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Assessment of physiological and biochemical recovery processes in four tree species following natural heat stress

Bewertung physiologischer und biochemischer Erholungsprozesse bei vier Baumarten nach natürlichem Hitzestress

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Keywords: Leaves, chlorophyll, carotenoids, effective quantum yield, catalase, phenolic compounds, water content

Schlüsselbegriffe: Blätter, Chlorophyll, Carotinoide, effektive Quantenausbeute, Katalase, phenolische Verbindungen, Wassergehalt

Abstract

The intensification of heat waves and extreme temperatures in recent years has negatively affected trees, reducing their growth processes and diminishing their capacity to withstand thermal stress. In this context, identifying the physiological and biochemical recovery processes in ecologically and economically valuable species is essential for sustainable forest management. This study investigated two forests dominated by *Quercus petraea* and by *Q. robur* located in the Plaiul Fagului Nature Reserve (Republic of Moldova). Detached leaves of *F. sylvatica*, *Q. petraea*, *Q. robur* and *Fraxinus excelsior* were collected and incubated under controlled conditions for ten days following natural exposure of the trees to thermal stress. The research assessed recovery processes based on functional parameters (photosynthetic pigment content, effective quantum yield of photosystem II), antioxidant activity (catalase and phenolic compounds), and leaf water retention capacity. *F. sylvatica* exhibited the highest tolerance to thermal stress, maintaining both photosynthetic function and leaf hydration. *Q. petraea* showed an intermediate response, characterized by elevated antioxidant activity. In contrast, *F. excelsior* recorded the lowest physiological and

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biochemical values, indicating increased vulnerability to thermal stress. Additionally, the physiological condition of *Quercus robur* trees revealed greater tolerance in individuals with green foliage compared to those with yellow-brown leaves. These findings suggest species-specific and condition-dependent differences in physiological adaptation mechanisms linked to foliar functional status.

Zusammenfassung

Die Zunahme von Hitzewellen und extremen Temperaturen in den letzten Jahren wirkt sich negativ auf Bäume aus, indem sie deren Wachstumsprozesse hemmt und ihre Fähigkeit zur Bewältigung von Hitzestress verringert. Vor diesem Hintergrund ist die Identifizierung physiologischer und biochemischer Erholungsprozesse bei ökologisch und wirtschaftlich wertvollen Baumarten von Bedeutung für die nachhaltige Forstwirtschaft. In dieser Studie wurden Blätter aus zwei Wälder dominiert durch *Quercus petraea* und *Q. robur* im Naturschutzgebiet Plaiul Fagului (Republik Moldau) gesammelt. Die Untersuchung bewertete die physiologischen und biochemischen Erholungsprozesse in abgetrennten Blättern von *F. sylvatica*, *Q. petraea*, *Q. robur*, *Carpinus betulus* und *Fraxinus excelsior*, die zehn Tage lang unter kontrollierten Bedingungen inkubiert wurden, nachdem die Bäume natürlichem Hitzestress ausgesetzt waren. Die Analysen zeigten signifikante Unterschiede zwischen den drei Arten hinsichtlich ihrer Erholungsfähigkeit, basierend auf funktionellen Parametern (Gehalt an photosynthetischen Pigmenten, Chlorophyllfluoreszenz), der Aktivität von Antioxidantien (Katalase und phenolische Verbindungen) sowie der Wasserhaltekapazität der Blätter. *F. sylvatica* zeigte die höchste Toleranz gegenüber Hitzestress und erhielt sowohl die photosynthetische Funktionalität als auch die Blattwasserhaltung aufrecht. *Q. petraea* wies eine mittlere Reaktion auf, die sich durch eine erhöhte antioxidative Aktivität auszeichnete. *F. excelsior* hingegen verzeichnete die niedrigsten Werte der physiologischen und biochemischen Indikatoren, was auf eine erhöhte Anfälligkeit gegenüber Hitzestress hinweist. Zugleich zeigte die Analyse des physiologischen Zustands von *Quercus robur* eine höhere Toleranz gegenüber Hitzestress bei den Individuen mit grünem Laub im Vergleich zu jenen mit gelb-bräunlichem Blattwerk. Diese Ergebnisse deuten auf artspezifische und zustandsabhängige Unterschiede in den physiologischen Anpassungsreaktionen in Abhängigkeit vom funktionellen Zustand des Blattapparates hin.

1 Introduction

The increasing frequency and intensity of heat waves and extreme temperatures over recent decades have profoundly affected tree physiological processes, reducing both forest productivity and natural regeneration capacity (Teskey *et al.* 2015; Duveneck *et* *al.* 2015; Aquilué *et al.* 2021). Understanding the mechanisms through which thermal stress impacts forest species has become essential for modeling ecosystem dynamics and anticipating the effects of climate change on forests (Ciais *et al.* 2005; Aquilué *et al.* 2021). However, the species-specific responses to extreme temperatures and their recovery capacity remain insufficiently understood, generating uncertainties regarding forest ecosystem resilience. In its most severe forms, thermal stress can lead to tree mortality, threatening the long-term stability and functioning of ecosystems (Thomas *et al.* 2002; Andersson *et al.* 2011; Teskey *et al.* 2014; Allen *et al.* 2015).

The effects of extreme temperatures vary considerably among species and provenances, with some exhibiting high tolerance, while others are severely affected, showing growth reduction, decreased photosynthetic efficiency, and increased vulnerability to pests (Dobbertin 2006; Pšidová *et al.* 2018). Forest tree species of major ecological and economic significance, such as sessile oak (*Quercus petraea*), pedunculate oak (*Q. robur*), European beech (*Fagus sylvatica*), and European ash (*Fraxinus excelsior*), play a crucial role in maintaining biodiversity and ecosystem functionality in Central and Western Europe (Peters 1997; Floren *et al.* 2025). Nevertheless, their vulnerability to thermal stress is increasingly evident, especially in the context of more frequent heatwaves and prolonged droughts, as highlighted by numerous studies on beech (*F. sylvatica*), one of the most thoroughly documented species in Europe (Gessler *et al.* 2007; Kramer *et al.* 2010).

Forest species have developed complex physiological mechanisms to cope with thermal stress, involving cellular and molecular-level adjustments. Exposure to extreme temperatures increases the fluidity of lipids in cellular membranes, affecting their integrity and promoting the denaturation of essential enzymes in chloroplasts and mitochondria. In chloroplasts, such disturbances may impair photosynthetic processes by altering the activity of carbon-fixing enzymes, while in mitochondria, the efficiency of cellular respiration is reduced, limiting the energy production required for metabolism of trees (Pflug *et al.* 2018). Recent studies show that under moderate thermal stress, trees can activate recovery mechanisms such as the restoration of photosynthetic pigments and photosystem II functionality, the up regulation of enzymatic antioxidants, and the accumulation of phenolic compounds, thereby contributing to the reduction of oxidative stress (Pšidová *et al.* 2018; Cuza *et al.* 2021; Visi-Rajczi *et al.* 2021; Húdoková *et al.* 2022).

Among the cellular organelles affected by thermal stress, chloroplasts are particularly sensitive, playing a central role in both photosynthesis and the regulation of physiological responses in forest species. High temperatures can disrupt their function by impairing electron transport along the photosynthetic chain, inhibiting chlorophyll biosynthesis, and reducing CO_2 assimilation, processes essential to tree metabolism (Kmiecik *et al.* 2016; Sun & Guo 2016).

Prolonged exposure to extreme temperatures accelerates chlorophyll degradation through oxidative processes, reducing the efficiency of photosystem II and disrupting the plant's energy balance. To mitigate these effects, chloroplasts activate protective and repair mechanisms, including the synthesis of stress-specific proteins and the reorganization of the thylakoid membrane. They also play a crucial role in cell signaling associated with thermal stress, contributing to the regulation of physiological responses to extreme environmental conditions (Yu *et al.* 2012; Chen *et al.* 2018). The relevance of these mechanisms is supported by experimental studies on the thermal stress responses of forest species. For instance, Çakmakçi and Güner (2024) demonstrated that reduced water availability in seedlings of *Fagus orientalis* led to a significant decrease in total chlorophyll content in the leaves ($p < 0.05$). However, drought tolerance varied among provenances, with biological material originating from Istanbul (180 m altitude) maintaining more stable chlorophyll content compared to other sources. Another study highlighted that the recovery of photosystem II functionality in oak leaves from the Republic of Moldova is strongly influenced by both the duration of thermal shock exposure and the post-stress incubation conditions. Although oak species have developed specific strategies for coping with high temperatures, excluding avoidance mechanisms and light-excess dissipation revealed a lower tolerance to thermal shock in downy oak leaves compared to those of pedunculate oak and sessile oak (Cuza *et al.* 2021).

Alongside the protective and repair processes occurring in chloroplasts, carotenoids play a crucial role in maintaining photosynthetic integrity under thermal stress conditions. These pigments are involved both in preventing oxidative stress, by neutralizing reactive oxygen species, and in stabilizing photosystems and sustaining post-stress recovery processes (Edge *et al.* 1997). Under high-temperature conditions, the accumulation of reactive oxygen species, particularly singlet oxygen, can lead to carotenoid oxidation. This process not only protects photosynthetic structures, but also acts as a molecular signal regulating the expression of genes involved in acclimation mechanisms, thereby contributing to plant adaptation to thermal stress (Havaux 2013).

Antioxidant enzymes play an essential role in maintaining redox balance in cells exposed to thermal stress. Among them, catalase is one of the main detoxifying agents of hydrogen peroxide (H_2O_2), a byproduct of intensified cellular metabolism under stress conditions (Zámocký & Koller 1999). This enzyme, found in peroxisomes, mitochondria, the cytosol, and chloroplasts, protects cellular components by rapidly converting H_2O_2 into water and oxygen, thereby preventing its accumulation to toxic levels. Catalase activation represents a crucial adaptive physiological response, essential for maintaining cellular homeostasis and protecting vital structures from oxidative damage (Michelet *et al.* 2013). Experimental studies have shown that the intensity of this response varies depending on tree species and provenance. For example, Sharma (2014) highlighted that catalase activity significantly increases in the presence of high H_2O_2 concentrations, underscoring its essential role in cellular metabolic protection. Notably, recent research indicates that both beech (*F. sylvatica*) and sessile oak (*Q. petraea*) exhibit strong catalase activation under drought conditions. In beech, the rise in enzymatic activity reflects an efficient antioxidant mechanism contributing to cellular protection and drought adaptation. In sessile oak, especially in provenances from more arid regions, the activation of catalase, alongside other antioxidant enzymes, suggests a superior capacity to maintain redox balance, facilitating more effective drought adaptation (Vukmirović *et al.* 2025).

