon drought and rewatering, but remained constant in fir needles. Foliar total N increased in both species upon drought and decreased upon rewatering. Accordingly, C:N ratios decreased in response to drought and recovered after rewatering. These results suggest that drought hardening may be achieved at least partially via osmotic adjustment by different compatible solutes in beech leaves and fir needles. No apparent effects of the number of neighbours were observed, although more fir neighbours tended to increase the RWC and total C contents of beech leaves. These results indicate that drought hardening in mixtures of beech and fir seedlings is largely independent of the number of interspecies neighbours.

Keywords: carbon and nitrogen contents; complementary effects; drought; osmotic adjustment; rewatering; saplings; stomatal control; water relations

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1. Introduction

During the last decades, global warming has severely affected seasonal distribution of precipitation in Central Europe by increased frequency, duration, and intensity of extreme events such as drought and flooding by heavy rainfall [1]. Under future climate conditions, these environmental constrains are projected to further increase, particularly in summer and autumn [2,3], thereby impacting plant–water relations, nutrient uptake, as well as numerous biochemical processes including photosynthesis and, hence, carbon fixation [4,5]. In this context, forests that account for approximately 45% of global terrestrial carbon stocks [6] are found to be highly sensitive to climate change including drought and heat events [7,8]. Therefore, full understanding of the response mechanisms and processes in trees to changing climate are of particularly importance for future forest development [9].

Drought is one of the most detrimental abiotic stresses strongly impacting morphological, physiological, and biochemical processes of plants, including the reduction of leaf water potential and sap movement as well as impaired photosynthesis due to stomatal closure [4,10,11]. European beech (*Fagus sylvatica* L.) is one of the dominant broadleaved species in Central Europe, being particularly sensitive to drought [12,13], with water availability as main limiting factor determining its natural distribution [12]. Silver fir (*Abies alba* Mill.) is a coniferous species native to Europe, sharing similar geographical distribution to beech, but is considered to be more resistant to warmer and drier climate due to its deep rooting system [14,15]. Previous studies have shown positive effects of admixing fir in beech forests or plantations, for instance, increasing soil organic carbon stocks [16], improving water relations of beech without affecting nitrogen nutrition [17–19], using less water and, hence, counteracting soil drying [20]. However, recent studies demonstrated that competition for water rather than facilitation concurrent with reduced sap flow in beech trees can take place in mixed beech-fir forests upon drying–wetting cycles [21], hydraulic redistribution of water by fir is restricted to severe soil drought [19] and depends on a sufficient amount of fir neighbours [17]. Schwarz and Bauhus [22] found that both beech and fir benefited from growing in mixed neighbourhoods, however, the complementary effects highly depended on tree size and neighbourhood density. These results are consistent with previous studies indicating that seedlings are more susceptible to environmental alterations and often respond faster to climate change than mature trees because of their limited root system and aboveground height, constraining their competitive ability [23]. Thus, it can be assumed that the benefit of mixed cultivation of beech and fir seedlings is limited.

In the present study, foliar water relations as well as carbon and nitrogen contents in response to water deprivation and rewetting were determined in mixed cultivations of beech and fir seedlings with different numbers of interspecies neighbours. Specifically, based on the results of published research, we hypothesized that: (i) mild drought induces drought hardening in beech and fir seedlings; (ii) admixing with beech will not sacrifice the water relations of fir, irrespective of water availability; (iii) foliar total N, but not total C contents are enhanced during drought and reduced upon rewetting; and (iv) facilitation effects of fir on beech are related to the number of neighbouring fir seedlings, i.e., more neighbouring fir will induce more significant effects on beech. The objectives of this study were to elucidate the physiological impacts of moderate drought on mixed beech and fir and to examine the effects of interspecific neighbourhood density.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

This study was conducted at the Chair of Tree Physiology, University of Freiburg, Germany (48°50.39′′ N/7°50′0.51′′ E). In March 2016, 10 two-year-old seedlings of European beech (*Fugus sylvatica* L.) and silver fir (*Abies alba* Mill.), each, were planted into mesocosms (size 120 × 75 × 60 cm; L × W × D) at 15 × 20 cm distance. The seedlings were obtained from a commercial tree nursery (Gustav Burger Forstbaumschulen, Harmersbach, Germany). The mean shoot lengths of beech and fir seedlings at planting were 21 cm and 31 cm, respectively; the average lengths of the roots were 14 cm for beech and 24 cm for fir seedlings. The planting design chosen resulted in 1 to 5 fir neighbours for beech seedlings and of 1 to 5 beech neighbours for fir seedlings at 8 (beech), 5–30 (fir) replicates, each, in 4 mesocosms (Figure 1). Firs with 6 neighbours of beech were excluded from analysis due not enough replicates (n = 2). This planting design was repeated 4 times, resulting in a total of 16 mesocosms.

The soil used for the mesocosms was collected in autumn 2015 in the black forest close to Emmendingen, Germany (48°02.013′′ N/7°96.888′′ E). It mainly consisted of mineral soil originating from Triassic sandstone and showed a sandy loam texture [17,18]. The soil material was mixed with perlite at 1:1 (*v*:*v*) to improve soil drainage and aeration. Leaf litter was collected from a mixed beech/fir forest close to the soil collection site and a leaf litter layer of 3 to 5 cm was placed on top of the soil in the mesocosms. Two soil moisture

sensors (Decagon Devices, Inc., Pullman, Washington, DC, USA) were vertically installed at 15 cm (shallow) and 37 cm (deep) depth in the mesocosms to determine volumetric soil moisture during the entire duration of the experiment.

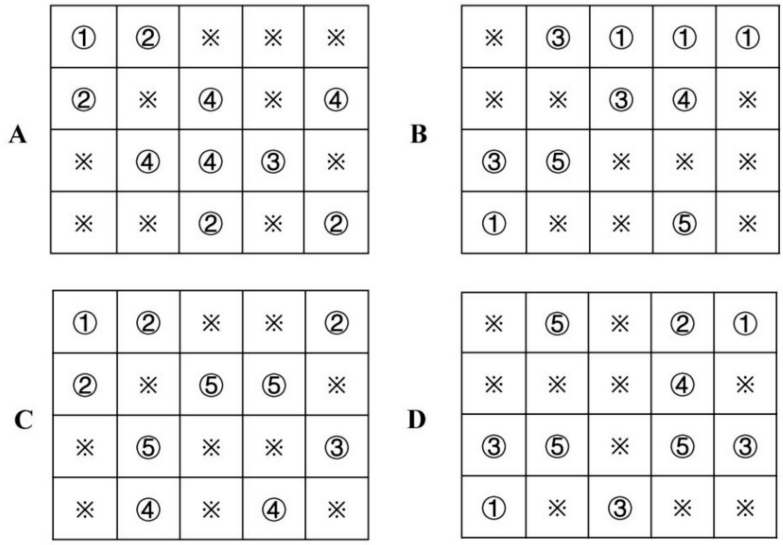


Figure 1. Mixed planting design of beech and fir seedlings in mesocosms (**A**–**D**). ※, silver fir; circle with number, beech. The numbers of 1–5 indicate the number of silver fir neighbours to the respective beech seedling.

2.2. Seedling Cultivation

For establishment of the seedlings, mesocosms were kept well-watered under greenhouse conditions from March to December 2016. Excess water was allowed to drain from bottom holes in the mesocosms. During the initial 4 weeks of plant establishment, leaf expansion of beech and fir seedlings took place. To simulate light availability in the understory of forest stands, mesocosms were illuminated at 150–250 µmol m^{-2} s^{-1} PAR (day/night = 16/8 h). During this time, the position and direction of the mesocosms were changed every month. In December 2016, mesocosms were transferred to field conditions and covered with roofs of UV transparent foil (greenhouse film transparent, RKW AGRI GmbH, Mannheim, Germany) ca. 45 to 70 cm above plant height to prevent uncontrolled water input by precipitation (Figure S1). Seedlings were watered with 30 L tap water per mesocosm per week reflecting the mean annual precipitation in Freiburg (DWD, Elzach-Fissnacht", 30-year long-term average: 1981–2010).

