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Small-Scale Environmental Heterogeneity Enhances Tree Recruitment through Carbon Recharge and Water Use Diversification

Éva Salamon-Albert [1,*] , Dénes Bartha [2] and Ágnes Csiszár [2]

1 Department of Plant Sciences, Institute of Biology, Faculty of Sciences, University of Pécs, Ifjúság útja 6, 7624 Pécs, Hungary
2 Institute of Environmental Protection and Nature Conservation, Faculty of Forestry, University of Sopron, Bajcsy-Zsilinszky u. 4, 9400 Sopron, Hungary
* Correspondence: albert@gamma.ttk.pte.hu

Abstract: Studying regeneration processes in oak-dominated forests requires a multi-faceted approach that considers local factors, disturbances, management actions, and tree responses. Our aims were to quantify the carbon and water-use responses of saplings and evaluate ecological consequences at the early tree regeneration phase of a pedunculate oak-hornbeam forest. We measured plant eco-physiological parameters using an open-chamber IRGA equipment in large experimental canopy gaps (instantaneous field data) and greenhouse (climate-controlled reference data) conditions. We used the non-parametric Kruskal–Wallis ANOVA test to analyze differences and similarities in the gas-exchange response. Functional fingerprints indicated shared resource use and efficiency functions at species-specific performance levels with temporal variations. Medium-level and seasonally balanced carbon uptake and water-use functions characterized pedunculate oak (*Quercus robur* L.) and European hornbeam (*Carpinus betulus* L.). In contrast, the response patterns in wild cherry (*Prunus avium* (L.) L.) and green ash (*Fraxinus pennsylvanica* Marshall) were dominated by water use. Goat willow (*Salix caprea* L.) had consistently elevated gas-exchange levels with the largest seasonal variation among the study species. We found that trees could be ranked on a relative isohydric-to-anisohydric scale regarding their species–environment interaction. According to the carbon-gain response pattern coupling with a non-structural carbohydrate exchange scheme, we were able to classify tree species as having medium- and long-term carbon resource management. In conclusion, spatially heterogeneous and temporally balanced canopy gaps facilitate tree species' development and mixed-stand regeneration by providing a functionally diversifying recruiting environment.

Keywords: alluvial oak forest; canopy-gap dynamics; carbon sequestration; functional response; local adaptation; water use

1. Introduction

Renewal dynamics in oak-dominated forests is currently disputed, in particular regarding its theoretical and management aspects, including species performance, environmental and biotic heterogeneity, natural and artificial environmental disturbances, and their interactions [1–4]. In mixed deciduous stands, studies and monitoring of regeneration are mainly focused on oak species, due to their abundance and high economic value [2,5–7]. Nevertheless, detailed knowledge on the local renewal of coexisting forest-forming trees is also necessary to maintain species diversity when climatic conditions are favorable and even more so when the climate is limiting [1,2,7–11]. Tree–environment relationships in mixed forest stands can differ based on age-specific phenology, environmental plasticity, and successional character [12–15], significantly contributing to the maintenance of plant functional diversity during regeneration. The ecological performance of tree saplings

should not be neglected, as saplings contribute to a large degree to plant functional diversity and can reshape the canopy layer [7,10]. For a more detailed understanding of local variance, it is worth examining and comparing the carbon- and water-exchange responses involved in early phase regeneration under canopy-gap conditions.

When initial conditions of regeneration are met and propagule availability and seedling emergence are sufficient [6,13,14,16–18], saplings face a multifactorial ecological challenge using their species-specific abilities and capacities [6,10,13,19]. In general, the formation of canopy gaps and forest type, especially the size and heterogeneity, has the largest influence on tree regeneration by inducing significant changes in environmental parameters compared to the closed canopy [1,20]. Within a large canopy gap, spatially and temporally heterogeneous local environments can form, providing diversified ecological ranges for regenerating plants [19]. These patches can offer a suitable starting point for both early and late successional forest-forming trees (e.g., *Quercus robur*, *Carpinus betulus*, and *Prunus avium*), intermittent pioneers (e.g., *Salix* spp.), and some alien introduced species (e.g., *Fraxinus pennsylvanica*) [1,11,14,19]. The level of irradiation (shade to sun) and soil and air humidity (wet to moist) are the most important abiotic factors, while the role of temperature is secondary [19,20]. Light-demanding species can regenerate in the central zone of the gaps under high irradiation and moderate air humidity, and shade-tolerant species grow under medium light and high air humidity conditions along the gap edges [14]. The soil-moisture level in large gaps is persistently high and more variable compared to that of closed forest stands [21], providing near continuous water-resource use during the tree circulation period.

Under natural environmental conditions, regeneration of pedunculate oak is highly variable and suboptimal at both local and regional scales [2,5,7,10,17]. Oak renewal is reported to be permanently reduced compared to neighboring forest stands and the development of coexisting trees [10,13]. Oaks can only maintain dominance if the local environment provides supporting or limiting interactions according to a multistage dynamic model [2,6,7]. Factors contributing to successful regeneration include large canopy gaps [5] with favorable abiotic conditions [6], moderate competitive pressure, and limited browsing activity [7], and shelterwood forest management that promotes propagule production [2]. Even so, the dominant oak shows long-term regeneration with continuous acclimation [13], and one of the most significant regeneration functions of young seedlings is the variation in the root–shoot ratio [22]. Common forest-forming trees, such as *Quercus*, *Carpinus*, and *Fagus* spp., and pioneers, such as *Betula*, *Salix*, and *Tilia* spp., could coexist with the dominant oak in alluvial forests [5,10,13], and *Fraxinus pennsylvanica* and other introduced species could sporadically occur [23]. At the same time, seedlings with moderate or strong shade tolerance can enjoy significant growth benefits during early regeneration [10,13]. Consequently, the functional responses of tree species to local (non-climatic) environmental traits are crucial in oak-dominated alluvial hardwood forests [6,7,9,16,19].

The goal of our study was to investigate the eco-physiological performance of recruiting trees in a mixed alluvial pedunculate oak-hornbeam forest. We studied the carbon- and water-exchange functions of the most important coexisting trees and some accessory species. We also explored the differences in gas-exchange responses and limitations that characterize the sapling-specific dynamics under extended canopy-gap conditions. In particular, we evaluated: (1) site-specific and factor-related variability in functional parameters, (2) canopy gap-related gas-exchange rates to detect field-acclimated plant-response functions compared to a reference performance, and (3) guided or shared gas-exchange response characters of the species using functional fingerprints. In summary, here, we describe and identify tree carbon and water-use profiles that may contribute to early regeneration success relevant to tree recruitment and close-to-nature forest management.

