

Article

Assessing Physiological Performances of *Quercus suber* L. After Cork Stripping and Kaolin Application

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Abstract

Cork oak (*Quercus suber* L.) forests play a crucial role in the Mediterranean region, providing essential ecological, social, and economic services. Increasing pressure from wildfires, pests, diseases, and climate change has led to a progressive decline of these ecosystems, making the development of innovative post-stripping management strategies urgent. This study evaluates the effectiveness of kaolin application on cork oak trees immediately after cork removal in a mixed forest in Sant Celoni (Barcelona, Spain). Short- and long-term physiological responses were assessed through stomatal conductance and chlorophyll fluorescence (OJIP test), while sap flux density (J_s) was continuously monitored over a four-month period (July–October 2023) using IoT-based TreeTalker® Cyber (Nature 4.0 s.r.l., Viterbo, Italy). Proximal vegetation indices (Normalized Difference Vegetation Index, NDVI; and Normalized Difference Red Edge, NDRE) were also evaluated but showed no significant differences among treatments ($p > 0.05$). Kaolin-treated trees (K) maintained significantly higher photosynthetic performance and stem water transport capacity compared to untreated stripped trees (nK), with effects persisting up to 140 days after stripping. These findings support kaolin application as a viable and low-cost tool for mitigating post-stripping physiological stress in cork oak forest management. Further research across multiple sites and consecutive harvesting cycles is recommended to fully assess its long-term implications for tree vitality and cork productivity.

Keywords: cork oak; kaolin; cork stripping; chlorophyll fluorescence; sap flux density; photosynthetic performance; Mediterranean forests; IoT monitoring



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1. Introduction

1.1. Cork Oak Forests: Distribution and Ecological Role

Cork oak (*Quercus suber* L.) forests, also known as woodlands, play a crucial role in the Mediterranean basin by providing essential ecological, environmental, and socio-economic services. Globally, these ecosystems cover approximately 2.2 million hectares, with a distribution primarily concentrated in the Mediterranean region [1,2]. Portugal (0.74 million ha) and Spain (0.6 million ha), which together account for over 50% of the global area and over 80% of global cork extraction. The remaining woodland is distributed

among other Mediterranean countries, including Morocco, Italy, Tunisia, Algeria, and France [3]. In these regions, the warm climate and ample sunshine create the ideal conditions for *Quercus suber* to thrive. The cork oak tree is the primary source of cork, a versatile and sustainable material that has been used for centuries in various industries.

1.2. Cork Stripping: Practice and Physiological Impact

Cork harvesting (cork stripping), a traditional practice in this region, involves skilled workers carefully removing the bark from mature trees every nine to fourteen years, depending on regional conditions and local regulations. The annual growth of cork oak trees typically occurs between spring and autumn, with the highest increments observed from June to August [4]. Stripping is performed from May to August, a period when the phellogen is physiologically active and the newly formed, thin-walled cork cells are swollen; these conditions facilitate the easy rupture and separation of the tissues from the inner bark [4,5]. The impact of stripping on tree physiology remains a subject of discussion, with conflicting perspectives regarding its magnitude: while early studies identified significant water loss from the exposed surface [6], more recent research suggests that trunk water loss is negligible when compared to total canopy transpiration [7]. Beyond these physiological concerns, the operation increases vulnerability to abiotic and biotic stress [4] and poses a risk of mechanical injury to underlying tissues. The cork oak area has continuously decreased over the past few decades in the Mediterranean region [6], driven by a combination of factors. These include wildfires [8], the impact of pests and diseases [9,10], poor or non-existent management [11], and intensifying summer droughts [12]. According to Leite et al. [13], the climate emergency represents one of the primary threats to the species, directly impacting its geographical distribution and potentially leading to a reduction in both tree diameter and cork growth in subsequent years [4]. These environmental pressures, particularly the synergy between water and temperature stresses, pose serious challenges to the natural regeneration of cork oak stands [14,15]. Beyond immediate physiological decline, the lack of recruitment combined with high adult mortality rates threatens the long-term sustainability of the entire ecosystem. Such degradation compromises both the quantity and the quality of cork production, making the development of innovative and adaptive management strategies an ecological and economic necessity.

1.3. Kaolin: Properties and Effects on Crop Physiology

One strategy to mitigate the effects caused by high temperature and water stress is the application of foliar reflective films, such as spraying with a kaolin-based aqueous suspension. Kaolin is a chemically inert, white aluminosilicate mineral $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ that, when sprayed on foliage, forms a protective powdery film. This coating increases leaf reflectance (albedo), thereby reducing leaf temperature and transpiration demand without impeding gas exchange. Applied directly to the exposed inner bark after cork stripping, kaolin may limit radiation-induced heating and evaporative water loss from the newly exposed tissues, potentially reducing thermal and drought stress during the critical phase of phellogen reconstruction. The physiological benefits of kaolin application have been documented across a wide range of horticultural and forestry species under Mediterranean and warm-climate conditions. In field-grown grapevine (*Vitis vinifera* L.) under summer drought, kaolin particle film reduced leaf temperature by up to 18% and increased predawn leaf water potential by up to 40.7%, while net photosynthesis and stomatal conductance increased by 58.7% and 28.4%, respectively, two months after application, alongside preserved chlorophyll content and reduced lipid peroxidation [16]. At the photochemical level, kaolin protected Photosystem II integrity in grapevine, resulting in significantly higher F_v/F_m values at midday under high irradiance and temperature, with improvements in

quantum yield, electron transport flux, and the overall PSII performance index (PI_{ABS}), particularly under the warmer and drier conditions [17]. In olive trees (*Olea europaea* L.) subjected to cyclic water deficit, kaolin reduced leaf sclerophylly, improved leaf relative water content and succulence, maintained higher stomatal conductance and net photosynthetic rate during drought periods, and reduced electrolyte leakage and investment in antioxidant defences, while also limiting nighttime water losses [18]. Under rainfed field conditions, kaolin further prevented chlorophyll degradation, reduced oxidative damage, and enhanced carbohydrate accumulation in stems, with gas exchange benefits persisting into autumn and winter months even after the particle film was no longer visible [19]. In apple trees (*Malus × domestica* Borkh.), kaolin particle film reduced canopy temperature under both clear and overcast conditions, increased stomatal conductance by reducing leaf-to-air vapour pressure deficit, and improved fruit mass and radiation use efficiency [20]. Across these species, a consistent pattern emerges: kaolin is most effective under moderate to severe abiotic stress, when it reduces radiation-induced heating, limits transpirational demand, and preserves photosynthetic machinery integrity. Spray applications of kaolin have also been shown to disrupt spruce budworm oviposition (*Choristoneura fumiferana* Clemens) [21], indicating additional pest management benefits.