2.3. Water Deprivation and Rewatering

During the growing season 2017, eight mesocosms were subjected to water deprivation from 9 May to 27 July 2017. During this time, soil water contents were monitored with the soil moisture sensors previously installed in 15 cm and 37 cm depth. The remaining 8 mesocosms served as controls and were watered regularly as before. For rewatering, the 8 water-deprived mesocosms were supplied with 75 L tap water per mesocosm from 28 July 2017 to 1 August 2017.

2.4. Sampling of Plant Material

Leaves of both species were sampled at the end of water deprivation and rewatering, from both controls and water deprived/rewatered mesocosms, i.e., from 27 to 28 July (Control 1, for "-water"), and from 8 to 9 August (Control 2, for "rewatered"), respectively. For leaf sampling, twigs were cut from the outer sun-exposed areas of the shoots. All samples were taken randomly between 10:00–14:00 to minimize influences of diurnal changes. For beech, three fully developed intact leaves were collected; for silver fir, both current-year and one-year needles were excised. Subsamples of leaves/needles were

immediately frozen in liquid nitrogen, then homogenized with mortar and pestle in liquid nitrogen and stored at −20 °C for further biochemical analyses. The remaining leaf material from beech and fir was weighted (Fresh weight, FW), dried in the oven at 60 °C for approximately 3 days until weight constancy (Dry weight, DW). The dried materials were used for total C, N contents and isotopic signature measurements. Foliar relative water content (RWC) was estimated as RWC (%) = [(FW − DW)/FW) × 100], as described by Peuke et al. [24].

2.5. Determination of Foliar $\delta^{13}C$, Total Foliar C and N Contents

The total N and total C contents as well as $\delta^{13}C$ signatures of the leaves were measured as previously described [25]. For this purpose, oven-dried samples were ground with a ball mill (Retsch MM 400, Retsch GmbH, Haan, Germany), aliquots (1.0–1.5 mg) were loaded into tin capsules (IVA Analysentechnik, Meerbusch, Germany), and measured in an isotope ratio mass spectrometer (Delta V Plus, Thermo Finnigan MAT, GmbH, Bremen, Germany) coupled via a Conflo III interface to an elemental analyser (NA2500, CE Instruments, Milan, Italy). A working standard (glutamic acid) was calibrated against primary standards and analysed after every tenth sample to account for a potential instrument drift over time as described by Simon et al. [26].

2.6. Statistical Analysis

The statistical significance of differences between neighbouring groups was investigated for the leaves of beech seedlings as well as for current (0yn) and one-year-old (1yn) needles of silver fir of the controls and treatments. The Shapiro–Wilk test was used to analyse data for normal distribution. If data followed normal distribution, one-way ANOVA was applied using Tukey's post hoc test ($p \leq 0.05$, $\alpha = 0.95$). If normal distribution of the data was not met, the Kruskal–Wallis one-way ANOVA was applied using Dunn's Method. Differences between controls and treatments for beech leaves as well as for 0yn and 1yn sliver-fir needles were analysed using Student's *t*-test if data were normal distributed. All statistical analyses were carried out with Sigmaplot 12.0 (Version 12.0, Systat Software, San Jose, CA, USA), figures were designed using Excel (Microsoft, Redmond, Washington, DC, USA) and Sigmaplot 12.0. Supplementary Tables S1 and S2 provide an overview on the number of replicates analysed for beech leaves and silver-fir needles, respectively.

3. Results

3.1. Foliar Relative Water Contents and $\delta^{13}C$ Abundance

As shown in Figure S2, the soil relative water content (RWC) at 37 cm depth ranged from 18.5% to 24% in control mesocosms throughout the growing season. In water deprived mesocosms, soil water content ranged from 18.2% to 22.5% before water deprivation and after rewatering, but decreased gradually to 10.7%–14.6% during water deprivation. For the shallow soil, RWC measured at 15 cm depth varied strongly, i.e., from 26.7% to 17.0% before water deprivation (Figure S2). Compared with control, the shallow soil RWC of treatments steadily decreased from 18.1%–17.1% to 6.0%–8.8% during water deprivation and obviously recovered to 12.1%–17.0% after rewatering. Apparently, the shallow soil RWC was more affected by microclimate and watering than RWC of deeper soil layers.

The foliar water status was characterized by the relative water content (RWC). It differed between beech leaves and fir needles as well as between the water deprivation and rewatering treatment (Figure 2). In general, the foliar RWC of fully developed beech leaves was similar to 1yn fir needles, but the RWC of 0yn fir needles was higher than the RWC of both, 1yn fir needles, and fully developed beech leaves. With prolonged growth, RWC of beech leaves declined, but RWC of 0yn and 1yn fir needles increased (compare: control 1 and control 2) (Figure 2a–c). Surprisingly, the water deprivation treatment significantly enhanced the foliar RWC of beech leaves and 0yn fir needles (Figure 2a,b). In beech leaves, but not in fir needles, this difference was maintained at subsequent rewatering (Figure 2a–c).