2. Materials and Methods

2.1. Investigation Protocols

To explore the gas-exchange performance of the saplings, we measured parameters in forest canopy gaps in a variable natural environment and under climate-controlled conditions to serve as a reference regarding the abiotic variables. Our study site was a mesophilous mixed forest, located in a lowland riparian landscape in southwest Hungary near Vajszló and Páprád (45°52′44.1″ N; 18°00′29.30″ E). This forest community (*Circaeo-Carpinetum* Borhidi 2003 em. Kevey 2006) formed a moderately dense (75%–85% cover) canopy with codominant pedunculate oak (*Q. robur*) and narrow-leaved ash (*F. angustifolia* Vahl), and European hornbeam (*C. betulus*) in the upper layer sparsely mixed with green ash, silver linden (*T. tomentosa* Moench), and wild cherry (*P. avium*) trees [23]. At this site, climate conditions were seasonally balanced with high annual precipitation (795 mm), high relative air humidity (80%), and a moderate mean annual temperature (11.5 °C). The soils were moderately acidic hystosols and luvisols with an alluvial deposit as the parent material [24]. We selected three artificial canopy gaps, where the study species were present and site parameters were ecologically comparable (Table 1). The experimental canopy gaps were larger (3000, 2500, and 2250 m^2) than the ones generally used in similar experiments, had no exposure, and presented high levels of illumination during the daylight hours. The vegetation cover in the gaps was medium to high (50%–70%), consisting of regrowth individuals of canopy trees (e.g., *Q. robur, C. betulus, P. avium,* and *F. pennsylvanica*), forest undergrowth (e.g., *Hedera helix* L. and *Rubus caesius* L.), and some pioneer species (e.g., *Salix caprea*). *Q. robur* had the highest regenerative abundance among the study trees, followed by *C. betulus, P. avium,* and *S. caprea*. Just before the beginning of the experiment, a large number of pedunculate oak seedlings were planted in the canopy gaps, selecting the most favorable positions under light-saturated conditions. This was necessary due to the lack of spontaneous regeneration, as well as the fact that plants regenerating from acorns often suffer severe winter frost damage during the early renewal period. In the case of other tree species, we provided natural environmental conditions across the canopy gaps with no human intervention.

Table 1. Climate parameters with [1] regional data and [2] microclimatic ranges in the measurement period of 2013–2014 (part 1). N = number of individuals and n = number of records in the experimental canopy gaps (part 2).

Climate Parameter/Sapling	Spring	Summer	Fall
[1] Temperature (mean, °C)	11.0	21.1	11.9
[1] Precipitation (sum, mm)	231	184	210
[1] Relative air humidity (mean, %)	76	71	84
[2] Photon flux density (µmol m^{-2} s^{-1})	16–2969	18–2449	28–2766
[2] Leaf temperature (min–max, °C)	15.6–35.7	18.5–37.0	14.5–28.8
[2] Carbon dioxide (min–max, ppm)	332–571	338–447	337–424
[2] Relative air humidity (min–max, %)	32.2–71.7	16.4–37.1	31.3–54.5
Quercus robur (N = 15)	n = 242	n = 334	n = 312
Carpinus betulus (N = 5)	n = 212	n = 297	n = 224
Prunus avium (N = 5)	n = 276	n = 205	n = 312
Fraxinus pennsylvanica (N = 8)	n = 227	n = 288	n = 235
Salix caprea (N = 8)	n = 243	n = 309	n = 236

The reference study site used to grow the experimental saplings was the outdoor greenhouse of the University of Pécs Botanical Garden (46°4′39.6″ N; 18°12′25.69″ E). The mean temperature at this site was 11.8, 22.6, and 12.8 °C, and precipitation was 282, 117, and 170 mm in spring, summer, and fall, respectively. The 2–4 years old saplings originated from a field site in a pedunculate oak-hornbeam forest in the Bükkhát Forest Reserve. We planted the trees into 18 cm tall and wide flowerpots containing a 6:2:2 soil–peat–sand mixture. Water was provided by natural rainfall and saplings received

additional weekly irrigation to prevent desiccation of the soil. A prolonged-release effect nutritional supplement (Osmocote Complete) was added at 20 g per container in March, at the beginning of the growth season. Light conditions of the outdoor greenhouse and the experimental forest gaps were similar, as the plants were exposed to total irradiation during daylight hours.

2.2. Sampling and Measurement Protocols

Plant gas-exchange parameters were measured both in the experimental canopy gaps and the reference site simultaneously along the vegetation seasons of 2013–2014. We selected saplings that were: (1) 3–4 years old, (2) persisted under a typical canopy-gap environment, (3) in the same phase of development, and (4) had unimpaired and mature shoots without any damage. We sampled fully developed leaves of expanded shoots of 5–15 individuals in the canopy gaps and 3–5 individuals from the reference plot. We recorded location-specific plant gas-exchange data for *Q. robur*, *F. pennsylvanica*, and *S. caprea* saplings regenerating in the fully illuminated area of the canopy gap (central zone with light-saturated irradiation). On the other hand, we measured *C. betulus* and *P. avium* individuals in the partially illuminated areas of the canopy gap (i.e., the transition zone with periodically saturated irradiation). Since narrow-leaved Hungarian ash (*F. angustifolia* subsp. *oxycarpa* (Willd.) Franco & Rocha Afonso) do not regenerate naturally in the early stage of canopy gaps, this species was excluded. We took 60 standardized measurements per response at the climate-controlled reference site.

We monitored gas exchange from mid-May to early June (spring), late July to early August (summer), and late September to early October (fall). We used portable and open-chambered IRGA equipment (LCA-2 and LCA Pro+; ADC BioScientific Ltd., Hoddesdon, United Kingdom) to record instantaneous and climate-controlled data. Among the parameters, we focused on the carbon uptake and water loss rates, which are synergistic via a shared stomatal pathway [25]. The detection functions and ranges of the measurement system were the following: 0–3000 μmol m^{-2} s^{-1} for Photosynthetic Photon Flux Density (PPFD), 0–50 °C for leaf and air temperatures (T), 0–2000 ppm for carbon dioxide concentration in the air (C_a), and 0%–100% for relative air humidity (RH). These instantaneous local climate parameters were also recorded during field measurements, near the studied plants. In reference measurements, climate parameters were standardized according to the defined protocol of light-response detection. We adjusted the level of irradiation (PPFD) to 0–2000 μmol m^{-2} s^{-1}, leaf temperature to 20, 25, and 30 °C, air humidity to 12 mBar, and carbon dioxide to 370 ppm. To increase the reliability of data, we measured plant gas-exchange responses along the total irradiation range both in forest gaps and climate-controlled greenhouse conditions. To obtain a real sense of gas-exchange rates, we measured plants that have not been exposed to significant abiotic stress, from sunrise until the midday depression. Finally, we highlighted assimilation (A; μmol CO_2 m^{-2} s^{-1}) as net carbon uptake, transpiration (E; mmol H_2O m^{-2} s^{-1}) as the level of plant water loss, and stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}) as the relative measure of stomatal opening. We also calculated the intrinsic (iWUE = A/gs) and photosynthetic (pWUE = A/E) water-use efficiencies to characterize leaf gas-exchange economy.