1.4. Study Aims and Hypotheses

This study follows the release of the EU patent EP3881993A1 ‘Method for improving the compliance with quality specifications of cork produced from *Quercus suber* by applying kaolin to the surface of the exposed inner bark of a tree, after extracting the cork plank’. In this study, a kaolin-based particle film (50%) was applied immediately after cork removal of *Quercus suber* L. Based on these premises, we hypothesized that kaolin acts through a dual mechanism: by increasing surface albedo and reflecting solar radiation, thereby reducing tissue temperature and vapour pressure deficit at the bark surface; and by functioning as a physical particle film that increases boundary layer resistance to water vapour diffusion from the exposed inner bark. Through these complementary pathways, kaolin application was expected to limit excessive evaporative water loss following cork stripping, thereby preserving stem water transport, canopy photochemical efficiency, and overall tree vigour in both the short and long-term post-stripping recovery. Preliminary results of this study were presented as a poster at the EGU General Assembly 2025, Vienna, Austria [22].

2. Materials and Methods

2.1. Study Area and Experimental Design

The study area is a cork oak (*Quercus suber* L.) forest located in the municipality of Sant Celoni (Province of Barcelona, NE Spain) (41°40′00.69″ N, 2°30′59.44″ E), within the Catalan Coastal Mountain Range, at an elevation of approximately 205 m a.s.l. The climate is Mediterranean, characterized by hot, dry summers and mild, wet winters, with precipitation concentrated between October and April. During the study period (June–November 2023), the site experienced typical summer drought conditions, with near-zero precipitation from June to August and a progressive increase in rainfall from September onwards. Mean annual temperature is approximately 14–15 °C. Approximately 94% of the area is forested. The experimental site is an uneven-aged mixed cork oak forest that has completed the typical 14-year cork harvesting cycle established for this region by local regulations and management practices. The longer harvesting interval compared to the more common 9–12 years observed in other cork-producing regions reflects the specific environmental and stand conditions of the Catalan Coastal Range, where slower cork growth rates require extended recovery periods. The dominant species is *Quercus suber* L., interspersed with scattered individuals of holm oak (*Q. ilex* L.), downy oak (*Q.*

pubescens Willd.), cherry (*Prunus avium* L.), and sweet chestnut (*Castanea sativa* Mill.). The understorey is characterized by bramble (*Rubus* spp.), butcher's broom (*Ruscus aculeatus* L.), and heather (*Erica* spp.), indicative of a mesic microclimate with moderate soil moisture availability. The presence of a stream in the lower part of the slope suggests potential access to shallow groundwater for trees in that area.

In June 2023, three adjacent experimental plots were established, each measuring 1500 m² (Figure 1). Within each plot, 12 cork oak trees were randomly selected, tagged, and assigned to one of three treatments: four trees served as non-debarked controls (nS); four trees were mechanically debarked by professional cork harvesters and subsequently treated with a kaolin suspension (K); and four trees were mechanically debarked but received no post-debarking treatment (nK). Mechanical debarking was performed following traditional cork harvesting techniques using standard cork axes, by certified professional harvesters employed by Correggi Cork Italia s.r.l. (Montecchio Emilia, Italy). Immediately after debarking, the exposed inner bark of K trees was uniformly coated with a kaolin suspension at a ratio of 2:1 (*w/v*) (Resacril s.l., Lalloso-Castellón, Spain) using a large brush, applying a single coat to achieve full coverage of the exposed surface. A representative photograph of the kaolin application procedure is provided in Figure S2 (Supplementary Materials). Key dendrometric characteristics recorded for each individual tree, including DBH before and after cork removal, total height, debarking height, and stripped surface area, are reported in Table S5a (Supplementary Materials). The locations of the trees within each plot were mapped using a Trimble Geo7x GNSS receiver (Trimble Inc., Sunnyvale, CA, USA) with VRS (Virtual Reference Station) differential correction, with a positioning accuracy of approximately 50 cm, operating in UTM WGS84 Zone 31N (EPSG: 32631) coordinate system.



Figure 1. Localization of trees in the three experimental plots and assignment of different treatments (K: stripped plants and kaolin application; nK: stripped plants not treated; nS: controls not stripped).

2.2. Physiological Data Acquisition

2.2.1. Leaf Level Physiology

Leaf-level physiological data included measurements of stomatal conductance (g_s) and chlorophyll a fluorescence (OJIP test indicators). Stomatal conductance was measured

using a Leaf Porometer (Decagon Devices, Pullman, WA, USA), a parameter proportional to CO_2 flow through the stomata, dependent on their density and aperture, ranging from 0 to $1000 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Low values indicate reduced gas exchange and stomatal closure, reflecting abiotic stress conditions. Chlorophyll *a* fluorescence was assessed through the OJIP test using a PAR-FluorPen FP 110 (Photon Systems Instruments, Drásov, Czech Republic), a non-invasive technique for evaluating photosystem II (PSII) efficiency. Measurements were taken at midday (approximately 12:00) on fully expanded, sun-exposed leaves at 4–5 m above ground level, accessed using a portable ladder, on 1 tree per treatment (K, nK, and nS) per plot (1 to 3), with 4 leaves measured per tree ($n = 12$ measurements per treatment).

The following OJIP parameters were selected:

- ϕ_{Po} : maximum quantum yield of primary photochemistry, expressing the probability that an absorbed photon is trapped by the PSII reaction centre;
- PI_{abs} : photosynthetic performance index, assessing the overall efficiency of light energy conversion into chemical energy [23];
- ET_o/RC : electron transport rate per reaction centre, reflecting the efficiency of the photochemical phase;
- DI_o/RC : energy dissipation per reaction centre, representing the proportion of absorbed energy lost as heat;
- TR_o/RC : trapped energy flux per reaction centre;
- ABS/RC : absorption per active reaction centre;
- ψ_{Eo} : probability that a trapped exciton moves into the electron transport chain beyond Q_A^- .

Two measurement campaigns were conducted: the first, one hour after bark stripping and kaolin application on 26 June 2023; The second campaign of measurements was carried out on 16 November 2023, approximately 140 days after cork removal. This timepoint was chosen to capture the long-term physiological response of trees well beyond the 60-day threshold after which scar tissues and the first new layers of the regenerating periderm are reported to be fully insulating [7].