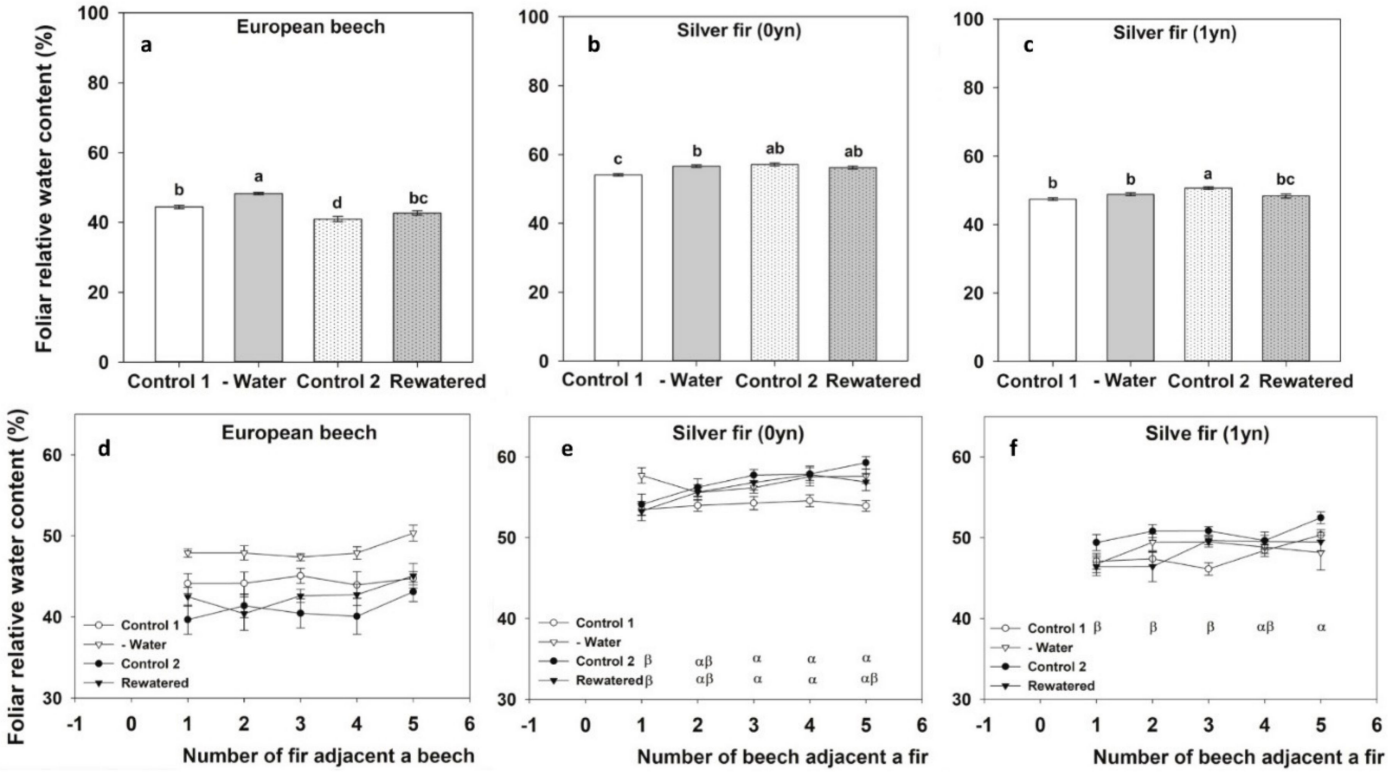


Figure 2. Foliar relative water content (RWC) of beech (*F. sylvatica* L.) and silver fir (*A. alba* Mill.) seedlings upon water deprivation (−water) and rewatering. Leaves/needles of control 1 were collected on the 27 to 28 July, leaves/needles of control 2 on the 8 to 9 August. 0yn, current−year needles; 1yn, one−year old needles. Significant differences between controls and treatments are indicated by different letters ($p \leq 0.05$; n = 80, (**a**−**c**)), effect of the number of neighbouring trees ((**d**−**f**), n = 5−30). Values shown are means ± SD.

The impact of fir on foliar water relations of beech and the impact of beech on foliar water relations of fir was characterized via the number of neighbouring trees of the respective other species. The foliar RWC of beech trees was not significantly affected by the number of adjacent fir trees irrespective of the treatment (Figure 2d). For 0yn fir needles, RWC was significant higher at 3 to 4 beech neighbours compared to 1 beech neighbour in August (control 2); this effect was largely maintained upon rewatering (Figure 2e). In July, RWC of 1yn fir needles was significantly higher in the presence of five beech trees (control 1), but this effect was no longer observed upon water deprivation or with prolonged growth (Figure 2f).

The foliar carbon isotope discrimination ($\delta^{13}C$) of beech seedlings decreased significantly with proceeding cultivation (compare control 1 and control 2) (Figure 3a), but this effect was not observed for 0yn and 1yn fir needles (Figure 3b,c). Water deprivation and subsequent rewatering did not affect the carbon isotope abundance of beech leaves and 0yn fir needles. However, water deprivation increased the carbon isotope discrimination of 1yn fir needles, but this effect was not maintained upon subsequent rewatering (Figure 3b,c). The number of neighbouring trees of the respective other species did not significantly affect the foliar carbon isotope discrimination, irrespective of species and treatment (Figure S3).

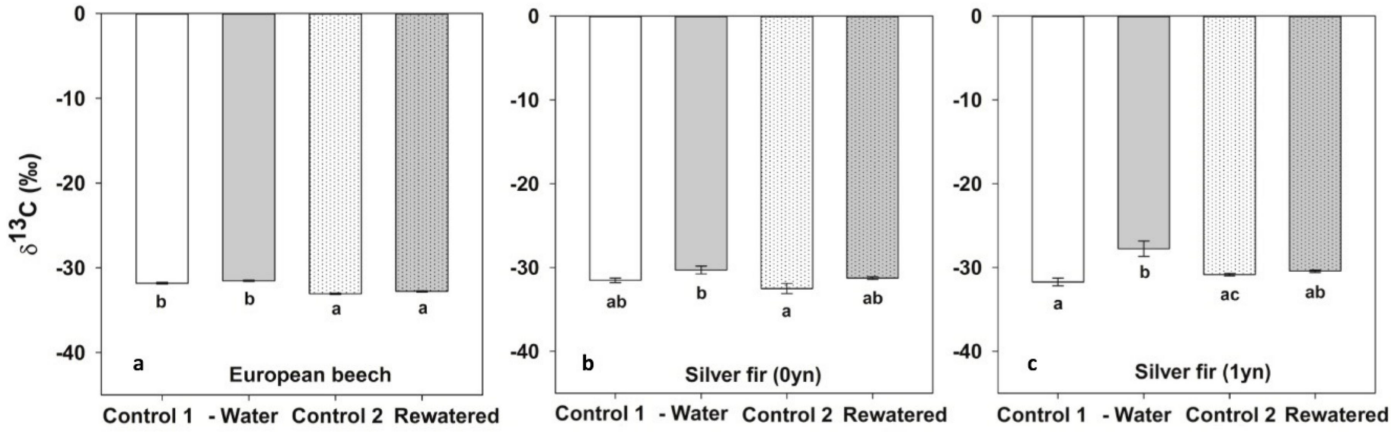


Figure 3. Foliar $\delta^{13}C$ abundance of beech (*F. sylvatica* L.) (**a**) and silver fir (*A. alba* Mill.) (**b**,**c**) seedlings. 0yn, current−year needles; 1yn, one−year needles. Significant differences between controls and treatments are indicated by different letters ($p \leq 0.05$; $n = 80$). Values shown are means ± SD.

3.2. Foliar Total C and Total N Contents

Water deprivation significantly enhanced the total C content of beech leaves, and this effect was maintained upon rewatering (Figure 4a). In contrast, neither water deprivation nor rewatering had significant effects on needle total C contents of fir (Figure 4b,c). Foliar total C contents varied significantly between species and treatments (Figure 4). In beech leaves, total C contents significantly declined with proceeding cultivation (i.e., control 1 compare to control 2) (Figure 4a). Similar effects were not observed for fir needles (Figure 4b,c).