2.3. Data Processing and Analyses

Based on light-response data collected under field and climate-controlled conditions, we calculated non-linear functional response curves by applying an algorithm with exponential decay (Equation (1)):

$$y = y_0 + A_1 \times e^{-x/t_1} \tag{1}$$

where, y_0 is the offset that refers to the upper limit, i.e., the maximum rate, A_1 is the amplitude that refers to the lower limit, i.e., the minimum rate, and t_1 is the decay constant that refers to the inflexion point. To verify the adequacy of the curve fitting, we calculated the statistical probability (P) of the corrected chi-square value (Chi^2 df^{-1}). Using Equation (1), we can define two subsets, data points under and over the inflection can be considered

low- and high-irradiated, respectively. We selected the high-irradiated subset for *Q. robur*, *F. pennsylvanica*, and *S. caprea*, as these seedlings were located in high-irradiated areas of the canopy gaps. On the other hand, the total response range, including both subsets, was applied for *C. betulus* and *P. avium*, as their saplings were located in partially shaded areas within the gaps. During the greenhouse measurements, we recorded climate-controlled datasets near the seasonal mean temperatures typical of canopy-gap conditions, i.e., 15 and 20 °C in the spring and fall and 25 and 30 °C in the summer.

We used the Origin program (Version 6.1, Northampton, MA, USA) to construct response curves and calculate the functional ranges. We evaluated gas-exchange variability using the Kruskal–Wallis nonparametric test in the Statistica software (Version 12.6, Irvine, CA, USA). We used this rank-based method as the measurement data followed a non-normal distribution. To examine the differences between the rank sums in the factors, we used the H-value as the test statistic of the Kruskal–Wallis ANOVA and the appropriate probability levels (P). We statistically compared the measurement sites (GAP and REF), the experimental tree saplings (*Q. robur, C. betulus, P. avium, F. pennsylvanica*, and *S. caprea*), and detected the seasonal variation in tree gas-exchange functions (spring, summer, and fall). For the final evaluation, we calculated the functional fingerprint to represent similarities and differences between the species by calculating relative resource use and efficiency levels [26].

3. Results

3.1. Site Factors and Gas-Exchange Heterogeneity

We identified the relative importance and performance of five important gas-exchange parameters comparing canopy gaps with the reference site (Table 2). In general, the gas-exchange function of all factors varied significantly in the location-specific light ranges under both measurement conditions ($p < 0.001$). We identified the largest differences between the gaps and the reference site in photosynthetic water use (1.36 ± 0.03 and 3.37 ± 0.02) and transpiration (4.01 ± 0.03 and 2.30 ± 0.03), respectively. In contrast, differences in stomatal conductance (0.24 ± 0.002 and 0.21 ± 0.002) and intrinsic water use (27.10 ± 0.35 and 39.04 ± 0.33) proved to be less important. Stomatal conductance was the most variable gas-exchange parameter among the species in canopy gaps, in *Q. robur* 0.27 ± 0.003, *C. betulus* 0.21 ± 0.004, *P. avium* 0.15 ± 0.003, *F. pennsylvanica* 0.14 ± 0.004, and *S. caprea* 0.43 ± 0.004 were detected. Assimilation and transpiration in *Q. robur* (8.95 ± 0.17 and 4.76 ± 0.06), *C. betulus* (4.43 ± 0.13 and 3.81 ± 0.05), *P. avium* (4.36 ± 0.13 and 2.91 ± 0.05), *F. pennsylvanica* (4.75 ± 0.17 and 2.95 ± 0.06), and in *S. caprea* (9.88 ± 0.19 and 6.79 ± 0.07) were also presented in wide functional ranges. On the other hand, both photosynthetic and intrinsic water use were much less variable in all species: *Q. robur* (1.73 ± 0.04 and 32.83 ± 0.77), *C. betulus* (0.89 ± 0.03 and 17.68 ± 0.65), *P. avium* (1.38 ± 0.13 and 31.46 ± 0.62), *F. pennsylvanica* (1.45 ± 0.03 and 32.34 ± 0.81), and *S. caprea* (1.58 ± 0.04 and 22.07 ± 0.90). Seasonal fluctuation was more balanced in canopy gaps than under reference conditions for both transpiration and water-use parameters. In particular, intrinsic water-use efficiency was the most attenuated tree response compared to other gas-exchange functions with values of 22.54 ± 0.62 in the spring, 28.52 ± 0.57 in the summer, and 29.77 ± 0.59 in the fall. On the other hand, stomatal conductance and assimilation proved to be much more temporally heterogeneous in the openings with values of 0.21 ± 0.004 and 4.42 ± 0.13 in the spring, 0.19 ± 0.004 and 4.41 ± 0.11 in the summer, and 0.31 ± 0.004 and 9.03 ± 0.12 in the fall, respectively. In the fall, assimilation showed the largest difference among species compared to other gas-exchange parameters. On the other hand, the smallest differences were detected in plant water-use parameters, especially in the carbon input-to-water output ratio. Seasonal changes in the reference measurements were more moderate in every parameter compared to species variability, except for photosynthetic water use. Overall, stomatal conductance showed the largest difference between the two measurement sites and intrinsic water-use efficiency was the most similar, considering both species and seasons.

Table 2. Relative importance of experimental factors in gas-exchange performance (A: assimilation, E: transpiration, gs: stomatal conductance, pWUE: photosynthetic water use, iWUE: intrinsic water use) under field (GAP) and reference (REF) conditions. Site = experimental sites (GAP, REF); Species = experimental trees; Season = experimental periods (spring, summer, and fall). H-values refer to Kruskal–Wallis ANOVA test statistics, n = 7949, all cases are significant at $p < 0.001$.

Gas Exchange Function	Factor	GAP	REF
A	Site	381.1	
	Species	805.2	1065.2
	Season	583.8	277.3
E	Site	1514.5	
	Species	1148.7	675.2
	Season	288.7	543.5
gs	Site	77.9	
	Species	1569.9	978.2
	Season	951.4	569.0
pWUE	Site	2777.8	
	Species	338.4	92.3
	Season	561.3	935.9
iWUE	Site	700.5	
	Species	472.2	423.2
	Season	177.3	225.4

3.2. Gas Exchange under Field and Reference Conditions

We compared the gas-exchange behavior of the study species in two environments, under canopy-gap (field) and climate-controlled (reference) conditions (Figure 1). Assimilation, photosynthetic, and intrinsic water use were significantly lower in canopy gaps for all species and in all seasons compared to the reference environment, except for the fall carbon uptake of saplings and the summer-to-fall intrinsic water use of wild cherry. The intrinsic water utilization of wild cherry reached a remarkably high value under field conditions in the summer. In comparison, summer transpiration and conductance of wild cherry and the spring and fall stomatal behavior of green ash showed the opposite pattern. Species differences in the canopy gaps were the most pronounced in photosynthetic water use in the spring, transpiration and intrinsic water use in the summer, and stomatal conductance in the fall. Carbon sequestration of the saplings differed the least among seasons and photosynthetic and intrinsic water use varied the least among species in each season. In terms of carbon dioxide uptake, gas conductance, and water-use efficiency, pedunculate oak and goat willow consistently had the highest values under canopy-gap conditions. Wild cherry and European hornbeam had the lowest values, with green ash being intermediate.