Alongside antioxidant enzymes, phenolic compounds, as secondary metabolites with an adaptive role, contribute to plant protection under extreme conditions. Due to their high antioxidant capacity, these compounds help neutralize reactive oxygen species, such as H_2O_2, OH^-, O_2^-, and $^1O_2$, which are produced in excess as a result of plant exposure to thermal stress conditions (Mellacheruvu *et al.* 2019). In a broader context, the effects of temperature on the phenolic profile have been investigated in plant species such as *Lavandula viridis* and *Thymus lotocephalus*, grown in vitro at varying temperatures (15, 20, 25, and 30°C). The results showed an increase in phenolic compound content and antioxidant activity in micropropagated plants exposed to higher temperatures. In contrast, in in vitro cultures, the opposite trend was observed, suggesting a differential influence of temperature on these compounds depending on the developmental stage of the plants and experimental conditions (Mansinhos *et al.* 2022).

In oak trees, multiple studies have highlighted high concentrations of phenolic compounds, positively correlated with antioxidant capacity (Ianni *et al.* 2020; Tanase *et al.* 2022). For example, research conducted by Sirgedaitė-Šėžienė *et al.* (2023) demonstrated significant variations in the phenolic content in the bark of *Q. robur* populations, influenced both by the provenance of the biological material and the solvent used for extraction, with methanol proving to be the most effective. Additionally, Ucar & Ucar (2011) identified β-sitosterol and quercitol as important phenolic derivatives from oak bark, emphasizing their role in cellular protection against oxidative stress.

However, the recovery processes of leaves in *Q. robur* and *Q. petraea* species after exposure to thermal stress caused by heatwaves and extreme heat remain insufficiently studied. This issue becomes even more relevant in the context of climate change in Europe, where prolonged heatwaves and drought episodes contribute to the contraction of the distribution area of *Q. robur*. The persistence and regeneration capacity of oaks under such conditions are essential for maintaining current forest habitats, yet it remains uncertain to what extent these adaptive processes will be sufficient to ensure the long-term survival of populations (Lorenz & Becher 2012). The importance of these two oak species is recognized both in forest ecology and in sustainable forest management practices in Central Europe, where oak plays a key role in the structure and functioning of forest ecosystems.

To analyze post-heat stress recovery processes, we used excised leaves collected from trees naturally exposed to heatwaves and subsequently examined them under controlled laboratory conditions. Although excised leaves are no longer supplied by the plant's vascular system, they temporarily retain the functional integrity of the foliar apparatus, allowing for the assessment of physiological processes that reflect the cumulative effects of stress. This method is acknowledged in the scientific literature as relevant for investigating hydraulic vulnerability and recovery capacity, particularly in the short term.

Thus, Blackman *et al.* (2009) analyzed drought responses in four tropical tree species by assessing leaf water potential (Ψ leaf) and transpiration (E) in intact plants, while leaf hydraulic conductance (K leaf) was determined on excised leaves to construct hydraulic vulnerability curves based on stress intensity. Similarly, Brodribb & Cochard (2009) demonstrated that evaluating post-drought hydraulic function (*e.g.*, conductivity loss) in leaves and stems is feasible through destructive methods such as centrifugation of excised samples to determine critical thresholds (Ψ_{50}, Ψ_{95}).

Furthermore, this approach has been previously applied in our studies on post-heat shock recovery dynamics in *Buxus sempervirens* and *Quercus* spp., where excised leaves were used to assess temporary physiological changes following exposure to high temperatures in the laboratory (Dascaliuc *et al.* 2007; Dascaliuc & Cuza 2011; Cuza *et al.* 2021; Dascaliuc *et al.* 2022). These results support the use of excised leaves as a relevant experimental model for investigating leaf recovery capacity, particularly in the early post-stress phases, when responses are already encoded at the cellular and biochemical levels.

Currently, there are few studies investigating the leaf recovery processes of *Fagus sylvatica*, *Quercus petraea*, *Q. robur*, and *Fraxinus excelsior* species after natural exposure to heatwaves and extreme heat. Through this study, we aim to achieve the following research objectives:

(I) Determining the physiological and biochemical variations associated with the post-stress recovery process in the studied species under controlled experimental conditions.
(II) Comparing the recovery capacity between species, highlighting the differences in tolerance to natural thermal stress.
(III) Establishing the level of tolerance to extreme temperatures based on the natural physiological responses of each species and assessing post-stress resilience.

2 Materials and Methods

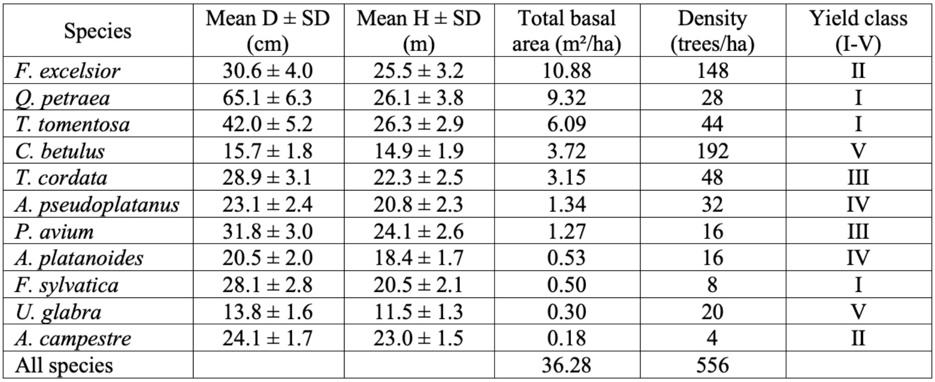
2.1 Sample Collection, Analyzed Parameters, and Experimental Conditions

In the Plaiul Fagului Nature Reserve, a single tree from each species was selected: European beech (*Fagus sylvatica*), sessile oak (*Quercus petraea*), and common ash (*Fraxinus excelsior*). All selected trees were approximately 80 years old and located in close proximity to each other (up to 40 meters apart) within a mixed forest of sessile oak (*Quercus petraea*), beech (*Fagus sylvatica*), and silver linden (*Tilia tomentosa*), with Carex brevicollis as the dominant ground vegetation (Doniță *et al.* 2007). The stand is situated on a north-facing slope with a 3-5° inclination, at an elevation of 340 meters (47.288°N, 28.014°E). The terrain is moderately fragmented, featuring micro-depressions that cause local humidity variations. The shaded slope is characterized by a cool and mesic microclimate. The soil is of the brown luvic type (corresponding to a luvisol in the WRB classification), with a loamy-sandy texture and a relatively homogeneous composition.

The selection of a single tree from each species aimed to reduce individual variability that could have introduced genetic differences unrelated to natural thermal stress. Data on the stand structure and the individual characteristics of the trees from which leaf samples were collected provide a reference framework for the comparative analysis of physiological and biochemical recovery processes following thermal stress (Tables 1 and 2).

Table 1: Stand structure in the mixed forest of Quercus petraea, Fagus sylvatica, and Tilia tomentosa with Carex brevicollis: dendrometric characteristics and vitality (per 1 ha).

Tabelle 1: Bestandsstruktur im Mischwald aus *Quercus petraea*, *Fagus sylvatica* und *Tilia tomentosa* mit *Carex brevicollis*: dendrometrische Merkmale und Vitalität (pro 1 ha).



Species	Mean D ± SD (cm)	Mean H ± SD (m)	Total basal area (m²/ha)	Density (trees/ha)	Yield class (I-V)
F. excelsior	30.6 ± 4.0	25.5 ± 3.2	10.88	148	II
Q. petraea	65.1 ± 6.3	26.1 ± 3.8	9.32	28	I
T. tomentosa	42.0 ± 5.2	26.3 ± 2.9	6.09	44	I
C. betulus	15.7 ± 1.8	14.9 ± 1.9	3.72	192	V
T. cordata	28.9 ± 3.1	22.3 ± 2.5	3.15	48	III
A. pseudoplatanus	23.1 ± 2.4	20.8 ± 2.3	1.34	32	IV
P. avium	31.8 ± 3.0	24.1 ± 2.6	1.27	16	III
A. platanoides	20.5 ± 2.0	18.4 ± 1.7	0.53	16	IV
F. sylvatica	28.1 ± 2.8	20.5 ± 2.1	0.50	8	I
U. glabra	13.8 ± 1.6	11.5 ± 1.3	0.30	20	V
A. campestre	24.1 ± 1.7	23.0 ± 1.5	0.18	4	II
All species			36.28	556	

The yield classes (I-V) presented in Tables 1 and 3 are based on a local classification system derived from yield tables, which assign productivity classes to tree species according to dominant height and stand age. This system is commonly applied in the region to assess forest productivity potential. It differs from yield class systems used in other countries, which typically rely on average annual volume increment.

Table 2: Dendrometric characteristics and vitality status of the analysed trees of Quercus petraea, Fagus sylvatica, and Fraxinus excelsior selected for the assessment of post-drought recovery processes.

Tabelle 2. Dendrometrische Merkmale und Vitalitätszustand der analysierten Bäume von *Quercus petraea*, *Fagus sylvatica* und *Fraxinus excelsior* zur Bewertung der Erholungsprozesse nach Trockenstress.

Species	D (cm)	H (m)	Crown width (m)	Crown length (m)	Social status	Vitality	Visible damage
F. sylvatica	36	25.3	4.9	14.1	Dominant	Good	No damage
F. excelsior	44	24.8	8.6	13.2	Dominant	Good	No damage
Q. petraea	42	25.6	7.4	13.8	Dominant	Good	No damage

Two pedunculate oak trees were selected, one with green leaves and the other with yellow brown leaves, from a mixed forest of pedunculate oak (*Quercus robur*) and hornbeam (*Carpinus betulus*) with *Rubus caesius*, in order to assess their physiological condition (Tables 3 and 4). The tree with yellow-brown leaves was chosen due to visible damage caused by the heat wave and prolonged drought. The stand is located at the base of the left slope of the Rădeni stream, at an altitude of 200 meters, with a gentle inclination of 2-3° and a south-eastern exposure (47.299°N, 28.061°E). The soil is a typical grey soil (corresponding to an albic luvisol in the WRB classification), with a loamy to clayey texture. The location of the forest stands from which leaf samples were collected from the mentioned species is illustrated in Figure 1, which shows their placement within the reserve.