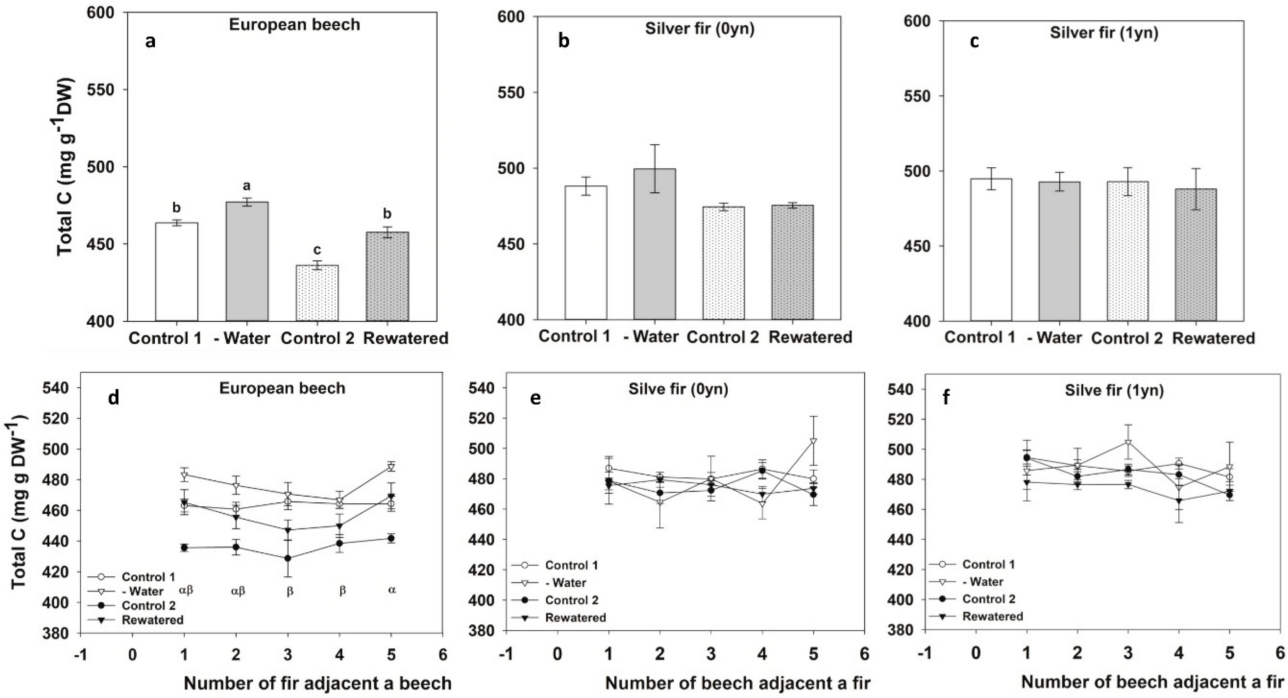


Figure 4. Foliar total C contents of beech (*F. sylvatica* L.) and silver fir (*A. alba* Mill.) seedlings. 0yn, current−year needles; 1yn, one−year needles. Significant differences between controls and treatments are indicated by different letters ($p \leq 0.05$; $n = 80$, (**a**−**c**)), effect of the number of neighbouring trees ((**d**−**f**), $n = 5$−30). Values shown are means ± SD.

Regarding the effect of the number of neighbouring firs on beech, foliar total C content was significantly higher at five fir neighbours compared to three and four fir neighbours

upon water deprivation; a similar pattern was observed after rewatering (Figure 4d). The number of adjacent beech trees had no significant effects on total C contents of current-year or one-year needles (Figure 4e,f).

Water deprivation significantly enhanced the total N content of both, beech leaves and 0yn as well as 1yn fir needles, but this effect was counteracted by subsequent rewatering (Figure 5). The number of neighbouring trees of the respective other species did not significantly affect foliar total N contents, irrespective of species and treatment (Figure S4). In general, total N contents of beech leaves were significantly higher than in 0yn and 1yn fir needles (Figure 5). Prolonged cultivation did not affect the total N content of beech leaves (compare: control 1 and control 2) (Figure 5a), but enhanced the total N content of 0yn and 1yn fir needles (Figure 5b,c).

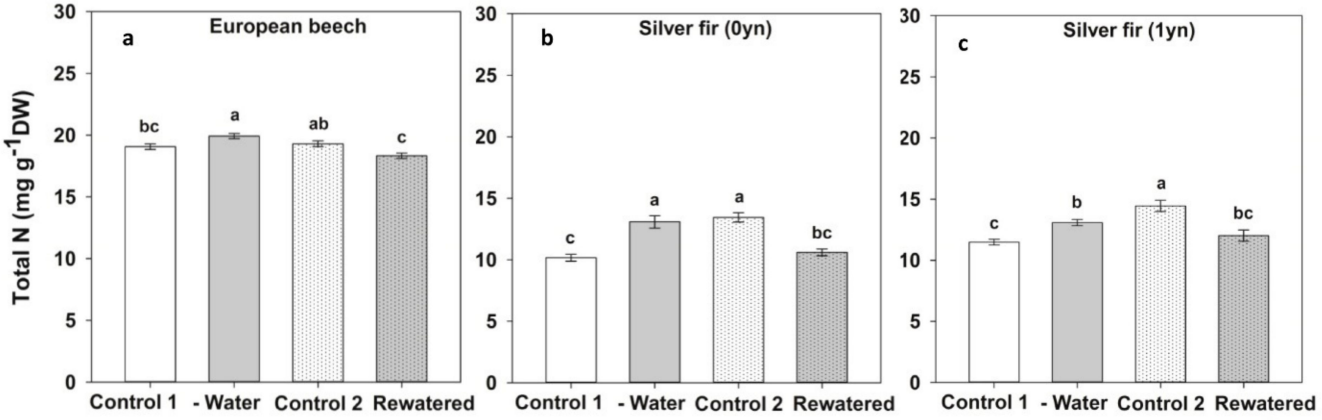


Figure 5. Foliar total N contents of beech (*F. sylvatica* L.) (**a**) and silver fir (*A. alba* Mill.) (**b**,**c**) seedlings. 0yn, current−year needles; 1yn, one−year needles. Significant differences between controls and treatments are indicated by different letters ($p \leq 0.05$; $n = 80$). Values shown are means ± SD.

The foliar C/N ratio was generally lower in beech leaves compared to 0yn and 1yn fir needles; it declined with proceeding cultivation (compare: control 1 and control 2) (Figure 6). Water deprivation reduced the foliar C/N ratio, but this effect was not significant for beech leaves. Rewatering significantly enhanced the foliar C/N ratio in both beech and fir seedlings. The number of neighbouring trees of the respective other species did not significantly affect foliar total N contents, irrespective of species and treatment (Figure S5).

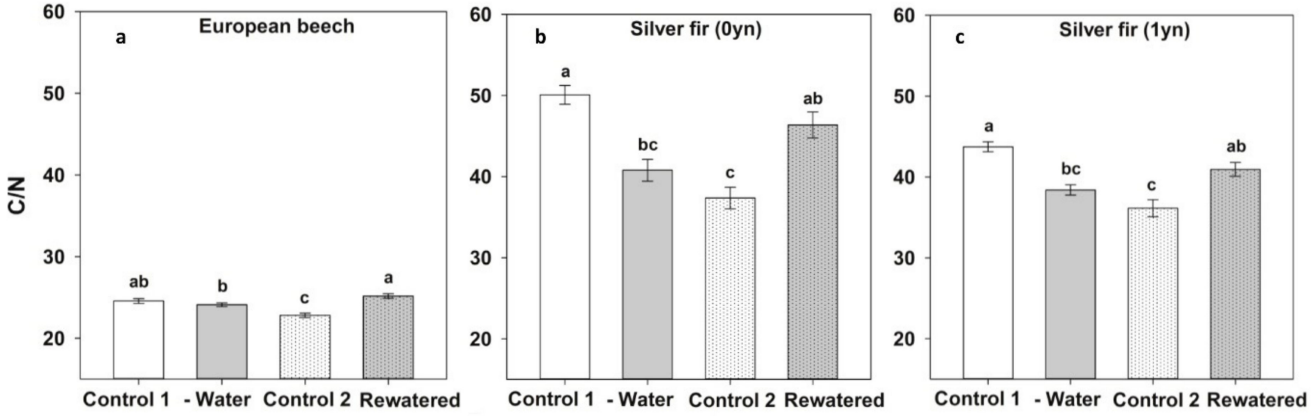


Figure 6. Foliar C/N ratio of beech (*F. sylvatica* L.) (**a**) and silver fir (*A. alba* Mill.) (**b**,**c**) seedlings. 0yn, current−year needles; 1yn, one−year needles. Significant differences between controls and treatments are indicated by different letters ($p \leq 0.05$; $n = 80$). Values shown are means ± SD.

4. Discussion

European beech is one of the dominant broadleaved forest tree species with both ecological and economic importance in Central Europe; however, it is particularly sensitive

to both high and low water availability [13]. Intercropping deep rooting silver fir has been proposed as a strategy to improve the resilience of beech forests towards intensified drying–wetting cycles under projected climate change conditions [16,17]. However, little is known about the responses of seedling mixtures of these species to drying–wetting cycles. In the present study we investigated to which extent moderate water deprivation and subsequent rewatering impacts foliar water relations as well as carbon and nitrogen accumulation of European beech and silver fir seedlings in mixed cultivation and the dependency of these responses on the number of neighbouring trees of the respective other species. We found that moderate water deprivation mediated drought hardening in both species and that this effect was partially counteracted by rewatering. Similar results were not obtained in previous studies with beech or fir seedlings in monocultures [24,27–40].