3.3. Carbon Uptake and Water Loss in Canopy Gaps

Using the five response variables, we constructed functional fingerprints that illustrate seasonal dynamics in canopy gaps based on the relative gas-exchange levels (Figure 2). According to preliminary cluster analyses (figures are not presented), resource use gas-exchange parameters (A, E, and gs) formed a well-articulated, strongly separated group compared to efficiency parameters (pWUE and iWUE). Among these competing gas-exchange domains, carbon- and water-exchange rates had a much higher interspecific variability than carbon-to-water efficiency functions. The relative levels of input and output parameters in the medium to high range showed large seasonal fluctuations for pedunculate oak and hornbeam. On the other hand, parameters in wild cherry and green ash varied from low to medium levels with moderate seasonal changes, and goat willow consistently showed the highest levels with large fluctuations. Carbon uptake increased from spring to fall in all species, with a summer decline in pedunculate oak and wild cherry. Transpiration markedly declined during the summer in most species, except for

European hornbeam and goat willow with somewhat elevated and remarkably high levels, respectively. Stomatal conductance varied in close relation to water-output response patterns of the saplings at lower (e.g., green ash), medium (e.g., pedunculate oak), and higher (e.g., goat willow) levels. Gas-exchange efficiency parameters were less variable among the species but with different levels and patterns, also showing contrasting seasonal performance in water use. These responses generally had medium-level variation, however, wild cherry showed consistently high levels, and goat willow had the largest seasonal fluctuation. In addition, photosynthetic water use with a continuous increase was the most favorable in the fall, with a significant summer decline in pedunculate oak and goat willow. On the other hand, intrinsic water use had largely different levels and various species-specific temporal patterns. Pedunculate oak and green ash showed a continuous increase, European hornbeam and wild cherry a summer maximum, and goat willow a summer decline.
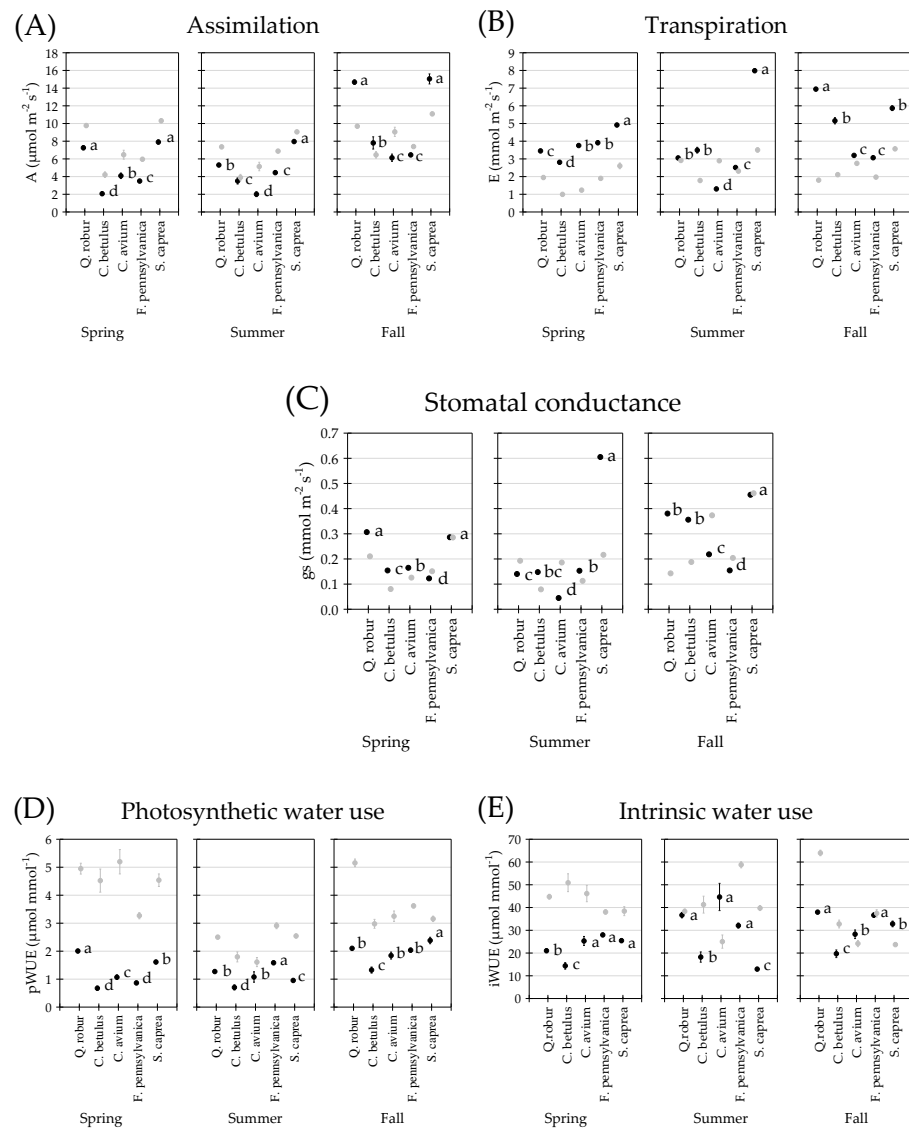

