Plant Physiology and Biochemistry 216 (2024) 109208

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Drought tolerance and recovery capacity of two ornamental shrubs: Combining physiological and biochemical analyses with online leaf water status monitoring for the application in urban settings

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ARTICLE INFO

Keywords:
Leaf sensor
Photinia x *fraseri*
Photosynthesis
Polyphenols
Viburnum tinus
Water relations
Water stress

ABSTRACT

When plants are transferred from nursery to urban environments, they often face drought stress due to inadequate maintenance, such as insufficient irrigation. Using drought tolerant species may help mitigate the adverse impact of drought stress in urban settings. Additionally, utilizing novel technologies for water status monitoring may help optimize irrigation schedules to prevent transplanting failures. This study investigated the physiological and biochemical responses of two ornamental shrubs, *Photinia* x *fraseri* and *Viburnum tinus*, subjected to water stress of increasing severity and rewatering. Water relations, gas exchanges, chlorophyll fluorescence and biochemical analyses were conducted alongside real-time monitoring of water status using leaf-water-meter sensors (LWM).

The progression of water stress had a notable negative impact on leaf gas exchanges and water relations in both species. Notably, *P. fraseri* avoided photoinhibition by reducing chlorophyll content and actual efficiency of PSII. Adjustments in leaf phenolic compounds played a significant role in enhancing drought tolerance of both species due to their antioxidant and photoprotective properties.

Upon rewatering, both species exhibited complete recovery in their physiological functions, underscoring their remarkable tolerance and resilience to drought stress. Additionally, LWM sensors efficiently tracked the dehydration levels, exhibiting a rising trend during the water stress progression and a subsequent decline after rewatering for both species. These findings confirm the reliability of LWM sensors in monitoring physiological status of plants in outdoor contexts, making them a suitable tool for use in urban settings.

1. Introduction

Urban green offers many ecosystem services, including improved air quality, CO_2 sequestration, and microclimate regulation, also increasing the aesthetic value of the surrounding urban areas (McPherson et al., 2018). The delivery of ecosystem services by urban green can be negatively affected by the multiple stressors typical of urban environments, such as poor soil conditions (*i.e.*, soil compaction and low soil fertility), high temperatures, and drought (Franceschi et al., 2023). Drought is considered the main factor threatening urban plants growth and vitality, by affecting both physiology (e.g., reduction in leaf water potential, stomatal closure, decrease in CO_2 assimilation, down-regulation in PSII photochemistry) and leaf biochemistry (e.g., chlorophyll reduction, increment in secondary metabolites production) (Toscano et al., 2019).

Before being planted in the urban environment, plants are typically cultivated in nurseries under high-density, self-shaded, or partially shaded conditions, receiving generous water and fertilizer applications (Smith et al., 2019). Consequently, when transferred to the urban conditions, they may experience significant drought stress (Wattenhofer et al., 2021). Indeed, local administrations very often fail to provide the

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https://doi.org/10.1016/j.plaphy.2024.109208
Received 24 April 2024; Received in revised form 19 September 2024; Accepted 16 October 2024
Available online 18 October 2024

right supply of irrigation to plants due to high costs of irrigation systems and mismanagement (Pincetl et al., 2013). This could exacerbate the mortality rates of urban trees and shrubs, particularly during their initial establishment phase. This problem can be partially mitigated by selecting drought tolerant species (Percival, 2023). An additional solution to avoid transplanting failures and to optimize irrigation scheduling to prevent drought stress in urban settings could be the application of new technologies that are able to monitor in real-time the physiological status of the plants (Fuentes et al., 2021). In recent years, new real-time monitoring devices, based on remote or contact sensors, have been developed (Brunetti et al., 2022; Bwambale et al., 2022). In particular, the use of the Leaf Water Meter (LWM) has been previously validated by Brunetti et al. (2022) in a controlled greenhouse experiment on four different Mediterranean woody species subjected to consecutive cycles of water stress and rewatering, showing promising results due to the high and significant correlation between the leaf dehydration levels (measured by the sensor) and leaf relative water content.

Photinia x *fraseri* and *Viburnum tinus* are widely used in the urban environment, especially for hedges. *Photinia* x *fraseri*, which belongs to the *Rosaceae* family, is a hybrid between *P. serrulata* and *P. glabra*, both native to Asia. It is an evergreen broad-leaved shrub, whose leaf color varies from bright red (young leaves) to dark green (old leaves) (Larraburu et al., 2010). *Viburnum tinus* L. belonging to the Caprifoliaceae family (or to the *Adoxaceae* family according to the APG classification), is an evergreen perennial shrub native of Mediterranean area with narrow leaves of a bright dark green color (Shao et al., 2019). Although these species are considered capable of thriving in urban conditions, only a few studies have investigated their responses to drought (Toscano et al., 2016, 2021; Bussotti et al., 2014).