4.1. Water Deprivation and Rewatering Affect Foliar Water Relations of Beech and Fir

Under water limitation, stomata play a pivotal role in plants via controlling both water losses and CO_2 uptake, as well as modulating the transpiration-driven water flow and water use efficiency [4,10]. Numerous studies have shown that carbon isotope discrimination by the leaves of C3 plants is highly related to photosynthetic gas exchange. Therefore, stable carbon isotope composition ($\delta^{13}C$) has been widely accepted as proxy of plant water relation, as well as indicator of plant water use efficiency and stomatal movement. Thus, this parameter provides insight into chemical, physical and metabolic process involved in carbon transformation [13,41,42]. Beech leaf $\delta^{13}C$ signature is a good indicator of stomatal conductance, vapour pressure deficit, and radiation availability of the current growing season [28]. Increased $\delta^{13}C$ and/or decreased leaf hydration are commonly observed in both pure beech [24,27,31,32,34–37] and pure mature beech trees [28,29,40] in response to drought. In the present study, surprisingly, moderate water deprivation significantly enhanced the relative water contents of beech leaves (Figure 2a), whereas $\delta^{13}C$ signatures were largely conserved (Figure 3a). The latter has previously been observed in mature trees of pure beech and beech–fir mixed forests across Europe [17,18]. Therefore, our first hypothesis is fully supported. The decreased leaf $\delta^{13}C$ with prolonged cultivation of beech in the present study is consistent with previously observed seasonal patterns [13]. The preserved $\delta^{13}C$ signatures observed upon water deprivation and subsequent rewatering in beech leaves (Figure 3a) indicate less impacted stomatal conductance during these treatments [43], as also documented in *Larix kaempferi* [41]. Limited sensitivity of stomatal regulation of beech was also observed in a mixture of beech and deep rooting oak (*Quercus petraea* (Matt.) Liebl.) compared to a pure beech stand [44,45]. This effect has been explained by improved water resource sharing in mixture compared to pure stands mediated by hydraulic lift of deep-water sources [17,19,45,46]. Drought impaired evapotranspiration, and transpiration observed in mature beech–fir forests [20] apparently did not happen in the current study with seedlings. The enhanced foliar water contents upon moderate water deprivation and its maintenance after rewatering may be attributed to other strategies to cope with reduced water availability, e.g., the accumulation of compatible solutes [24]. However, this assumption requires further analyses.

Similar to beech leaves, water deprivation also enhanced foliar water contents in current-year, but not in previous-year needles of silver fir. However, this effect was not maintained upon rewatering. Different to beech leaves, water contents of fir needles increased with prolonged growth (Figure 2b,c). Silver fir saplings have previously been reported to maintain their water balance at low water availability to some extent, either by increasing photosynthesis or by diminishing stomatal conductance [46,47]. In evergreen conifers, the older needle generations serve as storage pools that support the new needle generation upon sprouting by remobilization [48]. Whether this function is restricted to nutrients or also includes water has so far not been elucidated. We observed a strong increase of $\delta^{13}C$ in one year, but not in current-year needles in response to water deprivation (Figure 3b,c), indicating reduced stomatal conductance [49,50]. The higher water contents of current-year needles would assure sufficient carbon assimilation even under these

conditions. Outcompeting of fir for water by beech was observed after drought in beech and fir mixed forests [21] but was not demonstrated in the current study with seedlings. Therefore, our second hypothesis that admixing with beech will not sacrifice the water relations fir was fully supported. Lebourgeois et al. [51] also found that cultivation in mixed stands reduced the sensitivity of *A. alba* to summer drought.

Thus, in the present study, seedlings of both beech and fir responded differently to water deprivation compared to mature trees [21], probably because of the use of deeper soil water resources by the fully developed root systems in the field [52].

4.2. Admixing Beech and Silver Fir Affects Foliar C and N Contents upon Water Deprivation and Rewatering

Concomitant with enhanced leaf water contents and the stable stomatal conductance, significantly increased total C contents were observed in beech leaves, but not in fir needles upon moderate water deprivation and rewatering (Figure 4a). A drought-triggered increase of total C was previously documented in beech leaves [53] and roots [54]. This effect was likely due to enhanced C assimilation [54–56], but reduced respiratory consumption or export of carbon from the leaves [32,57–60] cannot be excluded. It suggests that osmotic adjustment by carbohydrates contributes [4,61,62] to drought hardening in beech leaves, but not in fir needles.

Consistent with results from mature mixed beech–fir forests [18], the foliar total N content of fir was significantly lower than that of beech in the present study, but foliar total N contents of both species reacted similar to water deprivation and rewatering (Figure 5). The increased total N contents upon water deprivation followed by a reduction after rewatering are likely to constitute a general response of the allocation of N-containing compounds between roots and leaves by long-distance transport (beech: [54,63]; fir: [50,64]; beech/fir mixture: [17]), but also suggest osmotic adjustment by transient accumulation of N containing compatible solutes such as amino acids during the drought treatment [4,29,63]. The present study also confirmed the close coupling of C and N metabolism previously reported [65–67].

Due to the changes of total N, C:N ratios of both species decreased in response to water deprivation and increased upon rewatering (Figure 6) in the range previously reported [17,53,68]. Decreasing C:N ratios upon drought were also demonstrated in beech and fir mixed forest [17], supporting the idea of drought-mediated changes in N allocation between roots and leaves and improved N use efficiency under these conditions [17]. This result and the stable C:N ratios of beech seedlings in monoculture upon drought [53] indicate facilitation of N use efficiency of fir on beech in mixed cultivation. The foliar C:N ratios upon water deprivation and, hence, projected climate change will also influence the soil C:N ratio, because litter with higher C:N ratio decomposes more slowly [69]. Overall, our third hypothesis has to be amended due to the significantly increased foliar total N contents upon water deprivation.

4.3. Little Effects of the Number of Interspecies Neighbours on Foliar Water Relations and C and N Contents

Against our fourth hypothesis, the number of interspecies neighbours did not significantly affect foliar water relations and C and N contents, although a tendency towards increased RWC and total C of beech leaves was observed with an increased number of surrounding fir (n = 5). This finding is consistent with a field study on mature trees [17]. Additionally, Schwarz and Bauhus [22] found that both beech and fir benefited from growing in mixed neighbourhoods; however, the complementary effects depended on tree size and density. In the mixture, larger trees gained more benefits irrespective of neighbourhood density, whereas smaller trees benefitted only in denser neighbourhoods. Apparently, at the seedling stage analysed in the present study, the effect of neighbouring species upon water deprivation was not strong enough to mediate changes in RWC and C and N contents.

5. Conclusions

To counteract the consequences of projected climate change with more frequent and intense drying–wetting cycles, the mixture of tree species has been proposed as an effective silvicultural strategy. In this study, we found drought hardening upon moderate water deprivation indicated by increased foliar water relations and C contents as well as modified N partitioning of beech seedlings grown in mixed cultivation with fir. Moreover, admixing with beech did not sacrifice these parameters in fir seedlings. The number of interspecies neighbours had few effects on these responses. In addition, drought hardening distinctly altered the foliar RWC and $\delta^{13}C$ signatures as well as C and N contents in a species-specific way, indicating that different mechanisms contributed to drought hardening in beech and fir seedlings. These mechanisms may include stomatal control in fir needles and osmotic adjustment by the accumulation of different compatible solutes such as carbohydrates and amino acids in beech leaves and fir needles. Based on these results, more detailed metabolic as well as molecular studies are required to elucidate the processes that mediate the observed responses of foliar C and N contents to mild water deprivation and rewatering. In addition, further research should be conducted to identify the long-term effects of drought hardening and drought resilience in mixtures of beech–fir seedlings under field conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13091386/s1, Figure S1: Photo of mesocosms covered with the UV transparent roof; Figure S2: Relative soil water content during the experiment; Figure S3: Foliar δ13C abundance of well-watered, water deprived and rewatered beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) seedlings; Figure S4: Foliar total N content of well-watered, water deprived and rewatered beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) seedlings; Figure S5: Foliar C/N of well-watered, water deprived and rewatered beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) seedlings; Table S1: Number of biological replicates of beech (*Fagus sylvatica* L.) leaves; Table S2: Number of biological replicates of silver fir (*Abies alba* Mill.) needles.