Figure 1. Seasonal gas-exchange patterns of the experimental saplings under canopy-gap (black symbols) and reference (grey symbols) conditions. Mean $\pm$ 0.95 confidence intervals are shown for the Kruskal–Wallis test at $p < 0.05$ (except for spring gs in *S. caprea*). Lower case letters indicate differences between the saplings' response functions with values decreasing from (a) to (d).
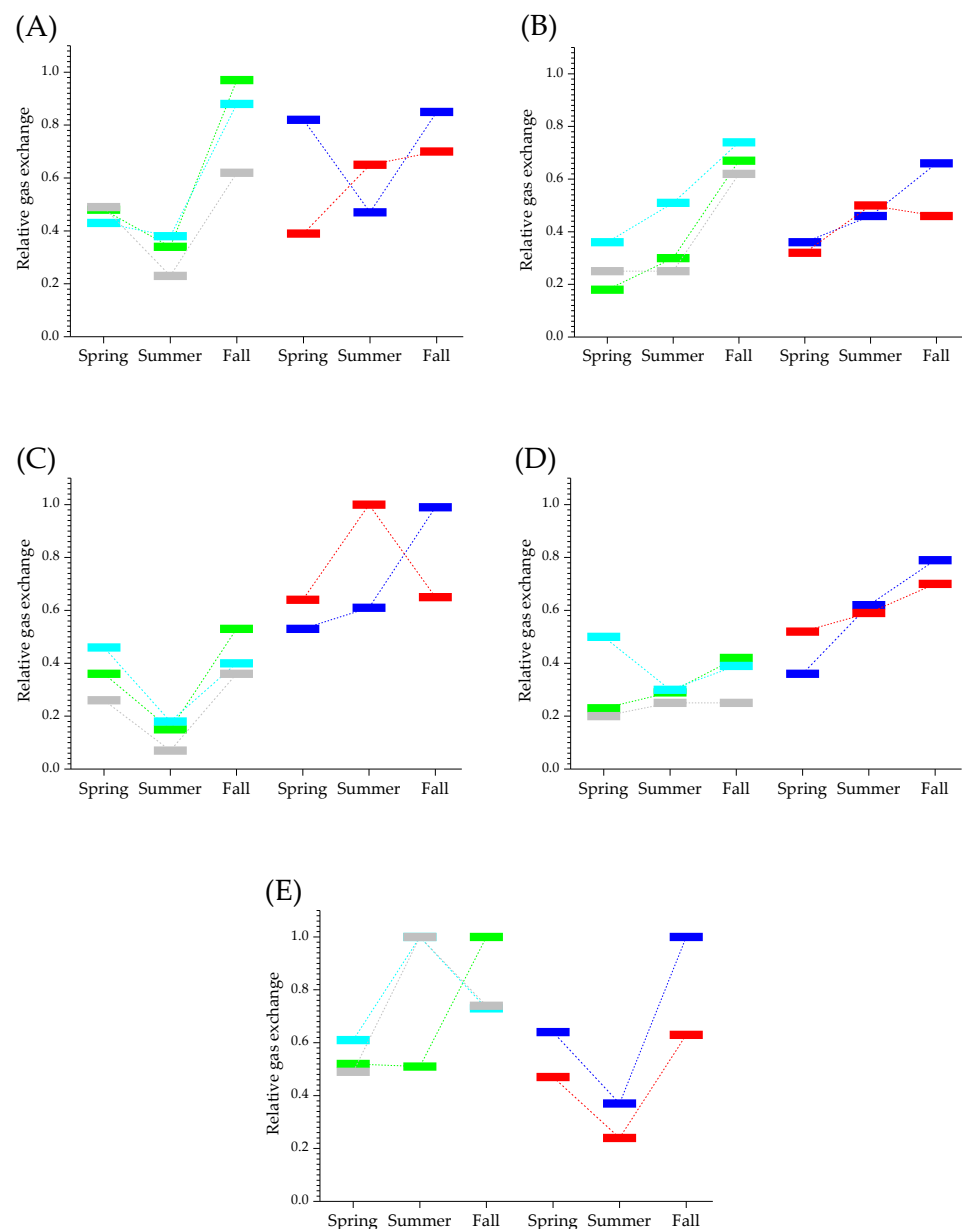
Figure 2. Functional fingerprints of (**A**) *Q. robur,* (**B**) *C. betulus,* (**C**) *P. avium,* (**D**) *F. pennsylvanica,* and (**E**) *S. caprea* saplings in canopy gaps. The relative gas exchange involves the standardized mean of a parameter. Assimilation (green), transpiration (cyan), and stomatal conductance (grey) are illustrated on the left, and photosynthetic (blue) and intrinsic water use (red) on the right.

4. Discussion

We studied carbon- and water-use responses of coexisting tree saplings in large canopy gaps to test plant functions to achieve close-to-nature forest regeneration. All gas-exchange functions were consistently less favorable in the canopy gap compared to the reference environment. Variations in carbon gain (resource use) versus water use (efficiency) functions were the most important tree gas-exchange response phenomena under field conditions. The relative levels of efficiency parameters (pWUE and iWUE) proved to be more heterogeneous than resource use functions (A and E). In addition, all species showed the highest carbon-input level in the fall along the seasonal response patterns. This increased assimilation during the late season can be essential to maintain year-round carbon management activity in regenerating saplings in canopy gaps, especially to keep the functional linkage between wintering and spring renewal.

Species-specific tree responses have been recommended as a research priority in mixed deciduous forests [2,3,12,19], as they can affect forest regeneration in different ways. Canopy-gap conditions in particular need to be considered, as they will determine the success of tree regeneration [1,20]. For this reason, we need to identify plant functional properties that can be used to characterize tree behavior and predict the success of a close-to-nature stand regeneration. The general basis can be the successional character at the community level, where trees can be classified as gap or forest competitors and colonizers regarding regeneration-related growth and life history [14]. Considering shoot and root development, intermittent and continuous types are characteristic, and the continuous types can vary among the seasons [8,27–30]. Assimilation rates refer to plant carbon-resource use, where the observed carbon sequestration is interpreted as related to the slow turnover dynamic in stored non-structural carbohydrates (NSC) [31]. Here, the plant reloads depleted reserves that have been used for development, defense, and energy-demanding physiological processes in its year-round activity [32]. We found diurnal and seasonal air humidity to be the most variable abiotic factor in the canopy gaps; therefore, it is worth evaluating the isohydric-to-anisohydric character of the saplings as the relative level of the plant–environment interaction [33]. We explain these aspects for each study species in detail in the following section.

Pedunculate oak (*Q. robur*) is a gap competitor that regenerates and grows best in canopy gaps with increased juvenile growth potential [14]. According to our findings regarding gas exchange, this species had variable carbon sequestration with a summer minimum and a fall maximum. Considering the spring–summer shoot and root growth period of the species [27], stored carbohydrates needed for the current growth must be accumulated during the previous fall. This late-season carbon allocation is also necessary to ensure the energy and organic matter available for bud break and early leaf development. The significant NSC fluctuation during the growing period can be consistently interpreted with the gas-exchange dynamics [34]. The two-pool NSC model of forest trees [35] suggests that under moderate abiotic stress, carbohydrates in long-term storage can also be used for growth. The contrast between maximum growth and minimal carbon uptake in the summer may result from the extensiveness and rapid mobilization of NSC reserves [36]. This is reinforced by the minimum summer conductivity as the most reactive component of the gas-exchange system, which may further limit atmospheric carbon dioxide availability [37]. The root–shoot ratio of the pedunculate oak seedlings that grow on permanently moist soil can shift towards a shoot biomass increase, as previously reported from a laboratory experiment [22]. Our results are consistent with the viable shoot development of the species, seen in canopy gap measurements in the field. Intrinsic water-use responses occurred at below-average (spring) and above-average (summer and fall) levels with moderate seasonal fluctuations. Based on the plant–environment interaction hypothesis [33], we recommend considering the pedunculate oak as a seasonal isohydric species.