Specifically, in light of the successful application of the LWM device in an indoor experiment (Brunetti et al., 2022); we hypothesize i) a reduction in water relations parameters and gas exchange performances in *P. fraseri* and *V. tinus* along the drought stress progression, followed by recovery under re-watering conditions; ii) an increase in the biosynthesis of secondary metabolites to counteract oxidative damages under drought stress conditions; iii) that Leaf Water Meter sensors may accurately monitor plant water status in outdoor conditions, resulting in a reliable tool to optimize irrigation strategies in urban contexts.

Thus, this study aims at investigating the tolerance of *P. fraseri* and *V. tinus* to drought of increasing severity, and their resilience and recovery ability after a period of rewatering. In detail, we assessed the most relevant physiological parameters and specific biochemical traits involved in photoprotection mechanisms. In addition, during the experiment, LWM sensors have been applied to monitor the water status of these two shrub species to assess their applicability in outdoor conditions simulating a possible application in urban context.

2. Materials and methods

2.1. Plant material

Three-year-old *Photinia x fraseri* Dress 'Red Robin' and *Viburnum tinus* L. plants were purchased from "Società Agricola F.lli Gorini piante s.s." were grown at the experimental station of GEA (Green Economy and Agriculture) located at 43°91′93″ N and 10°90′72″ E, Pistoia Tuscany, Italy. In July 2021, a total of forty-eight plants (twenty-four per species) were transplanted in 10-L (24 cm Ø) plastic pots containing a substrate of peat and pumice (4-1, w/w) added with 4.5 g L^{-1} of Basacote® Plus (12M; 15N-15P_2O_5-15K_2O). Before the beginning of the experiment, plants were daily watered to pot capacity for two weeks, then, preliminary measurements of net photosynthesis (A, μmol m^{-2} s^{-1}), stomatal conductance (g_{sw}, mmol m^{-2} s^{-1}), maximum photochemical efficiency of photosystem II (F_v/F_m), and leaf water potential (Ψ_{leaf}, MPa) were conducted to ensure the homogeneity of plant material.

2.2. Experimental conditions

The experiment lasted for a total of twenty-one days (from July 1, 2021 to July 21, 2021). Twelve plants per species were irrigated daily to pot capacity and used as control plants. The remaining twelve plants per species were treated with progressive water stress imposed by providing 80 % of the fraction of transpirable soil water (FTSW) (Brunetti et al., 2018) from 1st July to 9th July, followed by a rewatering of eleven days (from 10th July to 21st July) in which plants were irrigated to pot capacity (100 % FTSW) Figure SM1. Four control and four treated plants were sampled at each sampling time ($n = 4$). Two samplings were performed on 7th and 9th July (T1 and T2, respectively) to compare treated plants under water stress (WS1 at T1 and WS2 at T2) with control plants (Figure SM1). Plants were sampled at two different times during the water stress progression to ensure the achievement of a water stress level characterized by stomatal closure and photoinhibition. Then, a third and last sampling was performed on 21st July (T3) to compare treated plants under rewatering (RW) with control plants to evaluate the reversibility of water stress damages (Figure SM1). For greater clarity, the figure of the sampling design is reported as SM1.

Samplings were performed at midday (from 12:00 to 14:00 h) under clear sky conditions (midday mean temperatures, (°C) values and mean solar irradiance (Wm^{-2}) available in Fig. SM2 and consisted of physiological measurements and biochemical analysis conducted on healthy and fully expanded leaves at the top of the canopy.

2.3. Application of leaf water meter sensor (LWM)

The water status of the plants was monitored on a *continuum* using Leaf Water Meter (LWM) sensors previously validated by Brunetti et al. (2022) and provided by PaStella Factory.

Briefly, LWM is an alkaline battery-powered device, composed of a plastic clamp connected to a readout system equipped with climate and soil water content sensors and a LoRa module. The clamp contained LEDs and photodiodes, which measured leaf water content by emitting analog signals at two wavelengths: 1450 nm for water status estimation and dry matter absorption, and 890 nm for dry matter absorption. The analogic signals were digitized and sent to the LoRa gateway every 5 min, with a maximum operating distance of 20 m. The data were thermally calibrated and normalized to determine the leaf's dehydration level as described in Brunetti et al. (2022).