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References

1. IPCC. Summary for Policymakers. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V.P., Zhai, A., Pirani, S.L., Connors, C., Péan, S., Berger, N., Caud, Y., Chen, L., Goldfarb, M.I., Gomis, M., et al., Eds.; Cambridge University Press: Cambridge, UK, 2021.
2. Ruosteenoja, K.; Markkanen, T.; Venäläinen, A.; Räisänen, P.; Peltola, H. Seasonal Soil Moisture and Drought Occurrence in Europe in CMIP5 Projections for the 21st Century. *Clim. Dyn.* **2018**, *50*, 1177–1192. [CrossRef]

3. Stefanidis, S. Ability of Different Spatial Resolution Regional Climate Model to Simulate Air Temperature in a Forest Ecosystem of Central Greece. *J. Environ. Prot. Ecol.* **2021**, *22*, 1488–1495.
4. Rennenberg, H.; Loreto, F.; Polle, A.; Brilli, F.; Fares, S.; Beniwal, R.S.; Gessler, A. Physiological Responses of Forest Trees to Heat and Drought. *Plant Biol.* **2006**, *8*, 556–571. [CrossRef]
5. Kreuzwieser, J.; Rennenberg, H. Molecular and Physiological Responses of Trees to Waterlogging Stress. *Plant Cell Environ.* **2014**, *37*, 2245–2259. [CrossRef] [PubMed]
6. Ciais, P.; Reichstein, M.; Viovy, N.; Granier, A.; Ogée, J.; Allard, V.; Aubinet, M.; Buchmann, N.; Bernhofer, C.; Carrara, A.; et al. Europe-Wide Reduction in Primary Productivity Caused by the Heat and Drought in 2003. *Nature* **2005**, *437*, 529–533. [CrossRef] [PubMed]
7. Schuldt, B.; Buras, A.; Arend, M.; Vitasse, Y.; Beierkuhnlein, C.; Damm, A.; Gharun, M.; Grams, T.E.; Hauck, M.; Hajek, P. A First Assessment of the Impact of the Extreme 2018 Summer Drought on Central European Forests. *Basic Appl. Ecol.* **2020**, *45*, 86–103. [CrossRef]
8. Senf, C.; Buras, A.; Zang, C.S.; Rammig, A.; Seidl, R. Excess Forest Mortality Is Consistently Linked to Drought across Europe. *Nat. Commun.* **2020**, *11*, 6200. [CrossRef]
9. Lindner, M.; Maroschek, M.; Netherer, S.; Kremer, A.; Barbati, A.; Garcia-Gonzalo, J.; Seidl, R.; Delzon, S.; Corona, P.; Kolström, M. Climate Change Impacts, Adaptive Capacity, and Vulnerability of European Forest Ecosystems. *For. Ecol. Manag.* **2010**, *259*, 698–709. [CrossRef]
10. Damour, G.; Simonneau, T.; Cochard, H.; Urban, L. An Overview of Models of Stomatal Conductance at the Leaf Level. *Plant Cell Environ.* **2010**, *33*, 1419–1438. [CrossRef]
11. Bhusal, N.; Han, S.G.; Yoon, T.M. Impact of Drought Stress on Photosynthetic Response, Leaf Water Potential, and Stem Sap Flow in Two Cultivars of Bi-Leader Apple Trees (Malus × domestica Borkh.). *Sci. Hortic.* **2019**, *246*, 535–543. [CrossRef]
12. Geßler, A.; Keitel, C.; Kreuzwieser, J.; Matyssek, R.; Seiler, W.; Rennenberg, H. Potential Risks for European Beech (*Fagus sylvatica* L.) in a Changing Climate. *Trees* **2007**, *21*, 1–11. [CrossRef]
13. Fotelli, M.N.; Nahm, M.; Radoglou, K.; Rennenberg, H.; Halyvopoulos, G.; Matzarakis, A. Seasonal and Interannual Ecophysiological Responses of Beech (*Fagus sylvatica*) at Its South-Eastern Distribution Limit in Europe. *For. Ecol. Manag.* **2009**, *257*, 1157–1164. [CrossRef]
14. Bosela, M.; Lukac, M.; Castagneri, D.; Sedmák, R.; Biber, P.; Carrer, M.; Konôpka, B.; Nola, P.; Nagel, T.A.; Popa, I.; et al. Contrasting Effects of Environmental Change on the Radial Growth of Co-Occurring Beech and Fir Trees across Europe. *Sci. Total Environ.* **2018**, *615*, 1460–1469. [CrossRef] [PubMed]
15. Jarzyna, K. Climatic Hazards for Native Tree Species in Poland with Special Regards to Silver Fir (*Abies alba* Mill.) and European Beech (*Fagus sylvatica* L.). *Theor. Appl. Climatol.* **2021**, *144*, 581–591. [CrossRef]
16. Rehschuh, S.; Fuchs, M.; Tejedor, J.; Schäfler-Schmid, A.; Magh, R.-K.; Burzlaff, T.; Rennenberg, H.; Dannenmann, M. Admixing Fir to European Beech Forests Improves the Soil Greenhouse Gas Balance. *Forests* **2019**, *10*, 213. [CrossRef]
17. Magh, R.-K.; Grün, M.; Knothe, V.E.; Stubenazy, T.; Tejedor, J.; Dannenmann, M.; Rennenberg, H. Silver-Fir (*Abies alba* Mill.) Neighbors Improve Water Relations of European Beech (*Fagus sylvatica* L.), but Do Not Affect N Nutrition. *Trees* **2018**, *32*, 337–348. [CrossRef]
18. Magh, R.-K.; Yang, F.; Rehschuh, S.; Burger, M.; Dannenmann, M.; Pena, R.; Burzlaff, T.; Ivanković, M.; Rennenberg, H. Nitrogen Nutrition of European Beech Is Maintained at Sufficient Water Supply in Mixed Beech-Fir Stands. *Forests* **2018**, *9*, 733. [CrossRef]
19. Töchterle, P.; Yang, F.; Rehschuh, S.; Rehschuh, R.; Ruehr, N.K.; Rennenberg, H.; Dannenmann, M. Hydraulic Water Redistribution by Silver Fir (*Abies alba* Mill.) Occurring under Severe Soil Drought. *Forests* **2020**, *11*, 162. [CrossRef]
20. Magh, R.-K.; Bonn, B.; Grote, R.; Burzlaff, T.; Pfautsch, S.; Rennenberg, H. Drought Superimposes the Positive Effect of Silver Fir on Water Relations of European Beech in Mature Forest Stands. *Forests* **2019**, *10*, 897. [CrossRef]
21. Magh, R.-K.; Eiferle, C.; Burzlaff, T.; Dannenmann, M.; Rennenberg, H.; Dubbert, M. Competition for Water Rather than Facilitation in Mixed Beech-Fir Forests after Drying-Wetting Cycle. *J. Hydrol.* **2020**, *587*, 124944. [CrossRef]
22. Schwarz, J.A.; Bauhus, J. Benefits of Mixtures on Growth Performance of Silver Fir (*Abies alba*) and European Beech (*Fagus sylvatica*) Increase with Tree Size without Reducing Drought Tolerance. *Front. For. Glob. Chang.* **2019**, *2*, 79. [CrossRef]
23. Lloret, F.; Peñuelas, J.; Prieto, P.; Llorens, L.; Estiarte, M. Plant Community Changes Induced by Experimental Climate Change: Seedling and Adult Species Composition. *Perspect. Plant Ecol. Evol. Syst.* **2009**, *11*, 53–63. [CrossRef]
24. Peuke, A.D.; Schraml, C.; Hartung, W.; Rennenberg, H. Identification of Drought-sensitive Beech Ecotypes by Physiological Parameters. *New Phytol.* **2002**, *154*, 373–387. [CrossRef] [PubMed]
25. Simon, J.; Waldhecker, P.; Brüggemann, N.; Rennenberg, H. Competition for Nitrogen Sources between European Beech (*Fagus sylvatica*) and Sycamore Maple (*Acer pseudoplatanus*) Seedlings. *Plant Biol.* **2010**, *12*, 453–458. [CrossRef] [PubMed]
26. Simon, J.; Dannenmann, M.; Gasche, R.; Holst, J.; Mayer, H.; Papen, H.; Rennenberg, H. Competition for Nitrogen between Adult European Beech and Its Offspring Is Reduced by Avoidance Strategy. *For. Ecol. Manag.* **2011**, *262*, 105–114. [CrossRef]
27. Tognetti, R.; Johnson, J.D.; Michelozzi, M. The Response of European Beech (*Fagus sylvatica* L.) Seedlings from Two Italian Populations to Drought and Recovery. *Trees* **1995**, *9*, 348–354. [CrossRef]
28. Keitel, C.; Matzarakis, A.; Rennenberg, H.; Gessler, A. Carbon Isotopic Composition and Oxygen Isotopic Enrichment in Phloem and Total Leaf Organic Matter of European Beech (*Fagus sylvatica* L.) along a Climate Gradient. *Plant Cell Environ.* **2006**, *29*, 1492–1507. [CrossRef]