2.2.2. Sap Flux Density Monitoring

Sap flux density (J_s) was continuously monitored using the TreeTalker[®] Cyber system (Nature 4.0 s.r.l., Viterbo, Italy) installed on one tree per treatment (K, nS, and nK) per plot ($n = 9$ trees total). The system employs the Heat Ratio Method (HRM; [24]) through a three-needle probe radially inserted into the north-facing xylem. Each probe consists of three vertically arranged needles (28 mm long, 2 mm diameter, 7.5 mm spacing), with a central heater needle flanked by upstream and downstream temperature sensors. Rather than converting J_s to volumetric water use (L day^{-1}), sap flux density was used directly as the response variable. This choice was motivated by the uncertainty associated with sapwood area (A_s) estimation in *Q. suber* at the dimensional range of the study trees (DBH 19–41 cm post-stripping). Sapwood depth in this species has been variously reported at approximately 32% of trunk radius [7,25], in the outer 2–4 cm of xylem [26], or at an average depth of 6.5 cm [27], reflecting substantial inter-individual and site-specific variability. In the absence of direct radial sap flow profile measurements, any allometric scaling of A_s would introduce a source of systematic uncertainty that could obscure treatment comparisons. Using J_s as a normalized, area-independent metric therefore provides a more robust and reproducible basis for assessing the relative effect of kaolin application on stem water transport capacity among treatments. Monitoring started one week after cork stripping and Kaolin application (7 D.A.S.), following the stabilization period required after probe insertion. Daily J_s values were computed as the mean of all measurements

recorded between 07:00 and 20:00 h (local solar time), corresponding to the daylight period at the study site (41°40' N) across the monitoring season (July–October). Continuous data were partitioned into three periods adapted from Costa-e-Silva et al. [7], based on the physiological stages of post-stripping recovery: (1) up to 35 D.A.S. corresponding to the initial phase of phellogen reconstruction, during which the original phellogen dies and a new traumatic phellogen is rapidly formed; (2) 35–60 D.A.S. coinciding with the end of the dry season, after which scar tissues and the first new layers of the regenerating periderm are reported to be fully insulating [7]; and (3) from 60 D.A.S. to the end of October during the progressive reduction of summer abiotic stress and the onset of autumn precipitation.

2.2.3. UAV Data Acquisition and Processing

A DJI Phantom 4 (Shenzhen DJI Sciences and Technologies Ltd., Shenzhen, China) Multispectral RTK (Real-time kinematic) was used to capture multispectral and RGB data. The drone is equipped with an RGB camera and a multispectral camera array featuring 5 sensors that cover the Blue (450 nm), Green (560 nm), Red (650 nm), Red Edge (RE—730 nm), and Near-Infrared bands (NIR—840 nm). Each sensor has a resolution of 2 MP and a global shutter. These are mounted on a 3-axis stabilized gimbal, and the drone also includes centimetre-level navigation and positioning systems.

Data were collected under clear, cloudless skies at midday on two dates: 26 June 2023, coinciding with the cork stripping date, and 16 November 2023, corresponding to the long-term physiological assessment. The two acquisition dates were designed to compare the immediate and delayed responses of tree canopy to cork stripping and kaolin application. The flight mission was planned to use the DJI GS Pro (DJI Ground Station Pro) App (SZ DJI Technology Co., Ltd., Shenzhen, China) on an iPad mini (Apple Inc., Cupertino, CA, USA). The software automatically calculates flight paths over the study area based on parameters such as flying altitude, overlap, and sensor characteristics. To ensure optimal ortho mosaics, the survey area for the flight mission was defined as rectangular, with 80% side and front overlap. The flying time required to complete the mission was 9 min at an altitude of 40 m above ground level (AGL).

After acquiring the UAV data for both sampling dates (June and November 2023), Agisoft Metashape Professional v. 2.2.1 (Agisoft LLC, St. Petersburg, Russia) was used to process the images. The software encompasses a series of RGB and multispectral data processing steps, which include aligning and optimizing the camera, generating a dense point cloud, building a Digital Elevation Model (DEM), generating an orthomosaic, and exporting the resulting data. Subsequently, the multispectral orthomosaics created were imported into QGIS software (version 3.34, QGIS Development Team, Open Source Geospatial Foundation, Beaverton, OR, USA) to calculate multispectral vegetation indices (VI). Two VIs were selected for this study: NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Red Edge Index) [28,29].

$$\text{NDVI} = \frac{\text{NIR} - \text{RED}}{\text{NIR} + \text{RED}}$$

$$\text{NDRE} = \frac{\text{NIR} - \text{REDEdge}}{\text{NIR} + \text{REDEdge}}$$

NDVI and NDRE are vegetation indices used in remote sensing to measure the relative chlorophyll content in plants. Subsequently, the photointerpretation of each tree crown was done for each plant in each plot. A Region of interest (RoI) of a 30 cm radius around the tree crown's centre was defined to calculate the vegetation index values (Figure S1).

2.2.4. Statistical Analysis

The effect of kaolin treatment on stomatal conductance (g_s) and photosynthetic performance was evaluated using one-way ANOVA with three treatment groups (K, nK, and nS), followed by Tukey's HSD post hoc test ($p \leq 0.05$) to identify significant pairwise differences among treatments. Photosynthetic performance was assessed through the OJIP test parameters described in Section 2.2.1. Vegetation indices (NDVI and NDRE) were extracted from defined regions of interest (ROIs) on both sampling dates. A repeated measures ANOVA was used to test the effects of treatment and time, with Tukey's post hoc tests applied at the plot level to assess pairwise differences. For sap flux density data, differences in mean daytime J_s (07:00–20:00 h) among treatments within each of the three post-stripping periods were assessed using a Linear Mixed Effects Model (LME) with tree identity as a random intercept, to account for temporal pseudoreplication arising from repeated daily measurements on the same individuals. The model structure was: $J_s \sim \text{Treatment} + (1 | \text{Tree})$, fitted by Restricted Maximum Likelihood (REML). Pairwise post hoc comparisons among treatments were performed using Tukey's HSD test ($\alpha = 0.05$). All statistical analyses were performed in Python (version 3.13.3) using the *statsmodels* package and the Spyder IDE (version 6.0.5).

3. Results

3.1. Plant Physiological Response

3.1.1. Short-Term Measurements

Stomatal conductance (g_s) measured one hour after bark removal and kaolin application did not significantly differ among treatments (K: 152.84, nS: 146.50, nK: 171.01 mmol $\text{H}_2\text{O m}^{-2} \text{ s}^{-1}$; Tukey post hoc test, $p \geq 0.05$; Table S4). The results of the OJIP test are summarized in Table 1.

Table 1. OJIP fluorescence parameters measured approximately one hour after cork stripping and Kaolin application (short-term response) by treatment ($n = 12$ measurements per treatment; 4 leaves per tree, 1 tree per treatment per plot, 3 plots). K: stripped trees with kaolin application; nK: stripped trees without kaolin application; nS: unstripped control trees. ϕ_{Po} : maximum quantum yield of primary photochemistry; PI_{abs} : photosynthetic performance index; TR_o/RC : trapped energy flux per reaction centre; DI_o/RC : energy dissipation per reaction centre; ABS/RC : absorption per active reaction centre; ET_o/RC : electron transport per reaction centre; ψ_{Eo} : probability of electron transport beyond Q_A^- . Different letters within each row indicate significant differences among treatments (Tukey HSD, $p \leq 0.05$).