An LWM sensor was installed on two plants per treatment for the duration of the experiment. The leaf clip of the sensor was installed on a mature and healthy leaf, avoiding the midrib, 20 cm below the top of the plant. LWM recorded the leaf dehydration level (DL, %) with a 15-min temporal resolution using an online data transmission system.

In addition, the soil water content was monitored using soil moisture sensors (S-Soil MT-02A, Seed Studio). For each species and treatment, three soil moisture sensors were inserted in the pot at 10 cm of depth and connected to the readout system.

Furthermore, for the entire duration of the experiment, g_{sw} was measured every two days on four plants per treatment using an open system LI-600 fluorometer/parameter (LI-COR Biosciences) on the same leaves where the LWM sensors were applied. The measurements were performed at midday (from 12:00 to 14:00 h) under clear sky conditions (midday mean temperatures, (°C) values, and mean solar irradiance (Wm^{-2}) available in Fig. SM2) and recorded at stable g_{ws} values.

2.4. Physiological measurements

2.4.1. Water relations

Three twigs per plant were collected at the experimental garden, put in a zip-locked plastic bags, stored in a cooler and transported to the laboratory to determine Relative Water Content (RWC, %). Leaves were immediately measured for Fresh Weight (FW) once in the laboratory using a digital analytical balance (Precisa® 125A, Dietikon,

Switzerland), and then leaves were hydrated overnight until saturation (constant weight) in the dark to determine Turgid Weight (TW). Afterwards, leaves were oven-dried at 80 °C for 72 h until constant weight to determine Dry Weight (DW). Finally, RWC was calculated using the following equation:

$$RWC\ (\%) = (FW - DW) - (TW - DW) \quad \text{(Eq. 2)}$$

Leaf water potential was measured at midday on three twigs per plant using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). Additionally, three leaves per plant were cut, wrapped in parafilm and aluminum foil, transported to the laboratory in a cooler and stored at −20 °C for the osmotic potential measurements (Ψ_π, MPa). The osmotic potential was measured on freeze-thaw leaf expressed sap using a thermocouple psychrometer (PSY1, ICT International, Armidale, NSW, Australi (Callister et al., 2006). Water and osmotic potential were measured on leaves collected from the same branch used for RWC and gas exchange measurements. Single leaf measurements were combined to make an individual plant replicate.

2.4.2. Leaf gas-exchanges

Net photosyntheis (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i, μmol m^{-2} s^{-1}) were measured on four plants per treatment at each sampling time. Leaf gas-exchange performances were measured on three leaves per plant using a portable infrared gas analyzer (Ciras-3; PP-systems, Amesbury, MA, USA), operating at a CO_2 concentration of 410 ppm, at light intensity of 1800 μmol m^{-2} s^{-1} (according to the ambient PAR) and leaf temperature set as external temperature. Single leaf measurements were combined to make an individual plant replicate.

2.4.3. Chlorophyll fluorescence

The same leaves used for gas exchange measurements were used for chlorophyll fluorescence measurement. Leaves were dark-adapted for 1 h and used to measure the maximum photochemical efficiency of PSII (F_v/F_m) using a portable fluorometer (Pocket Pea, Hansatech, Norflolk, United Kingdom). The quantum yield of photosystem II (Φ_{PSII}) was measured under ambient light conditions using an LI-600 fluorometer/porometer (LI-COR Biosciences). Additionally, the ratio of electron transport rate to net photosynthesis (ETR/A) was calculated to measure the excess electron transport that can be deviated from photosynthesis to alternative electron sinks (Alderotti et al., 2020). Specifically, ETR was calculated as:

$$ETR = \Phi_{PSII} * PPFD * \alpha * \beta \quad \text{(Eq. 1)}$$

where Φ_{PSII} is the quantum yield of photosystem II, PPFD was the photosynthetic photon flux density, α was leaf absorbance (0.84) and β was the partitioning factor between photosystems I and II (0.5).

2.5. Destructive biochemical measurements

2.5.1. Leaf polyphenols extraction

Three leaves per plant were collected, frozen in liquid nitrogen and transported in a cooler to the laboratory where they were stored at −80 °C until the moment of the extractions for biochemical analyses. Leaves from the same plants were pooled together to make individual replicates.