29. Nahm, M.; Matzarakis, A.; Rennenberg, H.; Geßler, A. Seasonal Courses of Key Parameters of Nitrogen, Carbon and Water Balance in European Beech (*Fagus sylvatica* L.) Grown on Four Different Study Sites along a European North–South Climate Gradient during the 2003 Drought. *Trees* **2006**, *21*, 79. [CrossRef]
30. Battipaglia, G.; Saurer, M.; Cherubini, P.; Siegwolf, R.T.; Cotrufo, M.F. Tree Rings Indicate Different Drought Resistance of a Native (*Abies alba* Mill.) and a Nonnative (*Picea abies* (L.) Karst.) Species Co-Occurring at a Dry Site in Southern Italy. *For. Ecol. Manag.* **2009**, *257*, 820–828. [CrossRef]
31. Rose, L.; Leuschner, C.; Köckemann, B.; Buschmann, H. Are Marginal Beech (*Fagus Sylvatica* L.) Provenances a Source for Drought Tolerant Ecotypes? *Eur. J. For. Res.* **2009**, *128*, 335–343.
32. Ruehr, N.K.; Offermann, C.A.; Gessler, A.; Winkler, J.B.; Ferrio, J.P.; Buchmann, N.; Barnard, R.L. Drought Effects on Allocation of Recent Carbon: From Beech Leaves to Soil CO_2 Efflux. *New Phytol.* **2009**, *184*, 950–961. [CrossRef]
33. Nourtier, M.; Chanzy, A.; Cailleret, M.; Yingge, X.; Huc, R.; Davi, H. Transpiration of Silver Fir (*Abies alba* Mill.) during and after Drought in Relation to Soil Properties in a Mediterranean Mountain Area. *Ann. For. Sci.* **2014**, *71*, 683–695. [CrossRef]
34. Arend, M.; Sever, K.; Pflug, E.; Gessler, A.; Schaub, M. Seasonal Photosynthetic Response of European Beech to Severe Summer Drought: Limitation, Recovery and Post-Drought Stimulation. *Agric. For. Meteorol.* **2016**, *220*, 83–89. [CrossRef]
35. Dziedek, C.; von Oheimb, G.; Calvo, L.; Fichtner, A.; Kriebitzsch, W.-U.; Marcos, E.; Pitz, W.T.; Härdtle, W. Does Excess Nitrogen Supply Increase the Drought Sensitivity of European Beech (*Fagus sylvatica* L.) Seedlings? *Plant Ecol.* **2016**, *217*, 393–405. [CrossRef]
36. Nguyen, Q.N.; Polle, A.; Pena, R. Intraspecific Variations in Drought Response and Fitness Traits of Beech (*Fagus sylvatica* L.) Seedlings from Three Provenances Differing in Annual Precipitation. *Trees* **2017**, *31*, 1215–1225. [CrossRef]
37. Pflug, E.E.; Buchmann, N.; Siegwolf, R.T.W.; Schaub, M.; Rigling, A.; Arend, M. Resilient Leaf Physiological Response of European Beech (*Fagus sylvatica* L.) to Summer Drought and Drought Release. *Front. Plant Sci.* **2018**, *9*, 187. [CrossRef]
38. Todea, I.M.; Gonzalez-Orenga, S.; Plazas, M.; Sestras, A.F.; Prohens, J.; Vicente, O.; Sestras, R.E.; Boscaiu, M. Screening for Salt and Water Stress Tolerance in Fir (*Abies alba*) Populations. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2019**, *47*, 1063–1072. [CrossRef]
39. Todea, I.M.; González-Orenga, S.; Boscaiu, M.; Plazas, M.; Sestras, A.F.; Prohens, J.; Vicente, O.; Sestras, R.E. Responses to Water Deficit and Salt Stress in Silver Fir (*Abies alba* Mill.) Seedlings. *Forests* **2020**, *11*, 395. [CrossRef]
40. Weithmann, G.; Schuldt, B.; Link, R.M.; Heil, D.; Hoeber, S.; John, H.; Müller-Haubold, H.; Schüller, L.-M.; Schumann, K.; Leuschner, C. Leaf Trait Modification in European Beech Trees in Response to Climatic and Edaphic Drought. *Plant Biol.* **2022**. [CrossRef]
41. Bhusal, N.; Lee, M.; Han, A.R.; Han, A.; Kim, H.S. Responses to Drought Stress in Prunus sargentii and Larix kaempferi Seedlings Using Morphological and Physiological Parameters. *For. Ecol. Manag.* **2020**, *465*, 118099. [CrossRef]
42. Dawson, T.E.; Mambelli, S.; Plamboeck, A.H.; Templer, P.H.; Tu, K.P. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 507–559. [CrossRef]
43. Gallé, A.; Feller, U. Changes of Photosynthetic Traits in Beech Saplings (*Fagus sylvatica*) under Severe Drought Stress and during Recovery. *Physiol. Plant.* **2007**, *131*, 412–421. [CrossRef] [PubMed]
44. Jonard, F.; André, F.; Ponette, Q.; Vincke, C.; Jonard, M. Sap Flux Density and Stomatal Conductance of European Beech and Common Oak Trees in Pure and Mixed Stands during the Summer Drought of 2003. *J. Hydrol.* **2011**, *409*, 371–381. [CrossRef]
45. Pretzsch, H.; Bielak, K.; Block, J.; Bruchwald, A.; Dieler, J.; Ehrhart, H.-P.; Kohnle, U.; Nagel, J.; Spellmann, H.; Zasada, M. Productivity of Mixed versus Pure Stands of Oak (*Quercus petraea* (M Att.) L Iebl. and *Quercus robur* L.) and European Beech (*Fagus sylvatica* L.) along an Ecological Gradient. *Eur. J. For. Res.* **2013**, *132*, 263–280. [CrossRef]
46. McDowell, N.G.; Licata, J.; Bond, B.J. Environmental Sensitivity of Gas Exchange in Different-Sized Trees. *Oecologia* **2005**, *145*, 9. [CrossRef] [PubMed]
47. Quero, J.L.; Sterck, F.J.; Martínez-Vilalta, J.; Villar, R. Water-Use Strategies of Six Co-Existing Mediterranean Woody Species during a Summer Drought. *Oecologia* **2011**, *166*, 45–57. [CrossRef] [PubMed]
48. Szymura, T.H. Concentration of Elements in Silver Fir (*Abies alba* Mill.) Needles as a Function of Needles' Age. *Trees* **2009**, *23*, 211–217. [CrossRef]
49. Peguero-Pina, J.J.; Camarero, J.J.; Abadía, A.; Martín, E.; Gonzalez-Cascon, R.; Morales, F.; Gil-Pelegrín, E. Physiological Performance of Silver-Fir (*Abies alba* Mill.) Populations under Contrasting Climates near the South-Western Distribution Limit of the Species. *Flora Morphol. Distrib. Funct. Ecol. Plants* **2007**, *202*, 226–236. [CrossRef]
50. Matías, L.; Gonzalez-Díaz, P.; Quero, J.L.; Camarero, J.J.; Lloret, F.; Jump, A.S. Role of Geographical Provenance in the Response of Silver Fir Seedlings to Experimental Warming and Drought. *Tree Physiol.* **2016**, *36*, 1236–1246. [CrossRef] [PubMed]
51. Lebourgeois, F.; Gomez, N.; Pinto, P.; Mérian, P. Mixed Stands Reduce Abies alba Tree-Ring Sensitivity to Summer Drought in the Vosges Mountains, Western Europe. *For. Ecol. Manag.* **2013**, *303*, 61–71. [CrossRef]
52. Bolte, A.; Roloff, A. Einfluß von Buchenüberhältern Auf Bodenvegetation Und Naturverjüngung. *Allg. Forst-u. J.-Ztg* **1993**, *164*, Q7-101.
53. Netzer, F.; Thöm, C.; Celepirovic, N.; Ivankovic, M.; Alfarraj, S.; Dounavi, A.; Simon, J.; Herschbach, C.; Rennenberg, H. Drought Effects on C, N, and P Nutrition and the Antioxidative System of Beech Seedlings Depend on Geographic Origin. *J. Plant Nutr. Soil Sci.* **2016**, *179*, 135–150. [CrossRef]
54. Peuke, A.D.; Rennenberg, H. Carbon, Nitrogen, Phosphorus, and Sulphur Concentration and Partitioning in Beech Ecotypes (*Fagus sylvatica* L.): Phosphorus Most Affected by Drought. *Trees* **2004**, *18*, 639–648. [CrossRef]