Treatment	ϕ_{Po}		PI_{abs}		TR_o/RC		DI_o/RC		ABS/RC		ET_o/RC		ψ_{Eo}	
nS	0.76	a	1.76	a	1878.44	b	0.53	a	1353.44	a	0.64	a	0.35	a
nK	0.76	a	4.14	b	1669.89	a	0.39	b	1279.22	a	0.74	a	0.48	b
K	0.69	a	1.78	a	1850.78	b	0.51	a	1339.22	a	0.69	a	0.37	ab

The immediate response of stripped untreated trees (nK) to cork removal was a significant increase in PI_{abs} compared to nS and K trees (4.14 vs. 1.76 and 1.78, respectively; $p < 0.05$), alongside a significant decrease in DI_o/RC (0.39 vs. 0.53 in nS; $p < 0.05$). No significant differences were observed among treatments for ϕ_{Po} , TR_o/RC , ABS/RC , ET_o/RC , and ψ_{Eo} . Stripped kaolin-treated trees (K) did not differ significantly from unstripped controls (nS) in any parameter. The polyphasic fluorescence curves (Figure 2) confirmed this pattern: nS and K curves overlapped closely, while the nK curve diverged markedly in the thermal phase (J–P).

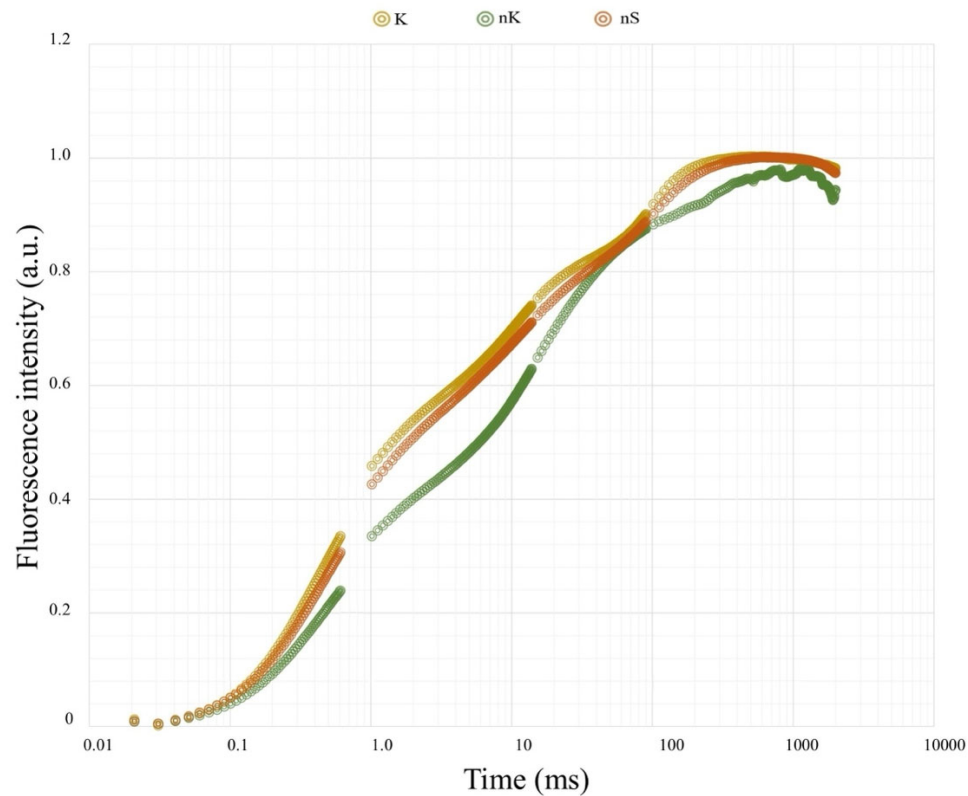


Figure 2. Polyphasic transient curves of chlorophyll a fluorescence (O-J-I-P test) measured approximately one hour after cork stripping ($n = 12$ measurements per treatment; 4 leaves per tree, 1 tree per treatment per plot, 3 plots): stripped trees with kaolin application (K, yellow); stripped untreated trees (nK, green); unstripped control trees (nS, orange). Fluorescence intensity is expressed in arbitrary units (a.u.) and normalized to the maximum value.

3.1.2. Long-Term Measurements

Stomatal conductance (g_s) measured 140 days after bark removal did not significantly differ among treatments (K: 123.8, nK: 138.0, nS: 148.6 mmol H₂O m^{−2} s^{−1}; Tukey post hoc test, $p \geq 0.05$). The results of the OJIP test are summarized in Table 2. At 140 D.A.S., stripped untreated trees (nK) showed significantly lower PI_{abs} and ψ_{Eo} compared to both nS and K trees ($p < 0.05$), indicating a persistent impairment of photosynthetic performance and electron transport efficiency. In contrast, kaolin-treated trees (K) displayed significantly higher TR_o/RC , ABS/RC , and ET_o/RC than both nS and nK trees ($p < 0.05$), suggesting enhanced energy capture and electron transport capacity at the reaction centre level. No significant differences were found among treatments for ϕ_{Po} and DI_o/RC .

Table 2. OJIP fluorescence parameters measured 140 days after cork stripping (long-term response) by treatment ($n = 12$ measurements per treatment; 4 leaves per tree, 1 tree per treatment per plot, 3 plots). K: stripped trees with kaolin application; nK: stripped trees without kaolin application; nS: unstripped control trees. Parameter abbreviations as in Table 1. Different letters within each row indicate significant differences among treatments (Tukey HSD, $p \leq 0.05$).

Treatment	ϕ_{Po}		PI_{abs}		TR_o/RC		DI_o/RC		ABS/RC		ET_o/RC		ψ_{Eo}	
nS	0.77	a	3.27	b	1265.33	a	0.38	a	1650.00	a	0.70	a	0.48	b
nK	0.73	a	1.76	a	1365.22	a	0.53	a	1891.67	a	0.65	a	0.38	a
K	0.79	a	3.10	b	1598.75	b	0.44	a	2035.38	b	0.88	b	0.46	b

3.1.3. Sap Flow Monitoring

Daily J_s data are shown in Figure 3, together with abiotic conditions (Figure 3a) and the daily J_s per treatment (Figure 3b). Mean daytime J_s per treatment for each of the three post-stripping periods are summarized in Figure 4 and mean diurnal J_s profiles for each period are presented in Figure 5. Data are presented from 7 D.A.S. onwards, as sensor stabilization required approximately one week following probe insertion. The three periods—phellogen reconstruction (0–35 D.A.S.), end of dry season (35–60 D.A.S.), and autumn recovery (from 60 D.A.S. to end of October)—were defined following the physiological timeline described by Costa-e-Silva et al. [7] and are indicated by vertical dashed lines in Figure 3a. The absence of pre-stripping baseline measurements represents a limitation of this study, as pre-existing biometric differences among trees cannot be fully excluded as a contributing factor to the observed differences in J_s .