Leaf material was ground in a mortar with liquid nitrogen, and 300 mg of powder was subsequently extracted with 3 × 5.0 mL ethanol 75% solution (pH 2.5 adjusted with formic acid) using an ultrasonic bath (BioClass® CP104, Pistoia, Italy) at a constant frequency of 39 kHz and power of 100 W. After that, extracts were centrifuged (5 min, 9000 rpm, ALC® 4239R, Milan, Italy), and the supernatants were partitioned with 3 × 5 mL of n-hexane to remove lipophilic compounds that could interfere with the analysis. The hydroethanolic phase was reduced to dryness using a rotavapor (BUCHI® P12, Cornaredo, Italy; coupled to a vacuum controller V-855), and the residue was resuspended with 1.0 mL of MeOH: Milli-QH_2O solution (1:1 v/v, pH 2.5 adjusted with HCOOH). All the analyses were conducted in triplicate for each species, and after the extraction procedure, the solution was characterized and quantified by HPLC-DAD/Q-TOF.

2.5.2. Leaf polyphenols characterization and quantification analysis by LC/Q-TOF and HPLC-DAD

In order to identify the total polyphenols content present in the extract, their characterization was made by LC-QTOF (Agilent 6530C, Agilent Techologies SpA, Milan, Italy) utilizing a quadrupole mass spectrometer operated in the electrospray ionization (ESI) negative mode coupled to a diode array detector (DAD). The applied ESI parameters were as follows: capillary voltage, 4000 V; fragmentor 180 V; skimmer 60 V; OCT 1 RF Vpp 750 V; pressure of nebulizer 20 psi; drying gas temperature 325 °C; sheath gas temperature 400 °C. For the stationary phase, an Agilent Poroshell 120 Aq-C18 column (2.7 μm) was used and the mobile phase consisted of (1% HCOOH, solvent A) and acetonitrile (1% HCOOH, solvent B). The flow rate was of 0.25 mL/min and the injection volume was 1 μL. Regarding the separation of the compounds, two different methods were used.

For *P. fraseri*, the separation was conducted using the following gradient: 1 min (3 % B), 2–57 min (3–40 % B), 58–60 min (40% B), and with a post-run of 60–61 min (40-3 % B), with 62 min of total analysis time.

Instead for *V. tinus* the following gradient was used: 1 min (3 % B), 2–57 min (3–40 % B), 58–60 min (40% B), 61–65 min (40–80% B), 66–68 min (80% B), and with a post-run of 68–71 min (80-3 % B) with 72 min of total analysis time.

The quantification of the polyphenols presents in those extracted obtained was performed by HPLC-DAD analysis using a Perkin® Elmer Flexar liquid chromatography equipped with a quaternary 200Q/410 pump and an LC 200 diode array detector (DAD) (all from PerkinElmer®, Bradford®, CT, USA). The injection volume of aliquots of 3 and 5 μL for *P. fraseri* and *V. tinus* were used respectively. The stationary phase consisted of a Zorbax® C-18 column (temperature 35 °C) while the mobile phases and the gradients were described above were applied. The flow rate was of 0.6 mL/min.

All the chromatograms were obtained at 280 and 330 nm. The identification and quantification of polyphenol contents were carried out based on the retention time, UV spectral characteristics, and comparison with standards, as well as based on literature data. Different standards (caffeic acid, ferulic acid, p-coumaric acid, oleuropein, myricitrin, and quercetin-3-*O*-glucopyranoside) were used to obtain five-point calibration curves. All the procedures were conducted in triplicate and the results were given in mg g^{-1} DW.

2.5.3. Malondialdehyde (MDA) analysis

The assessment of lipid peroxidation was conducted spectrophotometrically, utilizing the thiobarbituric acid (TBA) reaction to detect the formation of malondialdehyde (MDA) (Velikova et al., 2000). Leaf material (~300 mg per plant, sampling four plants per treatment at each time point) was firstly powdered in liquid nitrogen and then added with 5 mL of TCA solution (0.1%, w:v). After the centrifugation, 0.5 mL of the supernatant was added to 1 mL 0.5% (w:v) TBA in 20% TCA. The mixture was incubated at 90 °C for 30 min, and the reaction was stopped by cooling in an ice bath. Then the obtained samples were centrifuged,