55. Liu, J.-F.; Arend, M.; Yang, W.-J.; Schaub, M.; Ni, Y.-Y.; Gessler, A.; Jiang, Z.-P.; Rigling, A.; Li, M.-H. Effects of Drought on Leaf Carbon Source and Growth of European Beech Are Modulated by Soil Type. *Sci. Rep.* **2017**, *7*, 42462. [CrossRef]
56. Aranda, I.; Sanchez-Gomez, D.; Cadahia, E.; de Simón, B.F. Ecophysiological and Metabolic Response Patterns to Drought under Controlled Condition in Open-Pollinated Maternal Families from a *Fagus sylvatica* L. *Population. Environ. Exp. Bot.* **2018**, *150*, 209–221. [CrossRef]
57. Chuste, P.-A.; Maillard, P.; Bréda, N.; Levillain, J.; Thirion, E.; Wortemann, R.; Massonnet, C. Sacrificing Growth and Maintaining a Dynamic Carbohydrate Storage Are Key Processes for Promoting Beech Survival under Prolonged Drought Conditions. *Trees* **2020**, *34*, 381–394. [CrossRef]
58. Blessing, C.H.; Werner, R.A.; Siegwolf, R.; Buchmann, N. Allocation Dynamics of Recently Fixed Carbon in Beech Saplings in Response to Increased Temperatures and Drought. *Tree Physiol.* **2015**, *35*, 585–598. [CrossRef] [PubMed]
59. Dannoura, M.; Epron, D.; Desalme, D.; Massonnet, C.; Tsuji, S.; Plain, C.; Priault, P.; Gérant, D. The Impact of Prolonged Drought on Phloem Anatomy and Phloem Transport in Young Beech Trees. *Tree Physiol.* **2019**, *39*, 201–210. [CrossRef]
60. Hesse, B.D.; Goisser, M.; Hartmann, H.; Grams, T.E.E. Repeated Summer Drought Delays Sugar Export from the Leaf and Impairs Phloem Transport in Mature Beech. *Tree Physiol.* **2019**, *39*, 192–200. [CrossRef]
61. Shi, L.; Guttenberger, M.; Kottke, I.; Hampp, R. The Effect of Drought on Mycorrhizas of Beech (*Fagus sylvatica* L.): Changes in Community Structure, and the Content of Carbohydrates and Nitrogen Storage Bodies of the Fungi. *Mycorrhiza* **2002**, *12*, 303–311. [CrossRef]
62. Leuschner, C. Drought Response of European Beech (*Fagus sylvatica* L.)—A Review. Perspectives in Plant Ecology. *Evol. Syst.* **2020**, *47*, 125576.
63. Fotelli, M.N.; Rennenberg, H.; Gessler, A. Effects of Drought on the Competitive Interference of an Early Successional Species (*Rubus fruticosus*) on Fagus sylvatica L. Seedlings: 15N Uptake and Partitioning, Responses of Amino Acids and Other N Compounds. *Plant Biol.* **2002**, *4*, 311–320. [CrossRef]
64. Guo, J.; Yang, Y.; Wang, G.; Yang, L.; Sun, X. Ecophysiological Responses of *Abies fabri* Seedlings to Drought Stress and Nitrogen Supply. *Physiol. Plant.* **2010**, *139*, 335–347. [PubMed]
65. Huppe, H.C.; Turpin, D.H. Integration of Carbon and Nitrogen Metabolism in Plant and Algal Cells. *Annu. Rev. Plant Biol.* **1994**, *45*, 577–607. [CrossRef]
66. Geβler, A.; Kopriva, S.; Rennenberg, H. Regulation of Nitrate Uptake at the Whole-Tree Level: Interaction between Nitrogen Compounds, Cytokinins and Carbon Metabolism. *Tree Physiol.* **2004**, *24*, 1313–1321. [CrossRef]
67. Joseph, J.; Luster, J.; Bottero, A.; Buser, N.; Baechli, L.; Sever, K.; Gessler, A. Effects of Drought on Nitrogen Uptake and Carbon Dynamics in Trees. *Tree Physiol.* **2021**, *41*, 927–943. [CrossRef]
68. Lasota, J.; Małek, S.; Jasik, M.; Błońska, E. Effect of Planting Method on C: N: P Stoichiometry in Soils, Young Silver Fir (*Abies alba* Mill.) and Stone Pine (*Pinus cembra* L.) in the Upper Mountain Zone of Karpaty Mountains. *Ecol. Indic.* **2021**, *129*, 107905. [CrossRef]
69. Cools, N.; Vesterdal, L.; De Vos, B.; Vanguelova, E.; Hansen, K. Tree Species Is the Major Factor Explaining C: N Ratios in European Forest Soils. *For. Ecol. Manag.* **2014**, *311*, 3–16. [CrossRef]