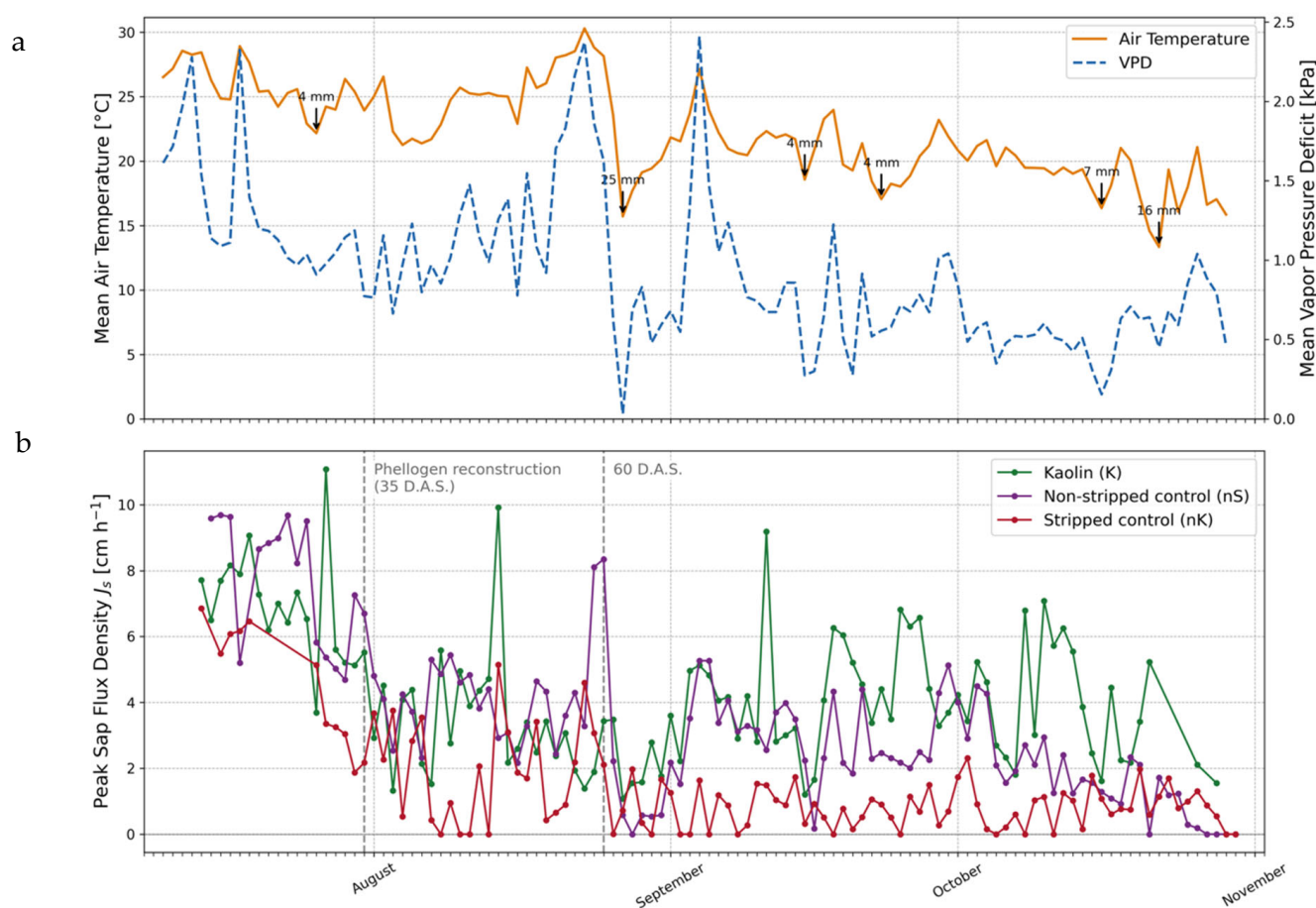


Figure 3. Sap flux density (J_s) monitoring of *Quercus suber* L. trees by treatment from 7 D.A.S. to the end of October 2023. (a) Daily mean air temperature (°C) and vapour pressure deficit (VPD, kPa); arrows indicate rainfall events with amount in mm. (b) Daily J_s (cm h^{-1}) per treatment over the monitoring period; vertical dashed lines mark the limits of the three post-stripping periods at 35 and 60 D.A.S. K: stripped trees with kaolin application (green); nK: stripped untreated trees (red); nS: unstripped control trees (purple). The three post-stripping periods are defined according to Costa-e-Silva et al. [7].

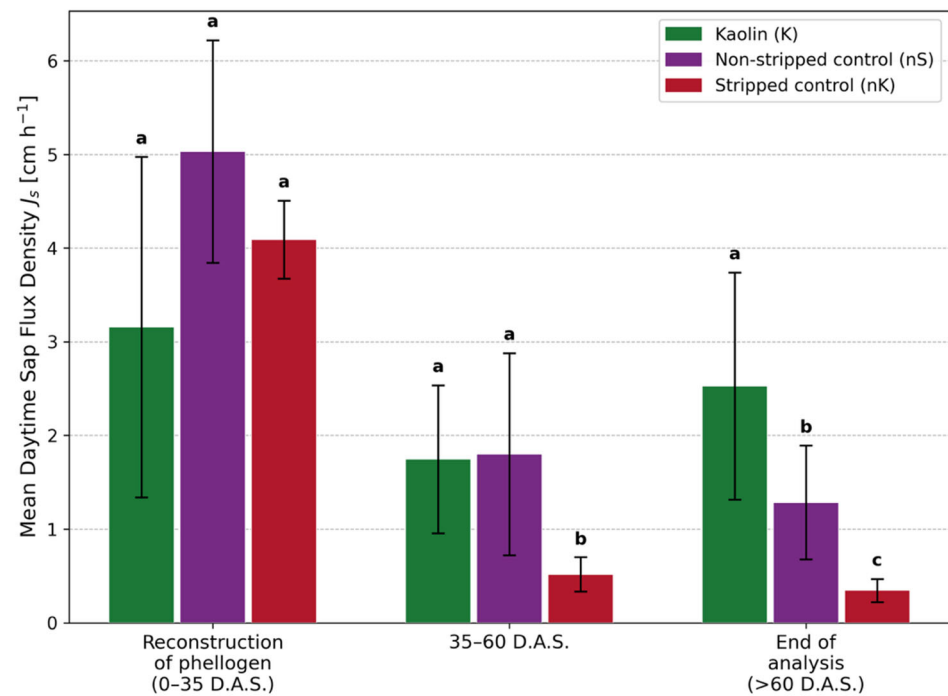


Figure 4. Mean daytime sap flux density (J_s , cm h^{-1} , 07:00–20:00 h) per treatment during the three post-stripping periods: phellogen reconstruction (0–35 D.A.S.), end of dry season (35–60 D.A.S.), and autumn recovery (>60 D.A.S.). K: stripped trees with kaolin application; nK: stripped untreated trees; nS: unstripped control trees. Error bars represent standard error of the mean (SEM, $n = 3$ trees per treatment). Different letters above bars indicate statistically significant differences among treatments within each period (Linear Mixed Effects Model + Tukey's HSD, $p \leq 0.05$).