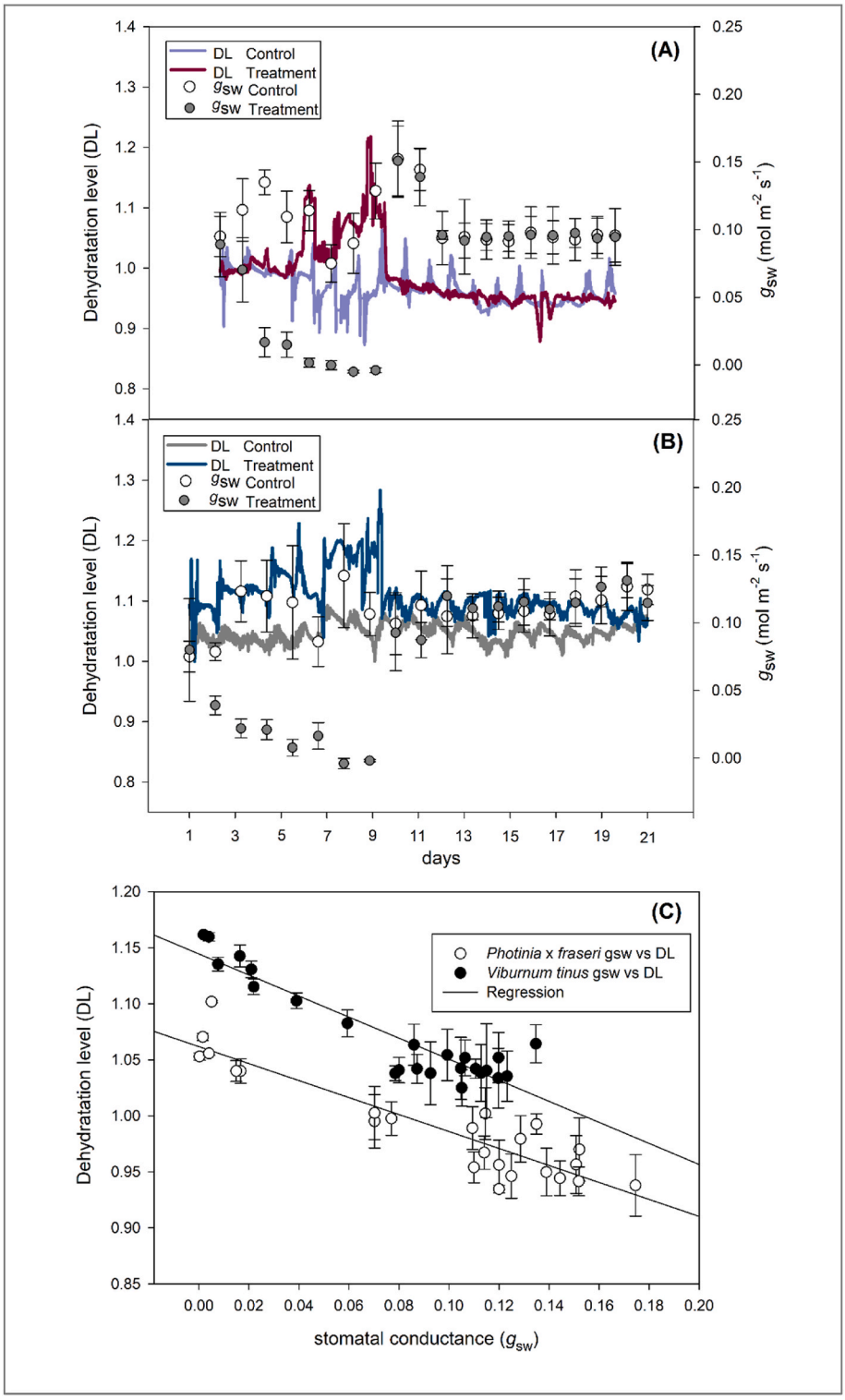

Fig. 1. Inverse linear relationship between leaf dehydration level (DL) measured by the Leaf Water Meter senso (LWM) and stomatal conductance (g_{sw}) *Photinia x fraseri* and *Viburnum tinus*. Data are in means ± sd ($n = 4$).

the supernatant was collected, and the absorbance was read at 532 nm. The amount of MDA–TBA complex was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

2.6. Non-destructive biochemical measurement

Three leaves per plant were measured with a Dualex® sensor (Dualex® Research, FORCE-A®, Orsay, France) to perform non-destructive biochemical measurements. The chlorophyll index (Chli) was obtained as the average of the adaxial and abaxial measurements. The Epidermal Flavonol index (Flavi) was calculated as the sum of both leaf sides measurements (Agati et al., 2016). Single leaf measurements were combined to make an individual plant replicate.

2.7. Statistical analysis

Data were checked for normal distribution and homoscedasticity. A *two-way* analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) was performed separately for each plant species to test the differences in physiological and biochemical parameters between treated and control plants at the different sampling times (T1, T2 and T3). Thus, water treatment and sampling time were considered as factors in the statistical analyses. All statistical analyses were performed using Sigma Plot 11.0 (Systat Software, Inc., San José, California, USA).