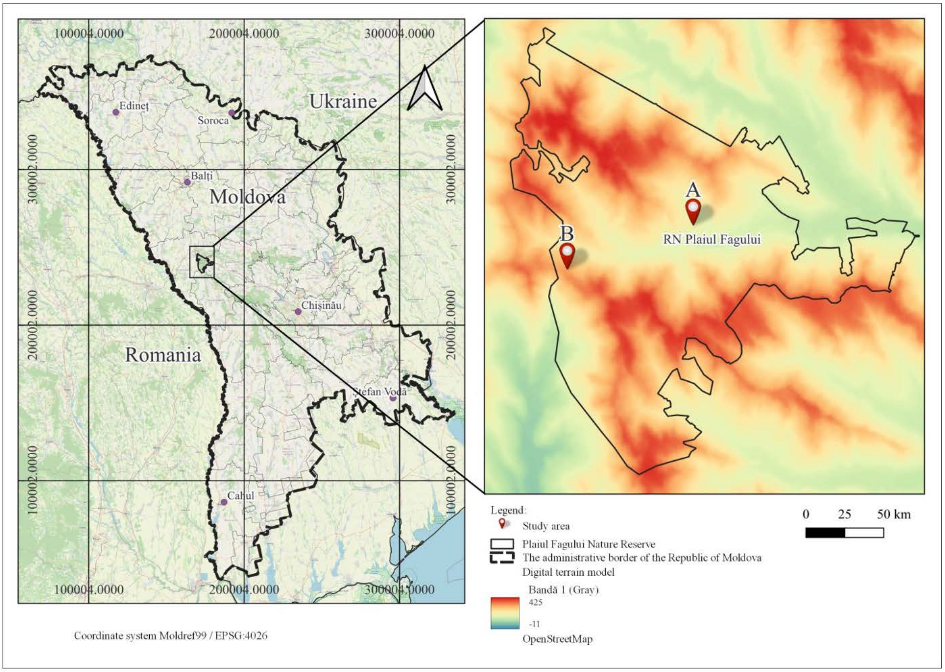
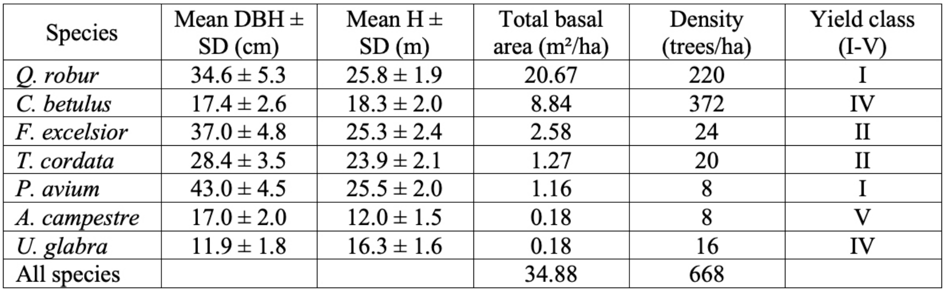


Figure 1: Map of the Plaiul Fagului Nature Reserve indicating the location of the stands from which leaf samples were collected. Stand A – mixed forest of Quercus robur and Carpinus betulus, with Rubus caesius. Stand B – mixed forest of Quercus petraea, Fagus sylvatica, and Tilia tomentosa, with Carex brevicollis.

Abbildung 1: Karte des Naturschutzgebiets Reservats Plaiul Fagului mit der Lage der Bestände, aus denen Blattproben entnommen wurden. Bestand A – Mischwald mit *Quercus robur* und *Carpinus betulus*, mit *Rubus caesius*. Bestand B – Mischwald mit *Quercus petraea*, *Fagus sylvatica* und *Tilia tomentosa*, mit *Carex brevicollis*.

Table 3: Stand structure in the mixed forest of Quercus robur and Carpinus betulus with Rubus caesius: dendrometric characteristics and vitality.

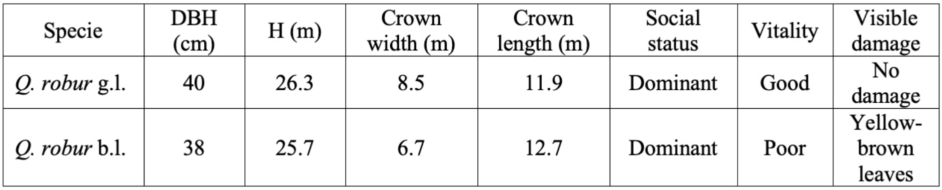
Tabelle 3: Bestandesstruktur im Mischwald aus *Quercus robur* und *Carpinus betulus* mit *Rubus caesius*: Dendrometrische Merkmale und Vitalität.



Species	Mean DBH ± SD (cm)	Mean H ± SD (m)	Total basal area (m²/ha)	Density (trees/ha)	Yield class (I-V)
Q. robur	34.6 ± 5.3	25.8 ± 1.9	20.67	220	I
C. betulus	17.4 ± 2.6	18.3 ± 2.0	8.84	372	IV
F. excelsior	37.0 ± 4.8	25.3 ± 2.4	2.58	24	II
T. cordata	28.4 ± 3.5	23.9 ± 2.1	1.27	20	II
P. avium	43.0 ± 4.5	25.5 ± 2.0	1.16	8	I
A. campestre	17.0 ± 2.0	12.0 ± 1.5	0.18	8	V
U. glabra	11.9 ± 1.8	16.3 ± 1.6	0.18	16	IV
All species			34.88	668	

Table 4: Dendrometric characteristics and vitality status of Quercus robur trees with green and yellow-brown leaves selected for assessing post-drought recovery processes, Q. robur g.l. – pedunculate oak with green leaves, Q. robur b.l. – pedunculate oak with yellow-brown leaves.

Tabelle 4: Dendrometrische Merkmale und Vitalitätszustand von *Quercus robur* Bäumen mit grünen und gelb-braunen Blättern, ausgewählt zur Bewertung der Erholungsprozesse nach Trockenheit *Q. robur* g.l. Stieleiche mit grünen Blättern, *Q. robur* b.l. Stieleiche mit gelb-braunen Blättern.



Specie	DBH (cm)	H (m)	Crown width (m)	Crown length (m)	Social status	Vitality	Visible damage
Q. robur g.l.	40	26.3	8.5	11.9	Dominant	Good	No damage
Q. robur b.l.	38	25.7	6.7	12.7	Dominant	Poor	Yellow-brown leaves

In early august 2024, at the end of a heatwave with extreme temperatures (reaching up to 35°C in the shade; see Figure 2) and prolonged drought, 30 leaves were collected per tree from the lower, south-facing part of the crown. The leaf samples constituted the primary biological material for all physiological and biochemical determinations. The leaves were excised, placed in paper envelopes, sorted by species, and transported in a portable cooler to maintain optimal temperature conditions.

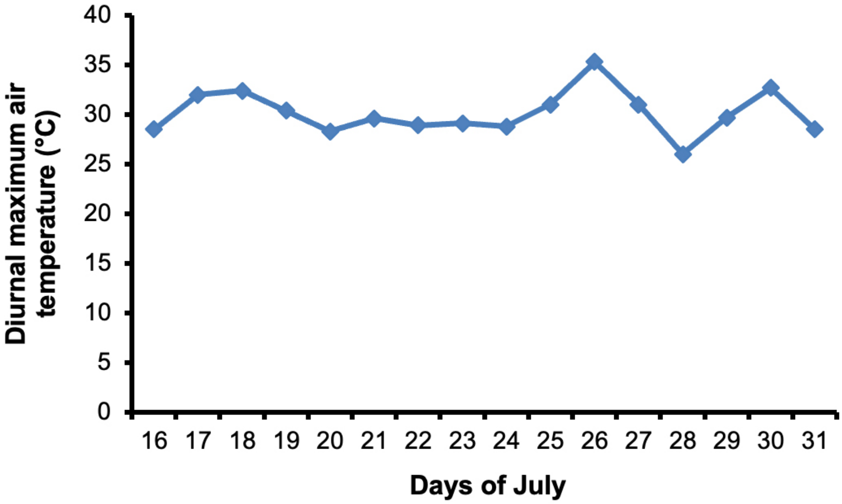


Figure 2: Variation in daily maximum temperatures during the period preceding leaf sampling for the analysis of physiological and biochemical indicators.

Abbildung 2: Verlauf der täglichen Höchsttemperaturen in der Zeit vor der Blattprobenahme zur Analyse physiologischer und biochemischer Indikatoren.

The use of detached leaves incubated under controlled laboratory conditions is a widely applied method in the scientific literature for studying post-heat shock recovery processes. This experimental approach enables a reproducible and standardized evaluation of physiological responses, eliminating the influence of fluctuating meteorological variables such as temperature, humidity, or solar radiation, which may compromise the accuracy of field-based assessments. The method is supported by numerous studies (Krause *et al.*, 2010; Dascaliuc *et al.*, 2022; Mihaljević *et al.*, 2024; González Argüello *et al.*, 2025), providing a robust methodological framework for the rigorous analysis of recovery processes under controlled conditions.

In the laboratory, the leaves were stored for ten days under controlled conditions: a constant temperature of 25°C, relative humidity of 85%, illumination of 200 lux, and a photoperiod of 16 hours of light and 8 hours of darkness. At intervals of 1, 3, 5, 7, and 10 days, samples were collected to determine the following physiological and biochemical parameters: chlorophyll *a* and *b* amount, carotenoid amount, effective quantum yield of photosystem II, catalase activity, total phenolic compound content, and leaf water content. The evaluation was performed comparatively, at each time interval, using the values from the first day as the initial reference. This procedure allowed for tracking the dynamics of the parameters depending on the duration of leaf storage under controlled conditions.

At each predetermined time interval, five leaves per species were taken from the controlled-environment chambers, cut into narrow strips, and pooled to obtain a composite sample. This sample was analyzed in two consecutive runs, each tested in three technical replicates.

2.2 Specific analysis methods

Chlorophyll extraction procedure

From this composite sample prepared according to the previously described protocol, 0.1 g of fresh plant material was weighed using an analytical balance. The plant material was ground thoroughly in a ceramic mortar with a porcelain pestle and homogenized in 10 mL of cold 80% (v/v) acetone, ensuring efficient pigment extraction. The resulting extract was stored in a refrigerated chamber at 5 °C for 4 hours to allow complete pigment solubilization. After incubation, the samples were centrifuged at 4000 × g for 10 minutes to separate the supernatant from the cell debris.

The absorbance of the clear supernatant was measured using a spectrophotometer (model CΦ-46), employing 1 cm path-length quartz cuvettes and 80% acetone as the blank. Absorbance readings were recorded at 662 nm and 644 nm to quantify chlorophyll *a* and chlorophyll *b*, respectively. Chlorophyll amounts were calculated using standard equations (Porra *et al.* 1989), and results were expressed as milligrams of pigment per gram of fresh weight (mg/g FW).

Carotenoid quantification

Carotenoids were determined from the same acetone extract used for chlorophyll analysis. Absorbance was measured spectrophotometrically at 440.5 nm.

Measurement of the effective quantum yield of photosystem II (PSII)

Chlorophyll fluorescence measurements were performed on excised leaves, ten leaves per tree, with three measurements taken on the same leaf under controlled conditions using a PAM-2100 fluorometer (H. Walz, Germany). For each species, ten leaves were examined under active light conditions. The device automatically recorded steady-state fluorescence (Ft) and maximum fluorescence (Fm'), induced by a saturating light pulse. Based on these measurements, the effective quantum yield of PSII was automatically calculated using the formula $\Phi PSII = (Fm' - Ft) / Fm'$ (Genty *et al.*, 1989). This parameter provides insight into the actual photochemical efficiency of PSII under illumination and was employed to monitor the recovery dynamics of the photosynthetic apparatus following stress exposure.

Catalase activity

Catalase activity was assessed using a modified version of the method described by Sinha (1972), in which residual hydrogen peroxide (H_2O_2) reacts with ammonium molybdate to form a yellow complex with maximum absorbance at 405 nm. For each species analyzed, 100 mg of leaf tissue were collected and homogenized in a mortar using 0.2 M Tris-glycine buffer. The homogenate was centrifuged at 15,000 × g for 15 minutes (Sigma 3K30), and 100 µL of the supernatant were used in the biochemical reaction. The reaction was initiated by adding 0.03% H_2O_2 and incubating the mixture at 37 °C for 10 minutes. The reaction was stopped by adding a 4% ammonium molybdate solution.