European hornbeam is a forest competitor that regenerates and grows best under shade conditions derived from the canopy layer with an average juvenile growth rate [14]. Our results support this observation, as individuals regenerated along the marginal zone of the experimental gaps, under restricted illumination. According to our measurements, carbon sequestration in this species was moderately variable between the spring minimum and the fall maximum. In general, the growth rate is known to decrease from spring to fall during the growing period [8]. Comparing the two dynamics, the assimilation maximum in the fall can mostly cover the spring carbohydrate demand originating from leaf emergence and early shoot growth. Consequently, the size of the stored carbohydrate reserves of the species may be limited, as previously suggested [8]. A temporal shift in the replenishment of NSC pools can also be assumed due to decreased atmospheric carbon dioxide availability derived from restricted stomatal conductance in spring and summer [37]. Reduced NSC productivity [38], possibly related to stomatal limitation, may result in a longer regeneration time of carbohydrate reserves. In concordance with the leaf-allocating behavior of the species [15], we detected a higher shoot mass and lower root mass in the field observations

regarding the root–shoot ratio. Water utilization of the plants was around average levels, moderately fluctuating along the seasons. Based on the plant–environment interaction hypothesis [33], this species can be considered an isohydric-to-anisohydric intermediate in the study area. It can also possibly tolerate moderate salt stress under seasonally fluctuating groundwater levels due to the salt tolerance of the species [39].

Wild cherry is reported to be a gap colonizer (pioneer) species that regenerates and grows best after gap formation with a juvenile growth maximum [14]. In our experiment, regrowth individuals regenerated in the marginal zone of the experimental units, in partially illuminated areas. The species had moderately variable carbon sequestration in the canopy gaps with a deep summer minimum at low-to-medium levels. The shoot and root growth of the saplings is characterized by a gradually decreasing rate from the early maximum [30]. Comparing the two dynamics suggests that the NSC reserves replenishing in the fall should cover the combined carbohydrate demand of spring and summer, in addition to the restrictive effect of poor summer stomatal conductivity [37]. Wild cherry primarily allocates carbohydrates to leaf growth and development [40,41], thereby becoming a successful pioneer species [42]. The root-to-shoot ratio is basically shoot-dominant [40,41], and root growth can vary through several maxima during the growing period [30]. Since carbon allocation capacity is determined by the environmental carbon dioxide oversupply [41], the competitive carbon sequestration activity of coexisting species can also affect the development of individuals. Water-use efficiency functions of the saplings dominate gas-exchange responses during the entire growing period at above-mean levels. Therefore, based on the interaction approach [33], this species can be classified as an isohydric water function respondent.

Goat willow is also considered a gap colonizer (pioneer) species that regenerates just after gap formation with a juvenile growth maximum [14]. Individuals were found in the permanently light-saturated zone of canopy gaps and the species had highly variable carbon sequestration with a medium level in the spring, a summer minimum, and a fall maximum. Both the shoot and root growth of young plants decrease from spring to fall [28]. A comparison of these dynamics suggests that the carbohydrate reserves that are replenished in the fall cover the needs of physiological events during spring and summer. The long-term medium–high stomatal conductance does not represent an important restrictive condition in terms of carbon-dioxide access in any season [37]. According to previous studies [28], under favorable field conditions, such as in the presence of a thick subsoil layer with a permanent moisture supply, this species primarily allocates to root development. The resulting extremely high root–shoot ratio [28] can create significant root competition with coexisting species [43], which gives an advantage to groundwater access. According to the levels of intrinsic water-use efficiency obtained in our experiment, the plants function as a fast water exchanger between the soil and air. Referring to its environmental interaction [33], this species clearly represents the anisohydric plant functional type.

Due to its successful early-phase gap regeneration and high juvenile growth potential, as also observed throughout the study area, green ash was classified as an early-phase gap-colonizing (pioneer) species [14]. Individuals were detected in the light-saturated area of canopy gaps, presenting moderately variable carbon sequestration at small-to-medium levels with a continuous increase from spring to fall. The shoot and root growth rates of the saplings continuously decrease from spring to fall under favorable environments over several periods [29]. The accumulation of non-structural carbohydrates is expected to increase under strong environmental stress, such as leaf or shoot loss [44]. These phenomena suggest that growth processes can be predominant in balanced environments, especially in the form of radial growth [29]. In our measurements, assimilation continuously increased from spring to fall, which suggests that the carbohydrate reserves that were replenished in the fall could cover most of the spring renewal and growth. After this period, the plant is forced to increase the observed carbon uptake to continuously rebuild NSC reserves. Permanently low stomatal conductance behaves as a co-restricting factor for the NSC reconstruction process by its carbon-uptake-limiting function [37]. Consequently, the plant

keeps its water-use efficiency at a higher level compared to carbon sequestration, which corresponds to an isohydric environmental response state [33]. This result is consistent with a previous finding that individuals adapted to mesic habitats present reduced gas-exchange rates compared to drought-adapted populations, including leaf-carbon gain and water loss [45]. Even though the species is widespread in floodplain forests [16], the measured gas-exchange functions indicate limited growth in regenerating individuals.

Our gas-exchange results suggest that assisted regeneration of pedunculate oak in large canopy gaps helps to maintain the community role of this species in the Central European region [3,4,6]. We strongly recommend the early planting of seedlings in light-saturated areas right after the canopy gap is opened [6,19], to prevent the underrepresentation of the species that occurs under natural conditions [13]. As an additional effect, the large number and biomass of pedunculate oak can inhibit the early invasion of non-native tree species [7,19], in our case the green ash [16], through biotic competitive pressure. From the economic point of view, the fast growth and large biomass production of *Q. robur* individuals with a favorable root–shoot ratio enables the formation of high-quality wood [2], which can promote sustainable forestry at a regional scale [3,4]. Moreover, these globally balanced and locally heterogeneous openings ensure the development of forest-forming trees at different spatiotemporal scales, resulting in adaptive stand regeneration.