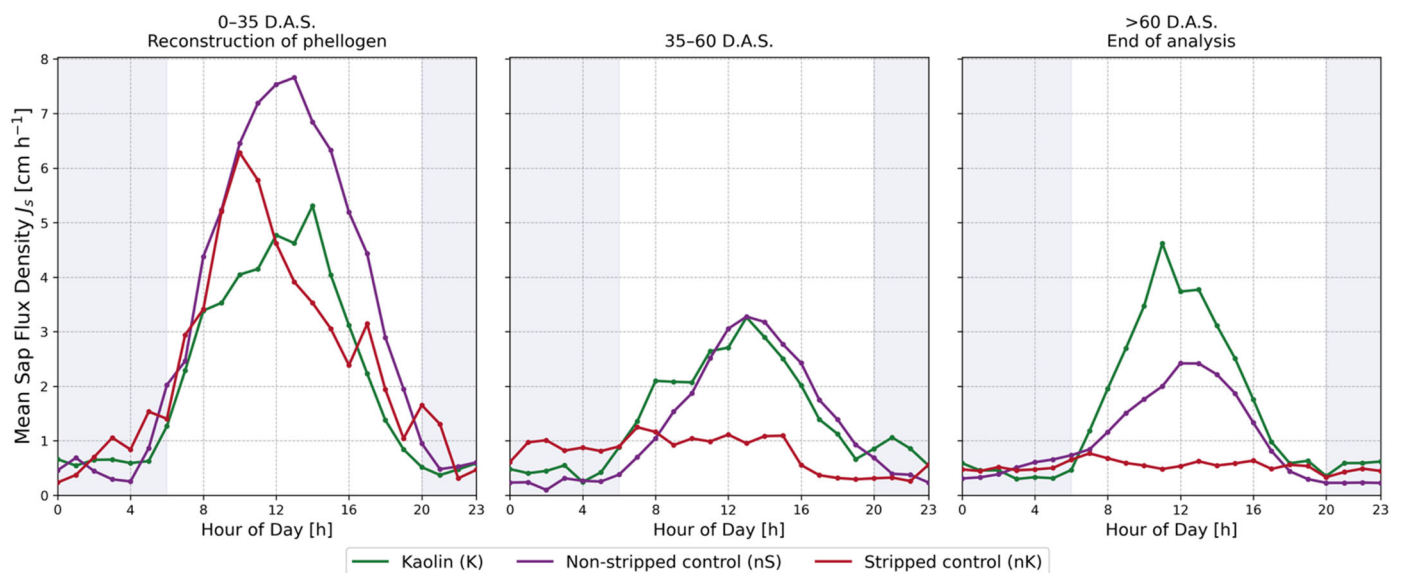


Figure 5. Mean diurnal J_s profiles (cm h^{-1}) for each treatment across the three post-stripping periods: phellogen reconstruction (0–35 D.A.S.), end of dry season (35–60 D.A.S.), and autumn recovery (>60 D.A.S.). Values represent hourly means averaged across all days within each period and across three individual trees per treatment. Shaded areas indicate nocturnal hours. K: stripped trees with kaolin application (green); nK: stripped untreated trees (red); nS: unstripped control trees (purple). The version with standard error of the mean is provided as Supplementary Figure S3.

No significant differences in mean daytime J_s were observed among treatments during the phellogen reconstruction phase (0–35 D.A.S.; Table S2). Mean J_s values were 3.78 ± 2.15 , 5.89 ± 1.52 , and $4.62 \pm 0.73 \text{ cm h}^{-1}$ for K, nS, and nK trees, respectively (Table S1). The

diurnal J_s profiles during this period showed a well-defined midday peak in all three treatments, with nS trees reaching the highest values around 12:00 h, followed by nK and K trees (Figure 5). The large inter-individual variability observed during this period is consistent with the absence of statistically significant treatment differences detected by the LME model. During the 35–60 D.A.S. period, nK trees showed a marked reduction in mean daytime J_s ($0.63 \pm 0.22 \text{ cm h}^{-1}$), remaining significantly lower than both K ($2.03 \pm 0.92 \text{ cm h}^{-1}$; $p = 0.0002$) and nS trees ($2.16 \pm 1.29 \text{ cm h}^{-1}$; $p < 0.0001$), while K and nS did not differ significantly ($p = 0.4827$). This pattern is clearly visible in the diurnal profiles (Figure 5), where K and nS trees maintained a defined midday peak while nK trees showed a flattened diurnal curve throughout the day, indicating a near-complete suppression of xylem water transport during the peak evaporative demand period.

In the final period (>60 D.A.S.), all three treatments differed significantly from one another (Table S2). K trees maintained the highest mean daytime J_s ($3.03 \pm 1.46 \text{ cm h}^{-1}$), significantly exceeding both nS ($1.57 \pm 0.75 \text{ cm h}^{-1}$; $p = 0.0001$) and nK trees ($0.36 \pm 0.14 \text{ cm h}^{-1}$; $p < 0.0001$). The difference between nS and nK was also significant ($p < 0.0001$). The diurnal profiles for this period show K trees maintaining a clear midday peak, while both nS and nK showed progressively lower values, with nK remaining essentially flat across all hours of the day (Figure 5). Across the monitoring period, K trees maintained J_s levels comparable to or exceeding those of unstripped controls (nS) from 35 D.A.S. onwards, suggesting that kaolin application effectively preserved xylem water transport capacity following cork stripping. In contrast, nK trees showed a progressive and persistent reduction in J_s throughout all three periods, consistent with a sustained impairment of stem hydraulic function following unprotected cork removal.

3.1.4. Proximal Vegetation Indices

NDVI and NDRE were extracted from crown-projected ROIs to assess the impact of cork removal and kaolin application on canopy vegetative status. Repeated measures ANOVA showed no significant differences among treatments (K, nK, nS) or between sampling dates (June and November 2023) for either index ($p > 0.05$; Table S3), suggesting that neither cork stripping nor kaolin application produced detectable changes in canopy greenness or chlorophyll content within the timeframe of this study.

4. Discussion

4.1. Short-Term Photochemical Responses and Systemic Shock Immediately After Stripping

This study evaluates the effects of kaolin stem application on cork oak physiology following cork removal, building on the claims of EU patent EP3881993A1, which proposed kaolin application as a method to mitigate the physiological stress induced by cork stripping, and provides empirical evidence supporting its use under natural forest conditions. Kaolin application post-debarking positively influenced the photochemical functionality of trees, measured through several PSII performance indices (PIs) assessing the structure and function of specific electron transport reactions in the thylakoid membrane [30]. These PIs provide a comprehensive assessment of plant responses to abiotic stressors such as drought, salinity, and temperature extremes [31,32].

In the short term, immediately following cork stripping, trees experience water loss from the exposed inner bark surface. Although early estimates suggested that this trunk water loss could equal canopy transpiration [6], direct measurements have since demonstrated that trunk evaporation represents only approximately 2% of canopy transpiration and does not significantly affect summer tree water balance [7]. Instead, cork removal directly impacts the tree's energy balance by triggering an immediate metabolic trauma and

generating an instantaneous, overwhelming carbon sink in the stem as the tree prepares for subsequent cell repair and periderm regeneration [7,33].