Optical density was measured at 405 nm using a spectrophotometer (model СФ-46), relative to a control sample, in three replicates. Catalase activity was expressed as micromoles (µMol) of H_2O_2 decomposed per minute per milligram of protein, according to the following formula:

$$Cat = \frac{C(H_2O_2)}{T \times C(protein) \times V} \qquad (1)$$

where:
Cat – catalase activity (µmol H_2O_2 min^{-1} mg^{-1} protein),
$C(H_2O_2)$ – hydrogen peroxide concentration (µMol),
T – incubation time (min),
C(protein) – protein concentration in the sample (mg/ml),
V – sample volume (ml).

Total phenolic compound content

The total phenolic content was determined using the colorimetric method described by Singleton and Rossi (1965), with slight modifications. To prevent the degradation of temperature-sensitive phenolic compounds and to standardize water content, the collected leaves were dried at a constant temperature of 40 °C. After drying, 20 mg of plant material from each species and sampling interval were finely ground and homogenized in a mortar with 2 mL of 80% ethanol. The suspension was incubated for 30 minutes in a water bath at 80 °C to facilitate the extraction of phenolic compounds. After centrifugation at 15,000 × g (Sigma 3K30) for 15 minutes, the resulting supernatant was collected and aliquoted into three replicates for each species.

For each replicate, 2.5 mL of Folin-Ciocalteu reagent were added to the supernatant, followed by a 3-minute preliminary reaction at room temperature. Then, 2 mL of 7.5% sodium carbonate solution were added to initiate the phenolic–reagent reaction under alkaline conditions. The samples were incubated at room temperature for 2 hours to allow the formation of blue complexes, characteristic of phenolic compounds.

The formed blue complexes were measured using a spectrophotometer (model СФ-46) at a wavelength of 765 nm. The phenolic compound content was calculated based on a calibration curve prepared with a gallic acid standard.

Leaf water content

The water content in leaves was determined by initially weighing the leaves, followed by dehydration in an oven (Thermostat No. 3) at 105°C until a constant dry weight was reached. The stability of the dry weight was confirmed through repeated weighings, considered constant after three consecutive measurements. To prevent moisture reabsorption, the dried leaves were placed in a desiccator with silica gel, connected to a vacuum pump that maintained a sub-atmospheric pressure. After returning to room temperature, the leaves were weighed using a precision balance (Kern 3100). As described by Barrs and Weatherley (1962), the water content in the leaves (Ca, %) was calculated based on the fresh weight (Mu) and dry weight (Ms) using the following formula:

$$Ca = \frac{Mu - Ms}{Mu} \times 100\% \qquad (2)$$

2.3 Statistical Methods

The Mann-Whitney test was applied to assess interspecific differences in the median values of physiological and biochemical parameters during the ten-day post-thermal shock recovery period. On the graphs, for each recovery interval, the standard deviations of the mean values of the analyzed physiological and biochemical parameters were presented to illustrate intraspecific variability and support the interpretation of statistical differences.

3 Results

3.1 Chlorophyll amount

During the ten-day incubation period of *Fagus sylvatica* leaves under favorable artificial conditions, the chlorophyll amount, particularly chlorophyll *b*, increased significantly, exceeding the initial values by 13.7%. This result indicates that, under optimized experimental conditions, the recovery of the processes responsible for maintaining chlorophyll amount in the leaves occurs, compensating for the deterioration induced by heat stress under natural conditions. In the case of *Quercus petraea*, chlorophyll *a* amount increased during the first three days of incubation and then remained relatively constant, reaching slightly higher values by the end of the experimental period. In contrast, chlorophyll *b* amount fluctuated throughout the incubation period, suggesting a slower and more variable recovery process (Figure 3). Thus, compared to *F. sylvatica*, *Q. petraea* exhibited a significantly lower and less stable capacity to recover chlorophyll amount in the leaves (Table 5).

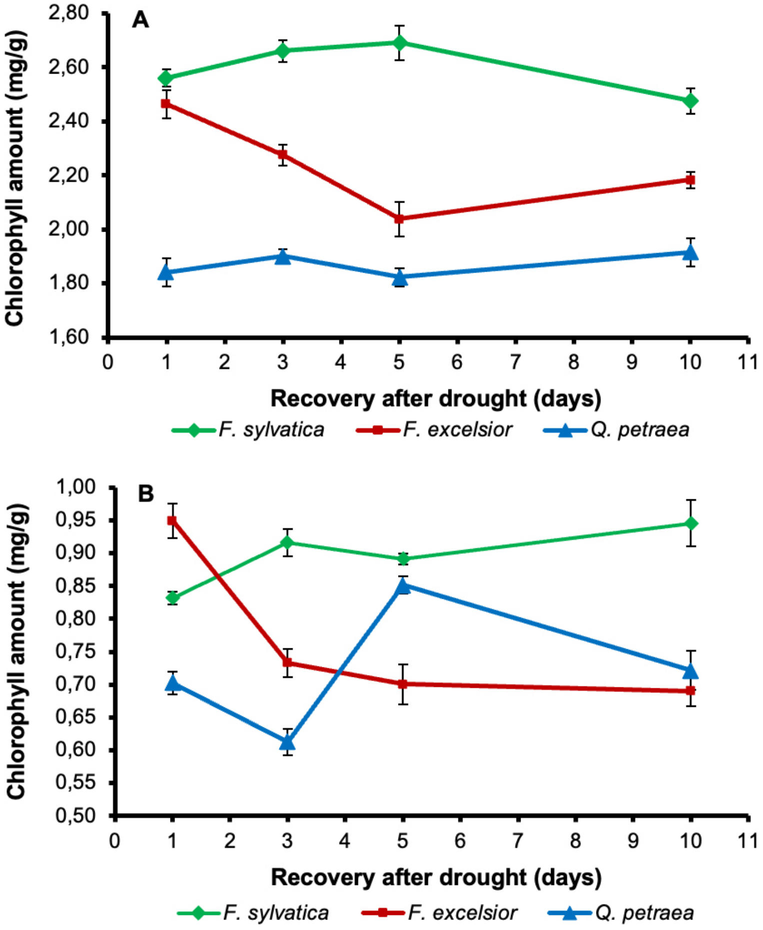
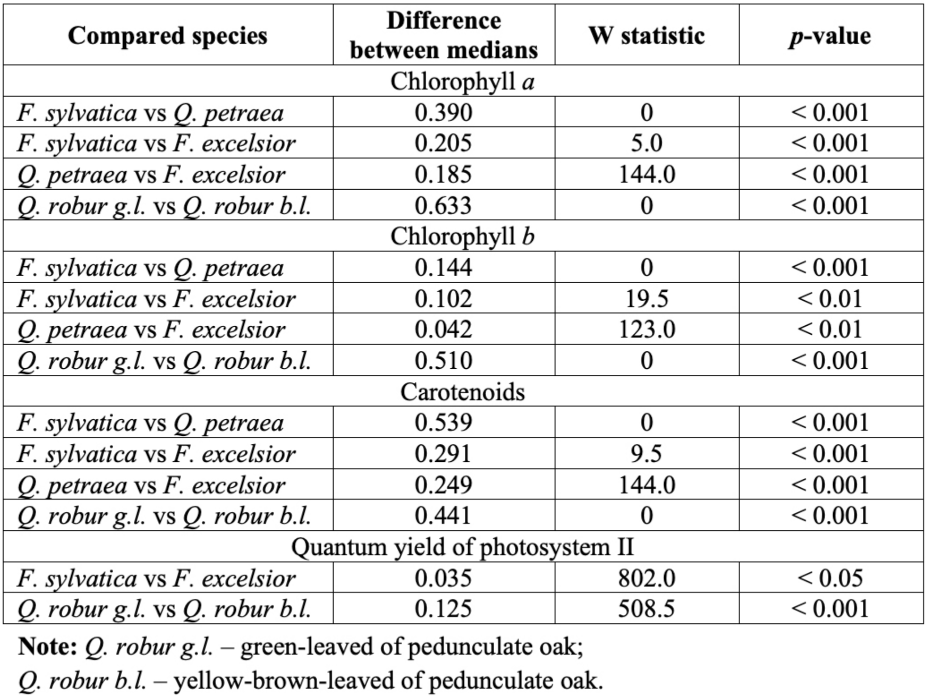


Figure 3: Dynamics of chlorophyll recovery in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period: A – chlorophyll a; B – chlorophyll b. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 3: Dynamik der Chlorophyllwiederherstellung bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Erholungsphase nach dem Stress: A – Chlorophyll *a*; B – Chlorophyll *b*. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

Table 5: Significant differences between species in terms of photosynthetic pigments and the quantum yield of photosystem II, as determined by the Mann-Whitney test.

Tabelle 5: Signifikante Unterschiede zwischen den Arten hinsichtlich der photosynthetischen Pigmente und der Quantenausbeute des Photosystems II, ermittelt mit dem Mann-Whitney-Test.



Compared species	**Difference between medians**	**W statistic**	***p*-value**
Chlorophyll *a*			
F. sylvatica vs *Q. petraea*	0.390	0	< 0.001
F. sylvatica vs *F. excelsior*	0.205	5.0	< 0.001
Q. petraea vs *F. excelsior*	0.185	144.0	< 0.001
Q. robur g.l. vs *Q. robur b.l.*	0.633	0	< 0.001
Chlorophyll *b*			
F. sylvatica vs *Q. petraea*	0.144	0	< 0.001
F. sylvatica vs *F. excelsior*	0.102	19.5	< 0.01
Q. petraea vs *F. excelsior*	0.042	123.0	< 0.01
Q. robur g.l. vs *Q. robur b.l.*	0.510	0	< 0.001
Carotenoids			
F. sylvatica vs *Q. petraea*	0.539	0	< 0.001
F. sylvatica vs *F. excelsior*	0.291	9.5	< 0.001
Q. petraea vs *F. excelsior*	0.249	144.0	< 0.001
Q. robur g.l. vs *Q. robur b.l.*	0.441	0	< 0.001
Quantum yield of photosystem II			
F. sylvatica vs *F. excelsior*	0.035	802.0	< 0.05
Q. robur g.l. vs *Q. robur b.l.*	0.125	508.5	< 0.001

Note: *Q. robur g.l.* – green-leaved of pedunculate oak;
Q. robur b.l. – yellow-brown-leaved of pedunculate oak.

In *Fraxinus excelsior*, chlorophyll *a* amount decreased rapidly during the first five days of artificial incubation of the leaves, and thereafter the recovery processes were slow, with chlorophyll values not returning to the initial levels. It is evident that, in the incubated leaves, the recovery of the processes responsible for maintaining chlorophyll amount was incomplete (Figure 3). These results suggest a lower tolerance of *F. excelsior* to high temperatures and drought compared to the other studied species.