3. Results

3.1. Application of LWM sensors

Leaf Water Meter (LWM) sensors successfully recorded changes in leaf water status in outdoor conditions during the study (Fig. 1). Indeed, DL constantly raised both in *P. fraseri* and *V. tinus* treated plants during the water stress progression compared to controls (Fig. 1A and B), thus, reflecting the decline in soil moisture observed in treated plants (Figure SM3). In the same period, g_{sw} was considerably reduced in treated plants compared to control in both species (Fig. 1A and B). However, after rewatering (T3), DL and g_{sw} in treated plants of *P. fraseri* and *V. tinus* recovered to controls' values (Fig. 1A and B), mirroring soil moisture values (Figure SM3). The suitability of LWMs was also confirmed by the significant and inverse relationship between leaf dehydration level (DL) and stomatal conductance (g_{sw}) observed both in *P. fraseri* ($R^2 = 0.84$) and *V. tinus* ($R^2 = 0.85$) (Fig. 1C).

3.2. Water relations

Water relations significantly differed between treated and control plants of both species at T1 and T2, while no differences emerged at T3. In *P. fraseri*, relative water content (RWC) and water potential (Ψ_w) were lower in treated plants compared to controls at T1, and further declined, reaching the minimum values at T2 (RWC = ~64 %; $\Psi_w = \sim -5$ MPa) (Table 1). Additionally, a significant reduction in leaf osmotic potential (Ψ_π) was observed in treated plants compared to controls only at T2. However, RWC, Ψ_w and Ψ_π fully recovered to control values at T3 (Table 1).

In *V. tinus,* RWC, Ψ_w and Ψ_π exhibited a similar trend to that observed in *P. fraseri,* showing lower values in treated than control plants at T1 and T2, with the minimum at T2 (RWC = ~54 %; $\Psi_w = \sim -5.5$ MPa; $\Psi_\pi = -5.9$ MPa) and no differences at T3 (Table 1).

Table 1
Leaf Relative Water Content (RWC), water potential (Ψ_w), and osmotic potential (Ψ_π) in treated and controls of *Photinia x fraseri* and *Viburnum tinus* plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

Photinia x fraseri						
Time	RWC		Ψw		Ψπ	
	Control	Treatment	Control	Treatment	Control	Treatment
T1	93.29 ± 0.53 a	84.10 ± 3.30 b	−2.46 ± 0.29 a	−3.65 ± 0.36 b	−3.34 ± 0.88 b	−4.26 ± 0.45 c
T2	92.62 ± 1.92 a	63.57 ± 2.81 c	−2.45 ± 0.21 a	−4.96 ± 0.19 c	−3.04 ± 0.26 ab	−5.30 ± 0.37 d
T3	94.34 ± 1.46 a	91.50 ± 0.82 ab	−1.83 ± 0.42 a	−1.79 ± 0.18 a	−2.31 ± 0.42 a	−2.25 ± 0.37 a
Viburnum tinus						
Time	RWC		Ψw		Ψπ	
	Control	Treatment	Control	Treatment	Control	Treatment
T1	86.62 ± 4.01 a	67.46 ± 7.30 b	−2.50 ± 0.48 a	−4.60 ± 0.48 b	−2.79 ± 0.23 a	−5.20 ± 0.36 b
T2	93.57 ± 1.62 a	54.31 ± 4.58 c	−2.09 ± 0.42 a	−5.50 ± 0.02 c	−2.60 ± 0.55 a	−5.92 ± 0.38 c
T3	92.56 ± 3.98 a	87.53 ± 5.49 a	−1.83 ± 0.35 a	−1.71 ± 0.31 a	−2.40 ± 0.18 a	−2.52 ± 0.34 a

3.3. Leaf gas-exchange

Treated plants of both species significantly differed from controls at T1 and T2, while weak differences emerged at T3.

In *P. fraseri*, treated plants showed a strong reduction in *A* (-99%) and g_{sw} (−67%) compared to controls both at T1 and T2, while at T3, treated and control plants did not differ for these parameters (Fig. 2A and B). Additionally, treated plants exhibited higher values of C_i compared to controls during the entire study period (Fig. 2C).

In *V. tinus*, *A* and g_{sw} had a similar trend to that observed in *P. fraseri*, with the exception that at T3 treated plants showed higher g_{sw} values than controls (+7%) (Fig. 2D and E). Moreover, C_i was significantly higher in treated plants than controls at T1 and T2 (+35%), while no differences emerged under rewatering at T3 (Fig. 2F).