The immediate response of nK trees to this stripping trauma was a significant increase in PI_{abs} , observed one hour after stripping. Rather than reflecting an adaptive improvement in performance, this transient response is interpreted as a signature of acute stress: a short-lived over-excitation of the photochemical apparatus triggered by the sudden hydric (and thermal) exposure of the freshly denuded living tissue. Such transient over-excitation, when coupled with a reduced capacity of the end electron acceptors, has been shown to precede photochemical damage rather than reflect successful acclimation [32]. Concurrently, the significant reduction in DI_o/RC indicates a decreased capacity to safely dissipate absorbed energy as heat, forcing a temporary reallocation of energy toward photochemical reactions: a pattern consistent with an apparatus overwhelmed by sudden stress rather than one operating in an ordered, regulated state, as described during the initial shock phases in other species [34].

Taken together, the short-term OJIP data on nK trees indicate a slowing of the electron transport rate in the photochemical phase (O-J) and a reduction in energy dissipation capacity, increasing the risk of photoinhibition in the thermal phase (J-P) and the accumulation of reduced Q_A^- . The polyphasic fluorescence curves confirmed these results: nS and K trees exhibited nearly identical patterns, consistent with the absence of significant differences between these treatments in all measured parameters (Table 1). In contrast, nK trees displayed a markedly different curve, particularly in the thermal phase (J-P), suggesting disruptions in the later stages of the electron transport chain. This divergence indicates that the transient over-excitation observed in unprotected trees was unsustainable, progressively giving way to chronic photoinhibition and a sustained impairment of photochemical efficiency over the long term.

4.2. Long-Term Hydraulic Dynamics and Sap Flux Responses

This severe energetic and physical burden led to consistently lower mean daytime J_s in nK trees compared to K and nS trees from 35 D.A.S. onwards (Figure 4). Given that stomatal conductance did not differ significantly among treatments at both sampling dates, the reduced J_s in nK trees likely reflects a localized hydraulic dysfunction and a generalized reduction in whole-tree metabolic coordination rather than stomatal regulation. It should also be noted that sap flux density and stomatal conductance were assessed at different temporal and spatial scales: J_s was monitored continuously at the stem level, whereas stomatal conductance was sampled on individual leaves at two discrete dates. Two comparable instantaneous readings of stomatal conductance do not imply equivalent integrated transpiration, and the divergence between a sustained reduction in stem-level J_s and unchanged leaf-level conductance is itself consistent with a hydraulic limitation arising upstream of the stomata, within the stem conductive pathway, rather than with stomatal closure. The absence of significant differences in stomatal conductance among treatments may appear to contrast with the early findings of Correia et al. [6], who reported marked alterations in plant water relations following cork stripping. However, this apparent discrepancy is explained by experimental conditions: the study by Correia et al. [6] was conducted under controlled conditions with limited soil water availability, whereas our measurements were taken in a natural forest setting where trees had continuous access to deep soil water resources, including potential groundwater contribution from a stream present in the lower part of the slope.

Under non-limiting soil water conditions, stomatal regulation is not the primary physiological response to cork stripping stress [7]. Instead, the progressive decline in J_s observed in nK trees suggests that without protective treatment, the high micro-local

vapour pressure deficit (VPD) at the exposed trunk surface permanently impairs peripheral hydraulic conductivity. In *Q. suber*, the most active water-transporting xylem vessels reside in the outermost annual rings, located near the cambial zone. The extreme environmental exposure of nK trunks is hypothesized to induce micro-embolisms and peripheral xylem cavitation, throttling water movement. This explains the explicit cause-effect relationship between debarking trauma and the decline in whole-tree sap flux (J_s): local water loss, although quantitatively minor compared to canopy transpiration, occurs directly above the most active xylem vessels, inducing a localized hydraulic dysfunction that effectively throttles water movement at the whole-tree scale.

In contrast, K trees displayed higher and more responsive J_s values, with peaks during midday hours consistent with maintained xylem hydraulic function, comparable to patterns reported by Costa-e-Silva et al. [7] up to 45 days post-stripping under conditions of no soil water limitation. The rapid recovery of J_s following rainfall events in September and October, particularly evident in K and nS trees, suggests that kaolin application successfully supported the maintenance of functional xylem water transport capacity throughout the post-stripping period. This interpretation is further supported by the long-term response of the photosynthetic apparatus, as assessed through the OJIP test parameters at 140 D.A.S., where K trees showed significantly higher energy fluxes per reaction centre compared to both nS and nK trees, including absorption (ABS/RC), trapped energy flux (TR_o/RC), and electron transport (ET_o/RC), indicating a superior long-term photochemical preservation of the kaolin-treated trees (Table 2).

Conversely, the remote canopy-scale indices (NDVI and NDRE) showed no significant differences between treatments and sampling dates. This suggests that the physiological effects of cork stripping and kaolin application were confined to internal hydraulic and photochemical parameters and were not yet detectable at the canopy scale within the short timeframe of this study. A longer monitoring period spanning multiple growing seasons would be needed to capture potential downstream effects on canopy development and leaf area [4].

4.3. Mechanistic Pathways of Kaolin Protection: Diffusion and Albedo Barriers

The physiological benefits observed in kaolin-treated trees can be attributed to the dual mechanism hypothesized in this study, which integrates both physical and metabolic pathways. These two mechanisms operate on distinct time scales and are therefore complementary rather than redundant: the physical barrier effect of the particle film acts immediately, from the moment of application, by sealing the exposed living tissue and limiting direct evaporative water loss, whereas the radiative and metabolic effects associated with reduced tissue temperature develop more progressively over the days and weeks following stripping. This temporal distinction is central to interpreting both the immediate divergence between treatments observed one hour after stripping and the sustained differences recorded throughout the monitoring period.

The first mechanism involves the physical barrier effect of the particle film on water vapour diffusion from the exposed bark surface to the atmosphere. Immediately after stripping, the freshly denuded cambial and phloem tissues are living, hydrated, and devoid of any protective barrier, and are therefore directly exposed to atmospheric water demand. By coating this surface at the moment of application, the kaolin film provides an immediate physical seal that limits direct evaporative loss before any temperature-mediated effect can develop, acting as a temporary functional surrogate for the removed cork insulation until the new periderm is reconstructed. This immediate sealing is the most plausible explanation for the divergence in photochemical performance already detectable one hour after stripping, when kaolin-treated trees behaved comparably to non-stripped controls

despite having undergone the same debarking wound. This barrier effect is physically distinct from stomatal regulation and operates independently of it, as documented in foliar kaolin applications: in walnut and almond, kaolin reduced leaf-to-air vapour pressure deficit by 0.1–0.7 kPa without affecting stomatal conductance [35], and similar results were reported in grapevine and olive [16,18]. On the exposed inner bark, which entirely lacks stomatal regulation, this physical barrier may play a proportionally greater role than in foliar applications: the particle film is hypothesized to increase the effective diffusion path length for water vapour between the evaporating tissue surface and the free atmosphere, thereby reducing evaporative conductance independently of any biological control. We acknowledge that this specific mechanism on exposed bark tissue has not been directly measured and remains a functional hypothesis requiring experimental verification through dedicated diffusion-resistance measurements on stripped and kaolin-treated cork oak surfaces.