Clear differences were observed in the recovery process between *Q. robur* with green leaves and that with yellow-brown leaves. In the green-leaved oak, the initial increase in chlorophyll *a* and *b* amount up to the third day of incubation suggests the occurrence of recovery processes. Subsequently, chlorophyll *b* amount showed a slight decrease (Figure 4). However, by the tenth day of observation, chlorophyll *b* amount had surpassed its initial value by 9.7%, indicating complete recovery of this physiological parameter in the leaves.

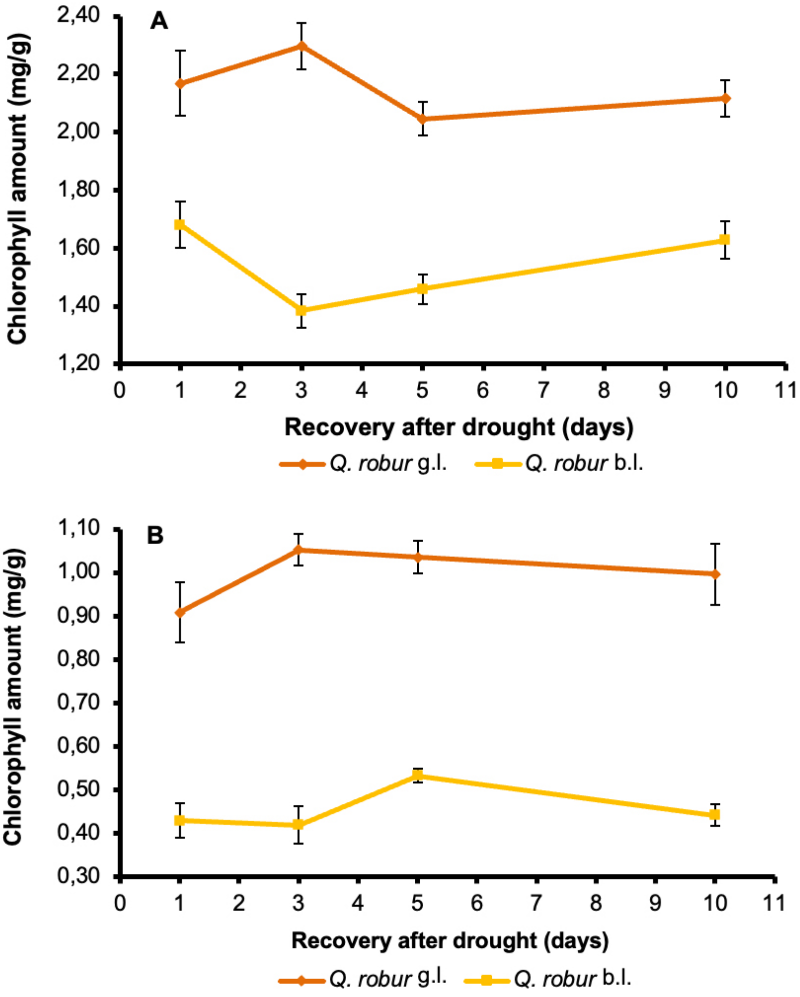


Figure 4: Dynamics of chlorophyll recovery in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period: A – chlorophyll a; B – chlorophyll b. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 4: Dynamik der Chlorophyllwiederherstellung bei *Quercus robur* mit grünen und gelb-braunen Blättern während der zehntägigen Erholungsphase nach dem Stress: A – Chlorophyll *a*; B – Chlorophyll *b*. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

In the case of *Q. robur* with yellow-brown leaves, the chlorophyll amount during recovery was significantly lower compared to that of the green-leaved variant ($p < 0.001$) (Table 5), indicating a pronounced degradation of photosynthetic pigments. On the first day of leaf incubation, the chlorophyll *a* amount was reduced and continued to decrease until the third day, while chlorophyll *b* remained relatively constant, with limited variation during the incubation period. By the end of the observation period, chlorophyll *a* showed moderate recovery, but at a level lower than on the first day of observation. Nevertheless, this process was slower and incomplete compared to the green-leaved (Figure 4). Thus, *Q. robur* with yellow-brown leaves exhibits a slower and less efficient response to natural heat stress.

3.2 Carotenoid amount

In the analyzed species, the carotenoid amount varied throughout the ten-day recovery period in leaves maintained under controlled laboratory conditions. In *F. sylvatica*, the carotenoid amount was initially high and showed a slight increase by the third day, followed by a moderate decline. Nevertheless, it remained higher than those observed in the other species. This trend suggests a higher physiological capacity to cope with natural heat stress and a sustained antioxidant potential during recovery. In contrast, *Q. petraea* consistently exhibited a lower carotenoid amount with minimal variation, indicating reduced tolerance to thermal stress and a limited recovery capacity (Figure 5). The significant differences between *F. sylvatica* and *Q. petraea* confirm this divergent physiological response ($p < 0.001$; Table 5).

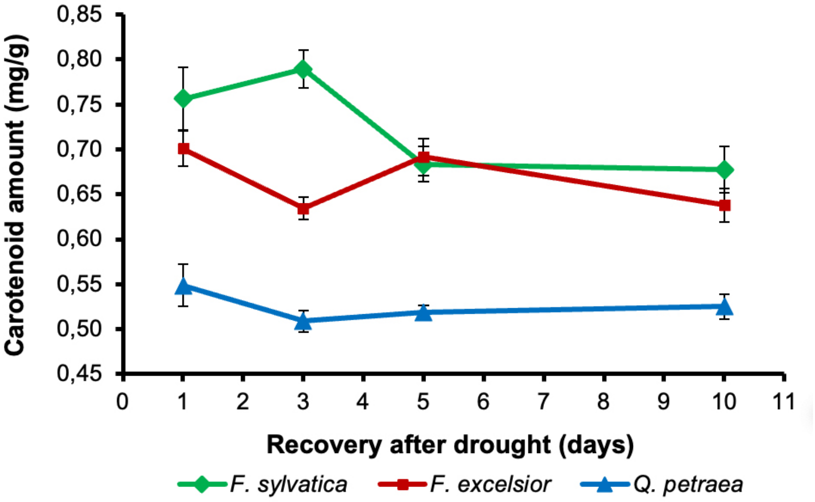


Figure 5: Dynamics of carotenoid recovery in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 5: Dynamik der Carotinoidrückgewinnung bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

In *F. excelsior*, the carotenoid amount was lower on the first day compared to *F. sylvatica*, showing a significant decline by the third day, followed by partial recovery and then another decrease. This fluctuating pattern suggests incomplete recovery and lower physiological stability than in *F. sylvatica* (Figure 5). Nevertheless, the values were generally higher than those recorded in *Q. petraea*, indicating an intermediate tolerance to natural heat stress (Table 5).

In *Q. robur* with green leaves, the initial carotenoid amount (0.538 mg/g) was higher than that of the yellow-brown leaves (0.489 mg/g). During the observation period, in *Q. robur* with green leaves, the carotenoid amount gradually decreased until the fifth day, followed by an increase towards the end of the observation period, indicating a partial recovery of this pigment. In contrast, in *Q. robur* with yellow-brown leaves, the carotenoid amount decreased rapidly during the first three days, and then fluctuated, suggesting an unstable recovery and a slower response after natural thermal stress (Figure 6). The significant differences between the green-leaved tree and the yellow-brown-leaved one ($p < 0.001$; Table 5) confirm an accentuated degradation of the pigment in the leaves affected by heat stress, indicating a reduced recovery capacity in the tree with yellow-brown leaves.

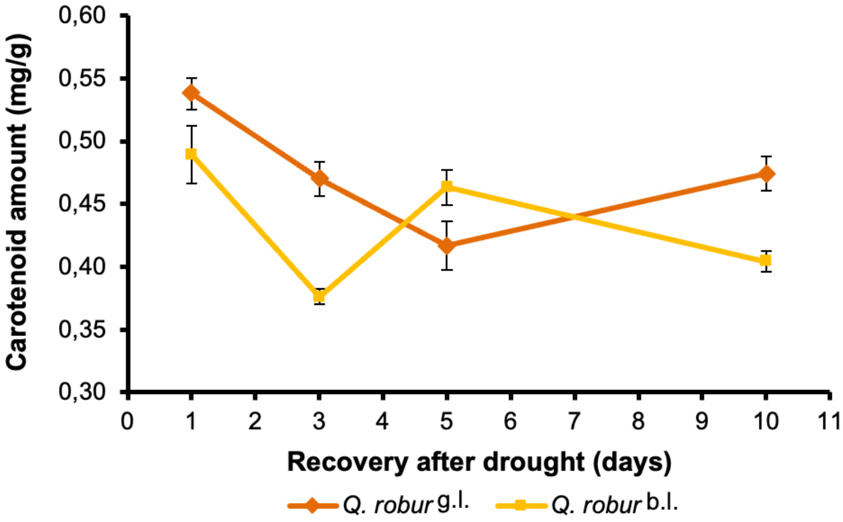


Figure 6: Dynamics of carotenoid recovery in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 6: Dynamik der Carotinoidrückgewinnung bei *Quercus robur* mit grünen und gelb-braunen Blättern während der zehntägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

3.3. Effective quantum yield of photosystem II

In leaves of *F. sylvatica* incubated under controlled conditions, a slight increase in the effective quantum yield of photosystem II was observed until day seven, followed by an intensification of the recovery process until the end of the observation period. In contrast, in leaves of *Q. petraea*, the quantum yield began to decrease moderately from the first day, maintaining this downward trend until day five, when it reached a minimum, followed by the initiation of slow recovery processes (Figure 7). However, compared to initial values, the functional recovery of photosystem II was incomplete.

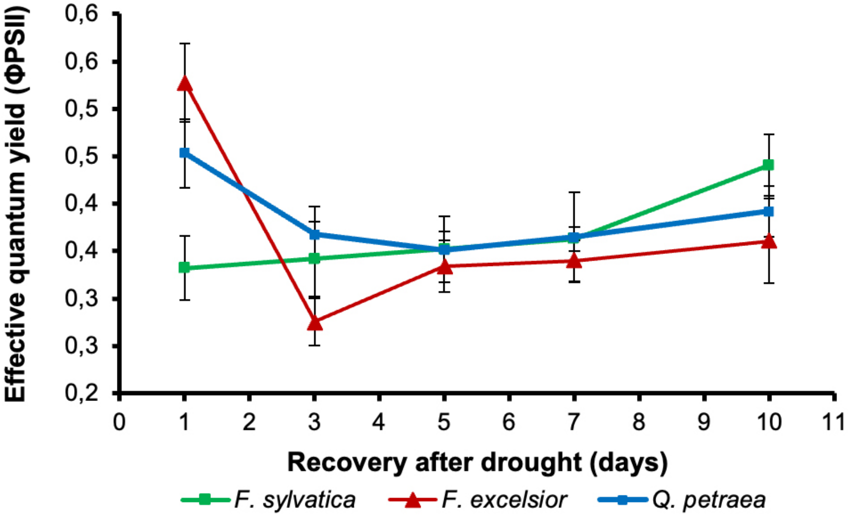


Figure 7: Dynamics of effective quantum yield recovery of photosystem II in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 10 excised leaves per species and recovery time point.