3.4. Chlorophyll fluorescence

The F_v/F_m parameter did not show significant differences between treated and control plants in *P. fraseri* during the whole study period (Fig. 3A). In *P. fraseri*, treated plants exhibited significantly lower values of the actual efficiency of photosystem II (Φ_{PSII}) compared to controls at both T1 and T2. Upon re-watering, *P. fraseri* treated plants displayed significantly higher values compared to controls (Fig. 3B). The Chl_i parameter was significantly reduced in treated plants compared to controls at T1 and T2, while no differences emerged among the plants at T3 (Fig. 3C).

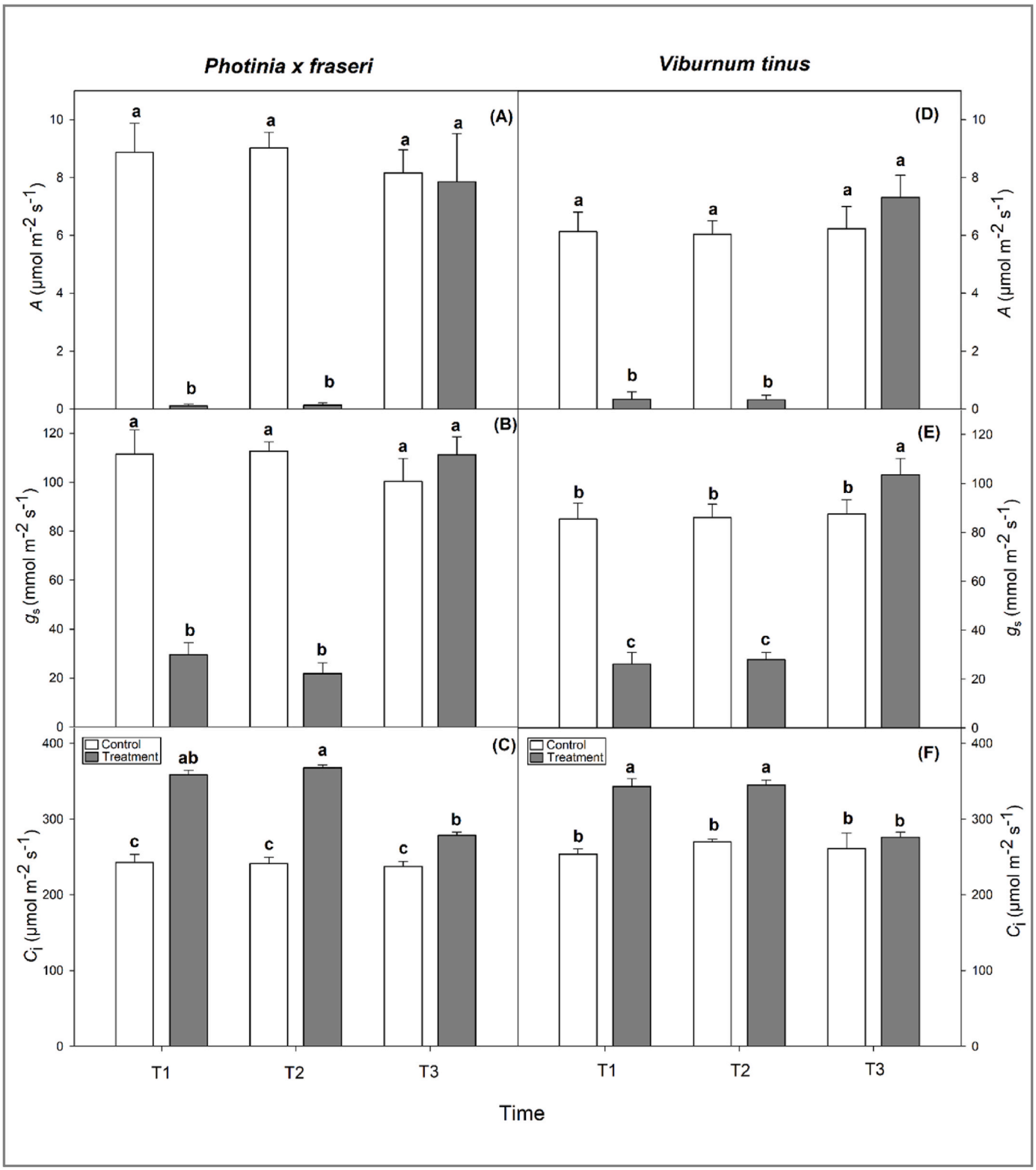

Fig. 2. Photosynthesis (*A*) (panels A and D), stomatal conductance (g_{sw}) (panels B and E), and intercellular CO_2 concentration (C_i) (panels C and F) in treated (dark grey bar) and control (white bar) *Photinia x fraseri* and *Viburnum tinus* plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are reported as means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