5. Conclusions

Based on the gas-exchange response results, we identified different eco-physiological acclimation strategies among the species, which were detected at various resource-use levels presenting temporal (seasonality) and spatial (allocation) patterns. Regarding the tree–environment interaction scheme of water use, wild cherry and green ash behaved as an isohydric, pedunculate oak as a seasonal isohydric, and common hornbeam as an intermediate species. Goat willow presented an anisohydric response pattern and could be considered the most suitable indicator of groundwater oversupply. Carbohydrate pools can be assumed to be stored long term (for three seasons, i.e., from winter to the next summer), which was characteristic in *Q. robur, P. avium,* and *S. caprea*. On the other hand, medium-term (two seasons, i.e., from winter to the next spring) carbon-storage capacity was confirmed in *C. betulus* and *F. pennsylvanica*. As most species regenerate with early shoot dominance, their later abundance can be predicted by the initial propagule set. As a result, we can predict an early leveling-off in the root–shoot ratio for all study species. Thus, the proportion of the most important forest-forming trees can be optimal even in regenerating stands. In pedunculate oak, we identified an overall intensive shoot growth and a fine-scale seasonal water-use pattern. Green ash displayed moderate resource use and efficiency functions in the experimental plots, even though this species is referenced to spread intensively in lowland higro-mesophilous habitats. In our opinion, the most significant abiotic-limiting factor of recruitment may be the local carbon dioxide limitation that could occur as a result of competition during the biotic phase of regeneration. In summary, forest sustainability can be achieved in floodplain pedunculate oak forests by creating large canopy gaps. Nevertheless, oak seedlings in particular must be planted at an early stage and protected from game browsing.

References

1. Zhu, J.; Lu, D.; Zhang, W. Effects of gaps on regeneration of woody plants: A meta-analysis. *J. For. Res.* **2014**, *25*, 501–510. [CrossRef]

2. Dobrovolný, L.; Martiník, A.; Drvodelić, D.; Oršanić, M. Structure, Yield and Acorn Production of Oak (*Quercus robur* L.) Dominated Floodplain Forests in the Czech Republic and Croatia. *South-East Eur. For.* **2017**, *8*, 127–136. [CrossRef]

3. Löf, M.; Ammer, C.; Coll, L.; Drössler, L.; Huth, F.; Madsen, P.; Wagner, S. Regeneration Patterns in Mixed-Species Stands. In *Dynamics, Silviculture and Management of Mixed Forests*; Managing Forest Ecosystems 31; Bravo-Oviedo, A., Pretzsch, H., del Rio, M., Eds.; Springer: Cham, Switzerland, 2018; pp. 103–130. Available online: https://link.springer.com/chapter/10.1007/978-3-319-91953-9_4 (accessed on 15 November 2022).

4. Pach, M.; Sansone, D.; Ponette, Q.; Barreiro, S.; Mason, B.; Bravo-Oviedo, A.; Löf, M.; Bravo, F.; Pretzsch, H.; Lesiński, J.; et al. Silviculture of Mixed Forests: A European Overview of Current Practices and Challenges. In *Dynamics, Silviculture and Management of Mixed Forests*; Managing Forest Ecosystems 31; Bravo-Oviedo, A., Pretzsch, H., del Rio, M., Eds.; Springer: Cham, Switzerland, 2018; pp. 185–253.

5. Bobiec, A.; Jaszcz, E.; Wojtunik, K. Oak (*Quercus robur* L.) regeneration as a response to natural dynamics of stands in European hemiboreal zone. *Eur. J. For. Res.* **2011**, *130*, 785–797. [CrossRef]

6. Annighöfer, P.; Beckschäfer, P.; Vor, T.; Ammer, C. Regeneration Patterns of European Oak Species (*Quercus petraea* (Matt.) Liebl., *Quercus robur* L.) in Dependence of Environment and Neighborhood. *PLoS ONE* **2015**, *10*, e0134935. [CrossRef] [PubMed]

7. Mölder, A.; Sennhenn-Reulen, H.; Fischer, C.; Rumpf, H.; Schönfelder, E.; Stockmann, J.; Nagel, R.-V. Success factors for high-quality oak forest (*Quercus robur*, *Q. petraea*) regeneration. *For. Ecosyst.* **2019**, *6*, 49. [CrossRef]

8. Klein, T.; Vitasse, Y.; Hoch, G. Coordination between growth, phenology and carbon storage in three coexisting deciduous tree species in a temperate forest. *Tree Physiol.* **2016**, *36*, 847–855. [CrossRef]

9. Ortmann-Ajkai, A.; Csicsek, G.; Lukács, M.; Horváth, F. Regeneration patterns in a pedunculate oak (*Quercus robur* L.) strict forest reserve in Southern Hungary. *Šumar. List* **2017**, *141*, 39–46. [CrossRef]

10. Demeter, L.; Bede-Fazekas, Á.; Molnár, Z.; Csicsek, G.; Ortmann-Ajkai, A.; Varga, A.; Molnár, Á.; Horváth, F. The legacy of management approaches and abandonment on old-growth attributes in hardwood floodplain forests in the Pannonian Ecoregion. *Eur. J. For. Res.* **2020**, *139*, 595–610. [CrossRef]

11. Tinya, F.; Kovács, B.; Aszalós, R.; Tóth, B.; Csépányi, P.; Németh, C.; Ódor, P. Initial regeneration success of tree species after different forestry treatments in a sessile oak-hornbeam forest. *For. Ecol. Manag.* **2020**, *459*, 117810. [CrossRef]

12. Hölscher, D. Leaf traits and photosynthetic parameters of saplings and adult trees of co-existing species in a temperate broad-leaved forest. *Basic Appl. Ecol.* **2004**, *5*, 163–172. [CrossRef]

13. Götmark, F.; Kiffer, C. Regeneration of oaks (*Quercus robur*/*Q. petraea*) and three other tree species during long-term succession after catastrophic disturbance (windthrow). *Plant Ecol.* **2014**, *215*, 1067–1080. [CrossRef]

14. Petrokas, R.; Baliuckas, V.; Manton, M. Successional Categorization of European Hemi-boreal Forest Tree Species. *Plants* **2020**, *9*, 1381. [CrossRef] [PubMed]

15. Konôpka, B.; Pajtík, J.; Šebe, V.; Surový, P.; Merganičová, K. Woody and foliage biomass, foliage traits and growth efficiency in young trees of four broadleaved tree species in a temperate forest. *Plants* **2020**, *10*, 2155. [CrossRef] [PubMed]

16. Drescher, A.; Prots, B. *Fraxinus pennsylvanica*—An invasive tree species in middle Europe: Case studies from the Danube Basin. *Contrib. Bot.* **2016**, *51*, 55–69. Available online: http://contributii_botanice.reviste.ubbcluj.ro/materiale/2016/Contrib_Bot_vol_51_pp_055-069.pdf (accessed on 15 November 2022).