Abbildung 7: Dynamik der Wiederherstellung der effektiven Quantenausbeute von Photosystem II bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Erholungsphase nach Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 10 exzidierte Blätter pro Art und Erholungszeitpunkt.

A rapid decrease in quantum yield was observed in *F. excelsior*, reaching a minimum on day three, after which recovery processes were initiated. Nevertheless, the recovery remained partial, suggesting a limited capacity to restore the quantum yield following natural heat stress. The difference compared to *F. sylvatica* was significant over the ten-day recovery period (Table 5, $p < 0.05$).

For *Q. robur* with green and yellow-brown leaves, the quantum yield initially exhibited similar values. However, photosynthetic processes evolved differently in these trees. In *Q. robur* with green leaves, the quantum yield declined until day three, after which recovery processes dominated until the end of the observation period. In contrast, in *Q. robur* with yellow-brown leaves, the quantum yield continued to decrease until the tenth day of the study, suggesting a low tolerance to heat stress (Figure 8).

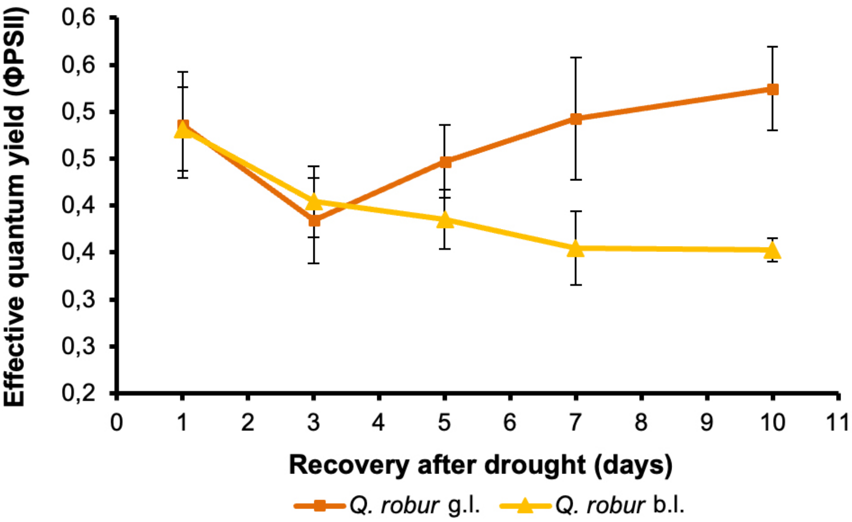


Figure 8: Dynamics of effective quantum yield recovery of photosystem II in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 10 excised leaves per species and recovery time point.

Abbildung 8: Dynamik der Wiederherstellung der effektiven Quantenausbeute von Photosystem II bei *Quercus robur* mit grünen und gelb-braunen Blättern während der zehntägigen Erholungsphase nach Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 10 exzidierte Blätter pro Art und Erholungszeitpunkt.

3.4 Catalase activity

The studied species exhibited distinct patterns in catalase activity during the ten-day incubation of leaves under controlled conditions. In *F. sylvatica*, catalase activity was significantly lower after the first day compared to *Q. petraea* and *F. excelsior*, suggesting a slower response to natural heat stress. By the fifth day, the catalase activity continued to decline rapidly, reaching a minimum on the tenth day. This trend indicates an incomplete recovery process and a reduced capacity of *F. sylvatica* to maintain antioxidant functions under prolonged thermal stress (Figure 9).

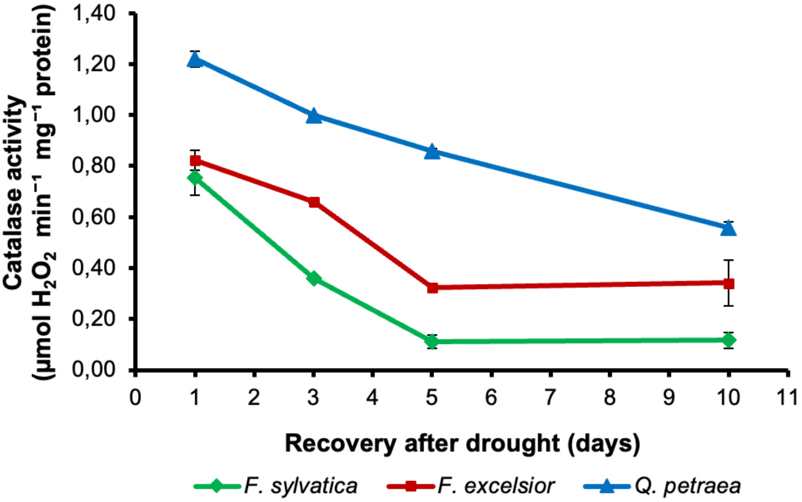


Figure 9: Dynamics of catalase recovery in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 9: Dynamik der Katalase-Erholung bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Post-Stress-Periode. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

In contrast, *Q. petraea* displayed catalase activity 1.6 times higher than *F. sylvatica* on the first day. Throughout the observation period, the decline in activity was slower than that observed in *F. sylvatica*, and by the tenth day, activity levels remained significantly higher. The Mann-Whitney test revealed statistically significant differences in catalase activity between *F. sylvatica* and *Q. petraea* (Table 6), suggesting that *Q. petraea* possesses a more effective defense mechanism against natural thermal stress by better sustaining its antioxidant functions.

Table 6: Significant differences between species in the activity of antioxidant substances, as determined by the Mann-Whitney test.

Tabelle 6: Signifikante Unterschiede zwischen den Arten in der Aktivität antioxidativer Substanzen, bestimmt mit dem Mann-Whitney-Test.

Compared species	**Difference between medians**	**W statistic**	***p*-value**
Catalase activity			
F. sylvatica vs *Q. petraea*	0.214	144	< 0.001
Q. petraea vs *F. excelsior*	0.241	0	< 0.001
Total phenolic compounds activity			
F. sylvatica vs *Q. petraea*	0.313	115	< 0.05
F. sylvatica vs *F. excelsior*	0.497	3	< 0.001
Q. petraea vs *F. excelsior*	0.810	1	< 0.001

In *F. excelsior*, catalase activity was lower than that measured in *Q. petraea*, but on the first day of recovery, it was slightly higher than in *F. sylvatica*. Throughout the experiment, the decline in enzymatic activity was slower than in *F. sylvatica*, and by the tenth day, its level was 0.23 µmol $H_2O_2 \cdot min^{-1} \cdot mg^{-1}$ protein higher (Figure 9). These observations suggest a more efficient recovery in *F. excelsior* compared to *F. sylvatica* in the dynamics of the response to natural heat stress between the two species.

In *Q. robur* leaves with green foliage, catalase activity after the first day of controlled incubation was 1.8 times higher than in those with yellow-brown leaves. By the fifth day, catalase activity decreased rapidly, and in the following period, it remained relatively constant. It is worth noting that, after ten days, the recovery processes of catalase activity in the green-leaved *Q. robur* remained high, similar to the levels observed in the yellow-brown leaves after the first day of observation (Figure 10). These results indicate that in *Q. robur* with green leaves, recovery was more complete compared to those with yellow-brown leaves, suggesting a more efficient antioxidant response and enhanced tolerance to natural heat stress.

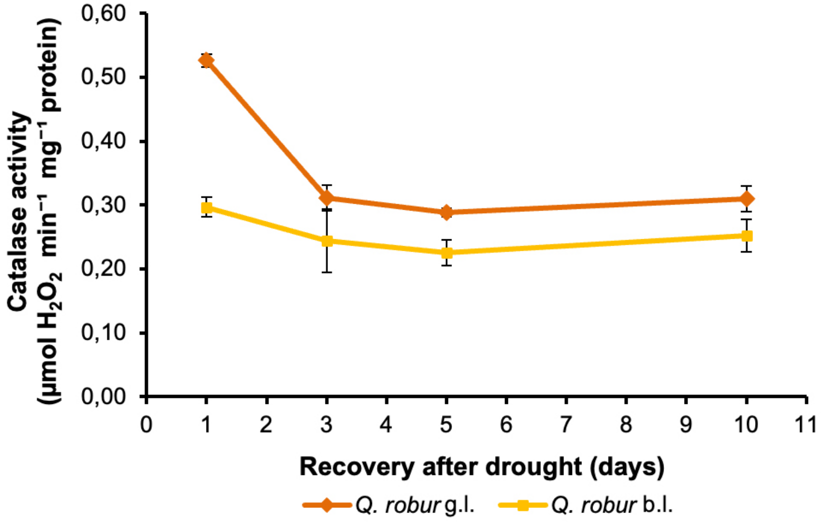


Figure 10: Dynamics of catalase recovery in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 10: Dynamik der Katalase-Erholung bei *Quercus robur* mit grünen und gelbbraunen Blättern während der zehntägigen Post-Stress-Periode. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

3.5 Total Phenolic compounds content

Following exposure to natural thermal shock, the total content of phenolic compounds in the leaf extracts of *F. sylvatica* remained relatively constant throughout the observation period. After ten days, the values were slightly lower compared to the first day, and fluctuations were minimal, suggesting a stable recovery of these metabolites in the leaves. In *F. excelsior*, the total phenolic content on the first day was slightly lower than that observed in *F. sylvatica*, and in the following days, the values gradually declined, with no signs of recovery by the end of the experiment (Figure 11). The results are supported by the Mann-Whitney test, which revealed significant differences between the two species (Table 6).

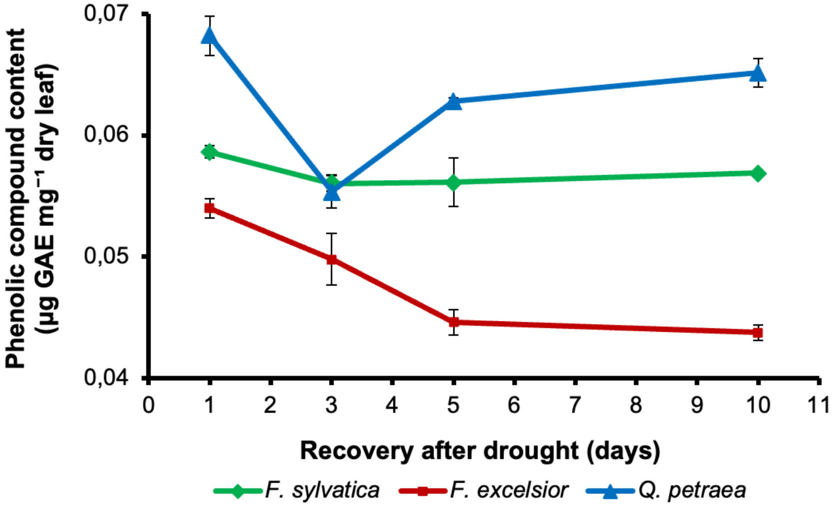


Figure 11: Dynamics of phenolic compound recovery in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 11: Dynamik der Wiederherstellung phenolischer Verbindungen bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

In *Q. petraea*, the initial content of phenolic compounds was 26.4% higher than that recorded in *F. excelsior*. After a decline observed by day three, signs of recovery became evident, with values gradually increasing until day ten, when they approached the initial level.