The second mechanism operates more progressively, through increased surface albedo. By reflecting incident solar radiation, kaolin reduces radiative energy absorption at the exposed inner bark surface, thereby lowering tissue temperature and mitigating the excessive vapour pressure deficit at the bark–atmosphere interface. While direct measurement of bark surface temperature using thermocouples or infrared sensors was not performed in this study, a methodological limitation we explicitly acknowledge and propose as a priority for future research, this gap reflects the novelty of the experimental context rather than an oversight. To our knowledge, no peer-reviewed study has evaluated the thermal or evaporative effects of kaolin applied directly to exposed woody stem tissue, as all existing literature on kaolin particle film technology concerns foliar or fruit surface applications. Cork itself is recognized as an exceptionally effective biological thermal insulator: with a thermal conductivity of approximately $0.045 \text{ W m}^{-1} \text{ K}^{-1}$, close to that of still air ($0.025 \text{ W m}^{-1} \text{ K}^{-1}$) and far below that of its own cell-wall material ($0.2 \text{ W m}^{-1} \text{ K}^{-1}$), it ranks among the lowest-conductivity biological materials [36]. Its removal during stripping therefore eliminates a critical protective barrier for the underlying meristematic tissues.

This progressive reduction in tissue temperature is in turn linked to water transport preservation through a metabolic pathway. High temperatures accelerate cellular respiration, a well-documented temperature dependence in plant physiology [37]. Following stripping, the original phellogen dies and a new traumatic phellogen forms in the deeper phloem tissues within 25–35 days [7]. This reconstruction process is metabolically extraordinarily demanding: the traumatic phellogen produces approximately 100 layers of cork cells per year, far exceeding the 3–6 layers produced by the original phellogen in the first years of growth [38]. The genes involved in suberin biosynthesis—QsCYP86A32, QsKCR1, and QsHCT—are significantly upregulated in traumatic differentiating cork [38], confirming that periderm regeneration represents one of the most carbon-expensive activities in the post-stripping tree. Furthermore, trees weakened by cork stripping damage show reduced diameter growth primarily in the first two years following stripping, with a delay of approximately one month in the onset of spring radial growth [4], reflecting a severe systemic competition for photosynthetic assimilates. By reducing the local temperature and the consequent respiratory burden on exposed stem tissues, kaolin prevents this carbon drain, freeing assimilates for cambial activity, xylem maintenance, and cellular repair, thereby preventing the hydraulic downregulation observed in nK trees.

The relevance of this evaporative protection is amplified under dry conditions. The effect of cork stripping on sap flow depends strongly on the interaction between stripping stress and soil water availability—in a wet year no significant differences in sap flow are observed between stripped and control trees, whereas in a dry year, cork stripping can reduce sap flow by up to 46% [7], indicating a synergistic interaction between traumatic

stress and soil water deficit. Our study was conducted under dry summer conditions in Catalonia, placing it precisely within this high-risk scenario. Under these conditions, even a modest reduction in evaporative conductance through the micro-porous kaolin particle film helps maintain peripheral tissue water potential above critical thresholds, preserving hydraulic continuity and supporting the sap flux density levels observed in K trees throughout the monitoring period.

We acknowledge that the relative contribution of each mechanism—diffusion resistance versus albedo effect—cannot be quantified from the current dataset, and that both proposed pathways represent functional hypotheses requiring direct experimental verification in future works.

4.4. Management Implications and Limitations

From a forest management perspective, these findings have relevant practical implications for cork oak woodland management, particularly in the context of increasing drought frequency and intensity projected for the Mediterranean region under climate change scenarios. Cork stripping, performed every 9 to 14 years, represents a critical vulnerability window during which trees are simultaneously exposed to high evaporative demand and deprived of their primary protective barrier. The application of kaolin immediately after stripping represents a low-cost, environmentally compatible intervention that could help maintain tree vitality and potentially safeguard cork production quality in subsequent harvesting cycles, as proposed by EU patent EP3881993A1. This is particularly relevant for stands where stripping coincides with dry years or occurs in trees already under water stress, conditions under which cork stripping can lead to a drastic 46% reduction in canopy transpiration [7].

However, the conclusions of this study should be interpreted with caution. The experimental design was limited to a single site and a single growing season, and the absence of pre-stripping baseline J_s measurements prevents full exclusion of pre-existing biometric differences among sampled trees as a contributing factor. Furthermore, the study did not encompass spring measurements, which represent a critical period for tree growth recovery in Mediterranean ecosystems. The absence of direct bark surface temperature measurements represents an additional methodological limitation, which future studies should address through thermocouples or infrared sensors applied directly to the exposed inner bark surface immediately after stripping. Future research should expand to multiple sites across different climatic conditions, monitor trees across consecutive cork production cycles, and include measurements of cork quality at the following harvesting turn to fully validate the potential of kaolin as a management tool for cork oak forest resilience.

5. Conclusions

Cork removal represents a critical management practice in cork oak forests, particularly under the increasing pressure of climate change in Mediterranean regions. This study provides empirical evidence that post-stripping kaolin application improves key indicators of photosynthetic performance and stem water transport capacity in *Quercus suber* L., suggesting a protective role during the critical post-stripping recovery period. These benefits were evident both in the short and long term, supporting a lasting protective effect of the treatment. Although canopy-scale vegetation indices did not show significant differences within the study timeframe, direct physiological measurements provide clear evidence of kaolin's protective action. These findings support kaolin as a viable and sustainable tool for cork oak forest management, with potential benefits for tree resilience and cork productivity. As discussed, conclusions should be interpreted in the context of a single-site study, and future

research across multiple climatic regions, forest types, and consecutive harvesting cycles will be essential to fully validate kaolin as a management tool.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f17070750/s1>. Figure S1: Identification of tree crowns by photo-interpretation and buffer definition for vegetation index calculation (Plot 1); Figure S2: Application of the kaolin suspension (2:1, w/v) on the exposed inner bark of a *Quercus suber* L. tree immediately after cork stripping; Figure S3: Mean diurnal sap flux density (J_s) profiles per treatment with standard error of the mean (SEM); Table S1: Mean daytime sap flux density (J_s) and SEM per treatment by post-stripping period; Table S2: Linear Mixed Effects Model (LME) and Tukey HSD results for mean daytime J_s among treatments within each post-stripping period; Table S3: ANOVA results for NDVI and NDRE proximal vegetation indices by treatment; Table S4: Stomatal conductance (g_s) per treatment measured approximately one hour after cork stripping; Table S5: Dendrometric characteristics of the 36 cork oak trees (*Quercus suber* L.) sampled across the three experimental plots.

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