17. Bobiec, A.; Reif, A.; Öllerer, K. Seeing the oakscape beyond the forest: A landscape approach to the oak regeneration in Europe. *Landscape Ecol.* **2018**, *33*, 513–528. [CrossRef]

18. Kiani, B.; Yegandoost, K. The study of parent-regeneration relationships for wild cherry (*Prunus avium* L.) in Hyrcanian forests. *J. For. Sci.* **2021**, *67*, 328–337. [CrossRef]

19. Terwei, A.; Zerbe, S.; Zeileis, A.; Annighöfer, P.; Kawaletz, H.; Mölder, I.; Ammer, C. Which are the factors controlling tree seedling establishment in North Italian floodplain forests invaded by non-native tree species? *For. Ecol. Manag.* **2013**, *304*, 192–203. [CrossRef]

20. Qiu, T.; Sharma, S.; Woodall, C.W.; Clark, J.S. Niche Shifts from Trees to Fecundity to Recruitment That Determine Species Response to Climate Change. *Front. Ecol. Evol.* **2021**, *9*, 719141. [CrossRef]

21. Kollár, T. Light conditions, soil moisture, and vegetation cover in artificial forest gaps in western Hungary. *Acta Silv. Lignaria Hung.* **2017**, *13*, 25–40. [CrossRef]

22. Deligöz, A.; Bayar, E. Drought stress responses of seedlings of two oak species (*Quercus cerris* and *Quercus robur*). *Turk. J. Agric. For.* **2018**, *42*, 114–123. [CrossRef]

23. Kevey, B. A baranyai Dráva-sík gyertyános-tölgyesei (*Circaeo-Carpinetum* Borhidi 2003 em. Kevey 2006). *Nat. Som.* **2007**, *10*, 41–71, (In Hungarian with English Summary). [CrossRef]

24. Dövényi, Z. *Magyarország kistájainak katasztere*, 2nd ed.; Hungarian Academy of Sciences Geographical Institute: Budapest, Hungary, 2010; p. 876. (In Hungarian)

25. Field, C.B.; Ball, J.T.; Berry, J.A. Photosynthesis: Principles and field techniques. In *Plant Physiological Ecology—Field Methods and Instrumentation*; Ehleringer, J., Mooney, H.A., Rundel, P.W., Pearcy, R.W., Eds.; Chapman and Hall: London, UK, 1991; pp. 209–253.

26. Salamon-Albert, É.; Csiszár, Á.; Bartha, D. Functional fingerprinting estimates renewal opportunities for tree species in a mixed Turkey oak forest. *Turk. J. Agric. For.* **2021**, *45*, 144–153. [CrossRef]

27. Hoffmann, G. Wachstumsrhythmik der Wurzeln und SproBachsen von Forstgeholzen. Growth Rhythms of Roots and Shoot Axis in Forest Trees. *Flora* **1972**, *161*, 303–319. [CrossRef]

28. Dušek, J.; Květ, J. Seasonal dynamics of dry weight, growth rate and root/shoot ratio in different aged seedlings of *Salix caprea*. *Biologia* **2006**, *61*, 441–447. [CrossRef]

29. Kremer, D.; Cavlovic, J.; Bozic, M. Growth characteristics of introduced green ash (*Fraxinus pennsylvanica* Marshall) and narrow-leaved ash (*F. angustifolia* L.) in lowland forest region in Croatia. *New For.* **2006**, *31*, 211–224. [CrossRef]

30. Bonomelli, C.; Bonilla, C.; Acuña, E.; Artacho, P. Seasonal pattern of root growth in relation to shoot phenology and soil temperature in sweet cherry trees (*Prunus avium*): A preliminary study in central Chile. *Cienc. Investig. Agrar.* **2012**, *39*, 127–136. [CrossRef]

31. El Omari, B. Accumulation versus storage of total non-structural carbohydrates in woody plants. *Trees* **2022**, *36*, 869–881. [CrossRef]

32. Davidson, A.M.; Le, S.T.; Cooper, K.B.; Lange, E.; Zwieniecki, M.A. No time to rest: Seasonal dynamics of non-structural carbohydrates in twigs of three Mediterranean tree species suggest year-round activity. *Sci. Rep.* **2021**, *11*, 5181. [CrossRef]

33. Hochberg, U.; Rockwell, F.E.; Holbrook, N.M.; Cochard, H. Iso/Anisohydry: A Plant–Environment Interaction Rather Than a Simple Hydraulic Trait. *Trends Plant. Sci.* **2018**, *23*, 112–120. [CrossRef]

34. El Zein, R.; Maillard, P.; Breda, N.; Marchand, J.; Montpied, P.; Gérant, D. Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. *Tree Physiol.* **2011**, *31*, 843–854. [CrossRef]

35. Richardson, A.D.; Carbone, M.S.; Keenan, T.F.; Czimczik, C.I.; Hollinger, D.Y.; Murakami, P.; Schaberg, P.G.; Xu, X. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytol.* **2013**, *197*, 850–861. [CrossRef]

36. Bessonova, V.P.; Chonhova, A.S. Influence of soil moisture level on metabolism of non-structural carbohydrates in *Quercus robur* leaves. *Regul. Mech. Biosyst.* **2021**, *12*, 628–634. [CrossRef]

37. Yan, W.; Zhong, Y.; Shangguan, Z. A meta-analysis of leaf gas exchange and water status responses to drought. *Sci. Rep.* **2016**, *6*, 20917. [CrossRef]

38. Weber, R.; Gessler, A.; Hoch, G. High carbon storage in carbon-limited trees. *New Phytol.* **2019**, *222*, 171–182. [CrossRef]

39. Zhou, Q.; Zhu, Z.; Shi, M.; Cheng, L. Growth and Physicochemical Changes of *Carpinus betulus* L. Influenced by Salinity Treatments. *Forests* **2018**, *9*, 354. [CrossRef]

40. Flore, J.A.; Layne, D.R. Photoassimilate production and distribution in cherry. *HortScience* **1999**, *34*, 1015–1019. [CrossRef]

41. Drută, A. Effect of long term exposure to high CO_2 concentrations on photosynthetic characteristics of *Prunus avium* L. plants. *Photosynthetica* **2001**, *39*, 289–297. [CrossRef]

42. Anev, S.; Marinova, A. Physiological adaptation of European beech (*Fagus sylvatica* L.) and wild cherry (*Prunus avium* L.) saplings after windthrow. *For. Ideas* **2021**, *2*, 436–445. Available online: https://oaji.net/articles/2022/6191-1642671454.pdf (accessed on 15 November 2022).

43. Mudrák, O.; Hermová, M.; Tesnerová, C.; Rydlová, J.; Frouz, J. Above-ground and below-ground competition between the willow *Salix caprea* and its understorey. *J. Veg. Sci.* **2016**, *27*, 156–164. [CrossRef]

44. Ramirez, J.A.; Vitali, V.; Martínez-Vilalta, J.; Handa, T.; Messier, C. Reserve Accumulation Is Prioritized over Growth Following Single or Combined Injuries in Three Common North American Urban Tree Species. *Front. Plant Sci.* **2021**, *12*, 715399. [CrossRef]

45. Abrams, M.D.; Kubiske, M.E.; Steiner, K.C. Drought adaptations and responses in five genotypes of *Fraxinus pennsylvanica* Marsh.: Photosynthesis, water relations and leaf morphology. *Tree Physiol.* **1990**, *6*, 305–315. [CrossRef] [PubMed]