Differences in the dynamics of phenolic compounds were observed between the *Q. robur* tree bearing green leaves and the one with yellow-brown foliage. In the tree with green leaves, phenolic content was higher on the first day of recovery compared to the tree with yellow-brown leaves. Subsequently, values declined until day five, after which recovery processes became noticeable. In the tree with yellow-brown leaves, phenolic content remained relatively constant during the first three days, followed by a gradual increase until day seven. On day ten, phenolic levels were slightly higher than those observed in the green-leaved tree, suggesting a slower but sustained recovery (Figure 12).

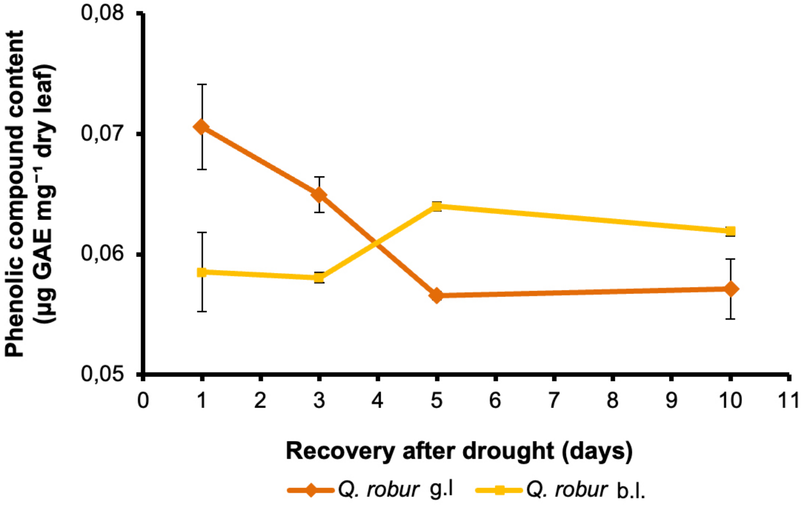


Figure 12: Dynamics of phenolic compound recovery in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 12: Dynamik der Wiederherstellung phenolischer Verbindungen bei *Quercus robur* mit grünen und gelb-braunen Blättern während der zehntägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

3.6 Leaf water content

According to the data presented in Figure 13, on the first day following natural thermal shock, leaf water content was highest in *F. excelsior* compared to F. *sylvatica* and *Q. petraea*. By the third day of observation, all three species exhibited an increase in leaf water content, suggesting an initial physiological response of adaptation. In the following days, F. excelsior continued to show a gradual increase, reaching by day twelve a value 21.0% higher than that recorded on the first day. This result indicates a greater capacity of this species to accumulate water in the leaves after exposure to heat waves and high temperatures.

In the other two species, *F. sylvatica* and *Q. petraea*, water content decreased slightly after day three, maintaining this downward trend until the end of the observation period (Figure 13). This pattern may indicate a lower capacity for long-term water accumulation in the leaves following exposure to natural thermal stress.

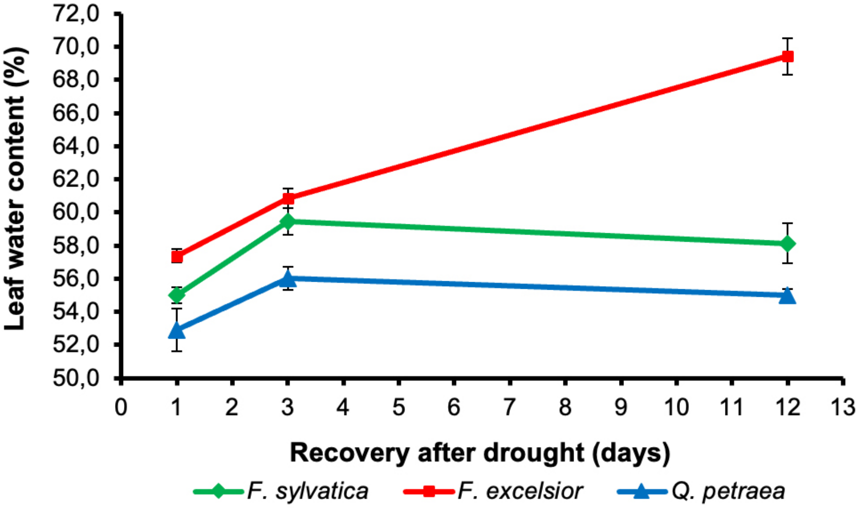


Figure 13: Dynamics of leaf water content recovery in Fagus sylvatica, Fraxinus excelsior, and Quercus petraea during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 13: Dynamik der Wiederherstellung des Blattwassergehalts bei *Fagus sylvatica*, *Fraxinus excelsior* und *Quercus petraea* während der zehntägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

In the *Q. robur* tree with green leaves, the initial leaf water content was 4.2% lower compared to the tree with yellow-brown foliage. By day tenth, water content had increased in both cases; however, in the tree with green leaves, it was 1.9% higher than in the yellow-brown leaves (Figure 14). These results suggest a differentiated dynamic of water accumulation in the leaves, influenced by their physiological state, indicating a variable response capacity to natural thermal stress between the green- and yellow-brown-leaved individuals.

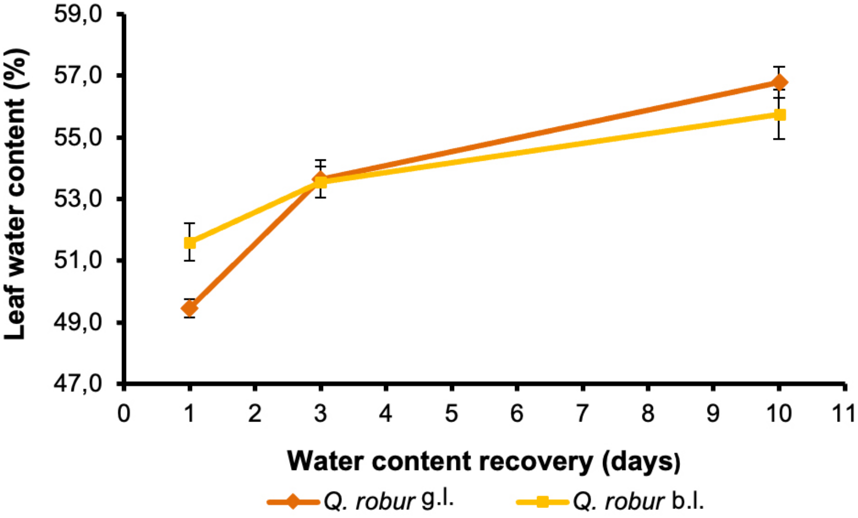


Figure 14: Dynamics of leaf water content recovery in Quercus robur with green and yellow-brown leaves during the ten-day post-stress period. Error bars represent standard deviation (SD); n = 6 technical replicates per species and recovery time point.

Abbildung 14: Dynamik der Wiederherstellung des Blattwassergehalts bei Quercus robur mit grünen und gelb-braunen Blättern während der zehn-tägigen Erholungsphase nach dem Stress. Die Fehlerbalken stellen die Standardabweichung (SD) dar; n = 6 technische Replikate pro Art und Erholungszeitpunkt.

4 Discussion

4.1. Response of photosynthetic pigments and effective quantum yield of photosystem II to heat stress

Forest species of high ecological and economic significance, such as *Quercus* spp. and *Fagus* spp., exhibit differential responses to elevated temperatures due to their specific bioecological traits (Dass *et al.* 2008; Lovreškov *et al.* 2022). The results of this study indicate that recovery processes in leaves incubated under controlled conditions following exposure to natural heat stress differed significantly among *Fagus sylvatica*, *Quercus petraea*, and *Fraxinus excelsior*, species characteristic of mixed forests dominated by *Q. petraea*, *F. sylvatica*, and *Tilia tomentosa*, with *Carex brevicollis* (Doniță *et al.* 2007). Significant differences were observed in the amounts of chlorophyll *a* and *b*, carotenoids, and in the effective quantum yield of photosystem II. During the incubation period, *F. sylvatica* maintained higher amounts of photosynthetic pigments and exhibited greater quantum yield compared to *Q. petraea* and *F. excelsior*, indicating a superior recovery capacity and enhanced tolerance to elevated temperatures. *Q. petraea* also demonstrated notable heat tolerance, though to a lesser extent than *F.*

sylvatica, with moderate fluctuations in the amount of chlorophyll *b*. In contrast, *F. excelsior* exhibited a more pronounced sensitivity to thermal stress, with more substantial reductions in photosynthetic parameters.

The literature indicates that the optimal temperature for photosynthetic processes ranges between 20-30°C, and can even reach up to 35°C, within which high photosynthetic rates are maintained (Teskey *et al.* 2015). In *Q. robur*, *Q. petraea*, and *Q. pubescens*, chlorophyll fluorescence recovers completely after thermal shock up to 49°C, suggesting a high thermal tolerance of the leaves of these species (Dascaliuc & Cuza 2011). However, surpassing a critical physiological threshold leads to a sharp reduction in photosynthetic efficiency, even in preadapted species (Song *et al.* 2014; Dascaliuc *et al.* 2022).

Kunert and Hajek (2022) observed that *Q. robur* and *Q. petraea* have a superior capacity to maintain the functionality of photosystem II under extreme temperatures compared to *F. sylvatica* and *F. excelsior*, suggesting a higher sensitivity in the latter species. Thermal tolerance is also associated with shade tolerance, with heliophilous species such as *Quercus* spp. being more resistant to heat waves, thus strengthening their role in climate-resilient forests. Similarly, Dass *et al.* (2008) demonstrated that *Q. robur* and *Q. petraea* respond to high temperatures by increasing critical temperatures measured through chlorophyll fluorescence, suggesting a common adaptation, but with limitations.

Our results indicate superior thermal tolerance in *Q. robur* compared to *Q. petraea*, as evidenced by the maintenance of effective quantum yield and high chlorophyll amount following natural thermal shock. However, this difference may be influenced by habitat variations in the *Plaiul Fagului* reserve, where the two species occupy distinct ecological niches. Our observations are complementary to those reported by Dreyer *et al.* (2001), who identified high photosynthetic efficiency in both species under controlled conditions. Additionally, the authors found that the critical temperature at which chlorophyll fluorescence increases sharply is similar (≈47°C) for both species, suggesting a comparable baseline tolerance. However, the differences observed in our study reflect a better response and recovery capacity post-stress in *Q. robur*. These findings highlight the importance of integrating local ecological variability into eco-physiological models applied to mixed forests, where trees of different species may exhibit distinct adaptive strategies to thermal stresses.

Following our analysis, we observed that thermal tolerance variability is not only evident between species but also among individuals of the same species, influenced by the physiological condition of the leaves. At *Q. robur*, the tree with healthy green leaves showed significantly higher thermal stress tolerance, reflected by a higher content of photosynthetic pigments compared to the tree whose leaves exhibited yellow-brown discoloration, indicating chlorophyll degradation. These differences suggest an impairment of photosynthetic function as a response to heat waves and prolonged extreme temperatures.

This finding is supported by the results of Lovreškov *et al.* (2022), who demonstrated in *Q. pubescens* and *Q. ilex* that defoliated oak trees (defoliation > 25%) exhibited significantly lower chlorophyll content compared to trees with lower defoliation (≤ 25%). These results align with our findings regarding *Q. robur*, highlighting that the physiological condition of the leaves strongly influences the tree's response to thermal stress as well as its subsequent recovery capacity.

Similarly, Húdoková *et al.* (2022) showed that light-dependent photosynthetic reactions are sensitive to supraoptimal temperatures. An evaluation of five species, including *F. sylvatica* and *Q. petraea*, revealed, through the OKJIP parameters, superior thermostability of photosystem II in broadleaf trees compared to conifers. *F. sylvatica* exhibited a smaller decrease in photosystem II maintenance compared to conifer species, while *Q. petraea* was found to be the most heat-tolerant, improving photosynthetic performance after thermal stress events. These results differ from our observations, which showed that, after ten days of recovery, the effective quantum yield of photosystem II was higher in *F. sylvatica* compared to *Q. petraea*.

4.2 Response of catalase, phenolic compounds, and leaf water content to thermal stress

Increased catalase activity and the accumulation of phenolic compounds are essential mechanisms through which trees protect their cells from oxidative damage caused by reactive oxygen species generated under thermal stress conditions (Visiné-Rajczia *et al.* 2023; Lovreškov *et al.* 2022). Research has shown that after incubating the leaves under controlled conditions, *Q. petraea* maintained a significantly higher level of catalase activity and phenolic compounds compared to the other species analyzed. Moreover, the recovery processes were more complete, suggesting a superior ability to adapt to thermal stress and, consequently, a higher tolerance to extreme conditions.

In *F. excelsior*, a more pronounced recovery of catalase activity was observed, while in *F. sylvatica*, phenolic compounds exhibited a more stable and uniform recovery response. These differences reflect the existence of distinct physiological strategies between species, based on the differential activation of enzymatic and non-enzymatic antioxidant systems under stress conditions. Thus, depending on the dynamics of these recovery processes, *Q. petraea* can be considered a species with high tolerance to extreme temperatures, while *F. excelsior* and *F. sylvatica* show a reduced capacity for physiological adaptation to stress.

Although research on post-thermal stress recovery processes in the leaves of the studied species is still limited, the literature provides some references for other oak species, particularly from the Mediterranean region. For example, following natural thermal stress, catalase activity showed significant variations between defoliated and non-defoliated trees across all species analyzed, except for *Q. ilex*. Increased catalase activity was recorded in leaf extracts from defoliated *Q. ilex* and *Pinus halepensis*, while the opposite trend was observed in *Q. pubescens* and *P. nigra* (Lovreškov *et al.* 2022). The authors suggest that the physiological response to thermal stress depends on each species' specific adaptive strategy and reflects varying degrees of functional plasticity in response to extreme conditions.

In the Republic of Moldova, *F. sylvatica* is a rare species with a limited distribution, increasingly exposed to heat waves and droughts. Under the conditions of the Plaiul Fagului Reserve, the species exhibited superior recovery of photosynthetic pigments and a high effective quantum yield after thermal stress, suggesting the maintenance of efficient photosynthetic functioning despite unfavorable conditions. However, compared to *Q. petraea*, the lower catalase activity indicates a physiological vulnerability to oxidative stress, despite a better ability to retain water in the leaves. These traits suggest the adoption of a conservative stress-tolerant strategy, focused on the efficient use of resources and maintaining essential metabolic functions, which may provide an adaptive advantage in the in situ conservation of this species within a protected forest ecosystem (Grime, 1977).

In extending our studies, Isah (2019) observed a significant increase in the production of secondary metabolites, including total phenolic compounds, in *F. sylvatica* under heat wave and drought conditions, as well as following cold stress, which is frequently associated with a decrease in biomass. These findings suggest a trade-off between antioxidant responses and the growth capacity of trees. Additionally, research by Visi-Rajczi *et al.* (2001) showed that *F. sylvatica* trees of different provenances may experience more intense stress under high temperatures and drought, mobilizing additional antioxidant resources to cope with the stress. Such biochemical responses are influenced by the genetic adaptation of the provenances and can be reflected in polyphenol concentrations and peroxidase activity, which are relevant indicators of acclimatization capacity. More recently, Visiné-Rajczia *et al.* (2023) correlated the polyphenolic composition of *F. sylvatica* leaves with the average stem diameter and associated climatic indicators, which are useful for predicting the effects of climate change on growth and the selection of breeding material for this species.

The interspecific differences presented indicate distinct physiological strategies for adaptation to thermal stress, as well as a strong link to water use efficiency, an essential mechanism for tree survival under drought conditions. Tolerance to high temperatures and water deficit are key adaptation mechanisms for plants in response to extreme climatic conditions (Fang & Xiong 2015). The maintenance of adequate water content in tissues is regulated through morphological adjustments and physiological mechanisms, such as water use efficiency (Farooq *et al.* 2009; Kooyers 2015). Our research indicates that the analyzed species exhibited differentiated strategies for maintaining water in leaves during the ten-day post-thermal shock recovery period. *F. excelsior* demonstrated the highest capacity for water retention by the end of the experimental period, while *Q. petraea* and *Q. robur* recorded lower levels. These interspecific differences suggest distinct physiological strategies for thermal stress adaptation, with significant implications for species selection based on environmental conditions in afforestation processes.

The scientific literature emphasizes that forest species' tolerance to thermal stress and drought, through enhanced water use efficiency, represents a key objective of sustainable and climate-smart forestry (Flexas *et al.* 2013). In this context, Stojnić *et al.* (2019) demonstrated that stomatal density and leaf dry mass per unit area directly influence intrinsic water use efficiency in *Q. robur*. Stomatal density showed the highest rate on the first principal component, where water use efficiency had the dominant weight, suggesting that stomatal regulation plays an essential role in controlling water use efficiency under moderate drought conditions.

Scientific data suggest that trees with higher water use efficiency adopt strategies that minimize water loss and/or allow for higher assimilation rates compared to those with lower efficiency. These traits can influence the species' competitiveness in terms of growth and survival, especially in regions prone to drought, where water availability plays a critical role in the competitive dynamics between species (Mészáros *et al.* 2007; Brendel *et al.* 2008).

4.3 Comparative assessment of species tolerance

Following the ten-day incubation of leaves from *F. sylvatica*, *Q. petraea*, and *F. excelsior* under controlled experimental conditions to evaluate recovery activities after natural thermal stress, the comparative analysis of physiological (chlorophyll *a* and *b*, carotenoids, and effective quantum yield of photosystem II) and biochemical (catalase activity, total phenolic compounds, and water content) traits indicated significant differences among the three studied species.

Fagus sylvatica exhibited the highest post-stress functional recovery capacity, as evidenced by a steady increase in photosynthetic pigment amount and a gradual yet continuous rise in the effective quantum yield of photosystem II, suggesting efficient restoration of photosynthetic performance. Although its antioxidant response, particularly catalase activity, was lower, beech maintained a relatively stable level of phenolic compounds, indicating a conservative yet effective defensive strategy.

Quercus petraea recorded intermediate values for photosynthetic parameters (chlorophyll, carotenoids, and effective quantum yield), but exhibited high antioxidant activity, maintaining elevated levels of catalase and phenolic compounds. This suggests an enhanced capacity for cellular protection against oxidative stress. However, its photosynthetic recovery efficiency was lower compared to *F. sylvatica*.

Fraxinus excelsior showed the lowest overall performance. Although it demonstrated a higher rehydration capacity, as evidenced by a progressive increase in water content, it failed to maintain functional levels of photosynthetic pigments and antioxidant activity. The continuous decline in chlorophyll and phenolic compounds indicates increased vulnerability to the applied stress.

In general, the results demonstrate that *F. sylvatica* exhibits the highest overall tolerance to thermal stress, followed by *Q. petraea*, which compensates through efficient antioxidant mechanisms. *F. excelsior* proves to be the most sensitive species, with a limited capacity for functional recovery during the analyzed period.

In the experiment, *Q. robur* exhibited variation in the physiological condition of its leaves, showing both green and yellow-brown leaves. Based on the analyzed physiological and biochemical parameters, trees with green (healthy) leaves displayed significantly higher tolerance compared to those with yellow-brown (degraded chlorophyll) leaves.

Quercus robur exhibited slightly higher tolerance to thermal stress than *Q. petraea*, particularly through the maintenance of photosynthetic pigments and effective quantum yield. However, *Q. petraea* demonstrated more intense antioxidant activity, reflected by higher levels of catalase and phenolic compounds, suggesting an adaptive strategy focused more on cellular protection than on maintaining photosynthesis. These differences reflect distinct modes of adaptation to thermal stress, influenced by the bioecological characteristics of each species. It is worth noting that the oak species analyzed naturally develop under different ecological conditions, which, while limiting direct comparisons, allows for highlighting their specific adaptive strategies.

Despite the valuable insights provided, this study presents certain limitations related to the sampling design, particularly the use of a single tree per species. This methodological choice was made to minimize intra-specific genetic variability, which could obscure species-specific physiological responses to natural heat stress. However, this approach constrains the ability to generalize the findings to broader populations, as it does not account for within-species variation. Future research should include a larger number of individuals per species and develop appropriate methodological frameworks to disentangle the effects of local environmental conditions from physiological responses to thermal stress. Achieving this would enable a more comprehensive assessment of the physiological and genetic variability involved, thereby enhancing the ecological relevance and applicability of the results to wider habitat contexts.

5 Conclusions

The comparative analysis of physiological (photosynthetic pigments, effective quantum yield of photosystem II) and biochemical (catalase activity, phenolic compounds, water content) parameters revealed significant differences among the studied species, whose leaves were collected from a mixed forest of *Q. petraea*, *F. sylvatica*, and *T. tomentosa*, with *C. brevicollis*. These differences were observed in terms of recovery capacity and tolerance to natural thermal stress. *F. sylvatica* demonstrated superior tolerance, maintaining high photosynthetic functionality and efficient rehydration capacity despite a more subdued activation of antioxidant systems. *Q. petraea* exhibited an intermediate response, characterized by moderate levels of pigmentation and effective quantum yield, which were consistently associated with the activation of antioxidant mechanisms, suggesting a compensatory strategy of cellular protection. In contrast, F. excelsior displayed the weakest recovery processes, with reduced values for both physiological and biochemical parameters, despite progressive rehydration, indicating a lower tolerance to thermal stress. Additionally, the individual physiological state of trees in a mixed stand of *Q. robur* and *C. betulus*, with *R. caesius*, was evaluated. Leaves were collected from two *Q. robur* trees: one with green, seemingly healthy foliage and the other with yellow-brown leaves showing visible signs of chlorophyll degradation. The analyzed parameters indicated more efficient physiological recovery in the tree with green foliage, suggesting significantly higher tolerance to natural thermal stress compared to the affected specimen.

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