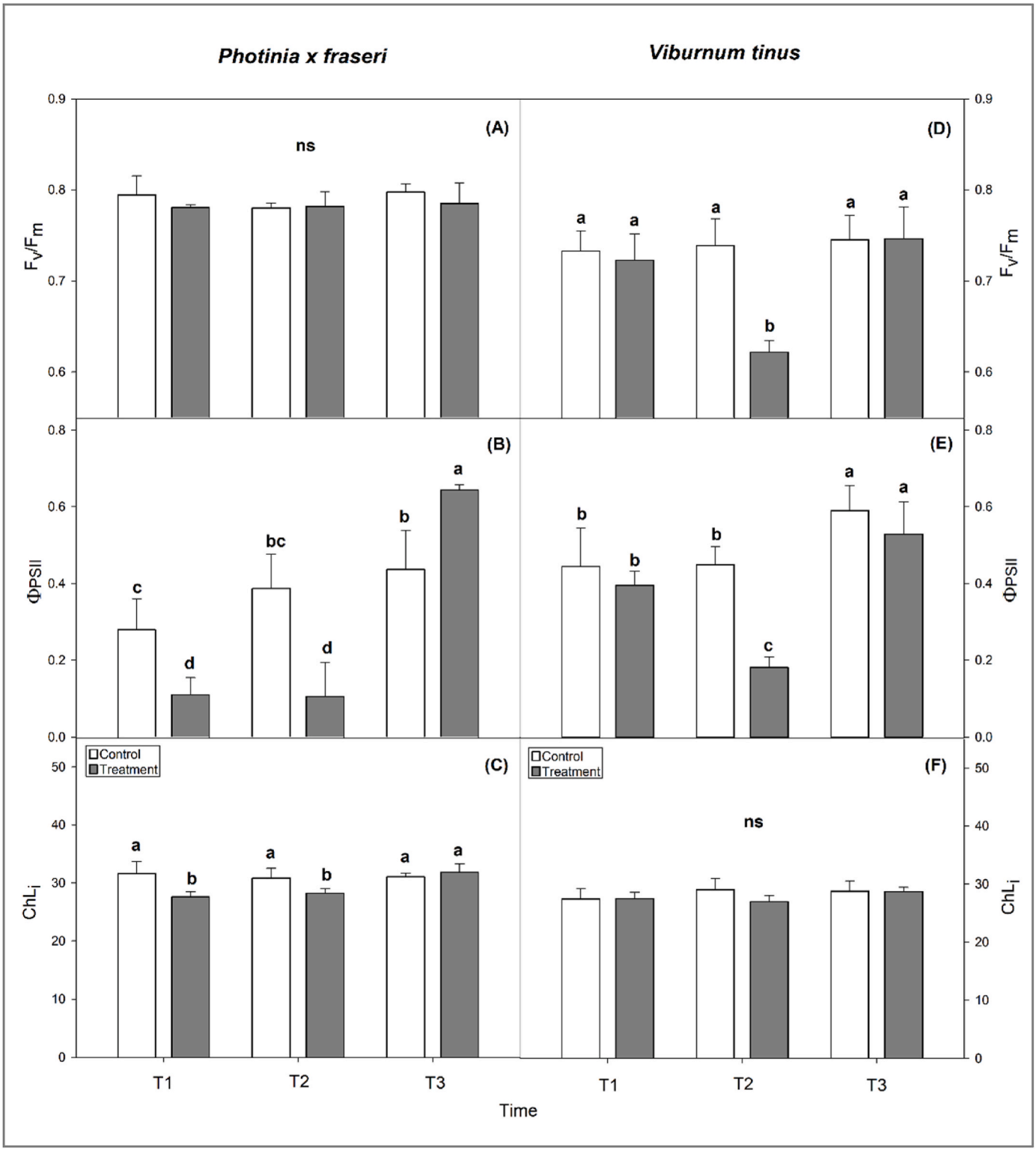

Fig. 3. Maximum efficiency of PSII photochemistry (F_v/F_m) (panels A and D), the actual efficiency of PSII (Φ_{PSII}) (panels B and E) and Chlorophyll index (Chl_i) (panels C and F) in treated (dark grey bar) and control (white bar) *Photinia x fraseri* and *Viburnum tinus* plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are reported as means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

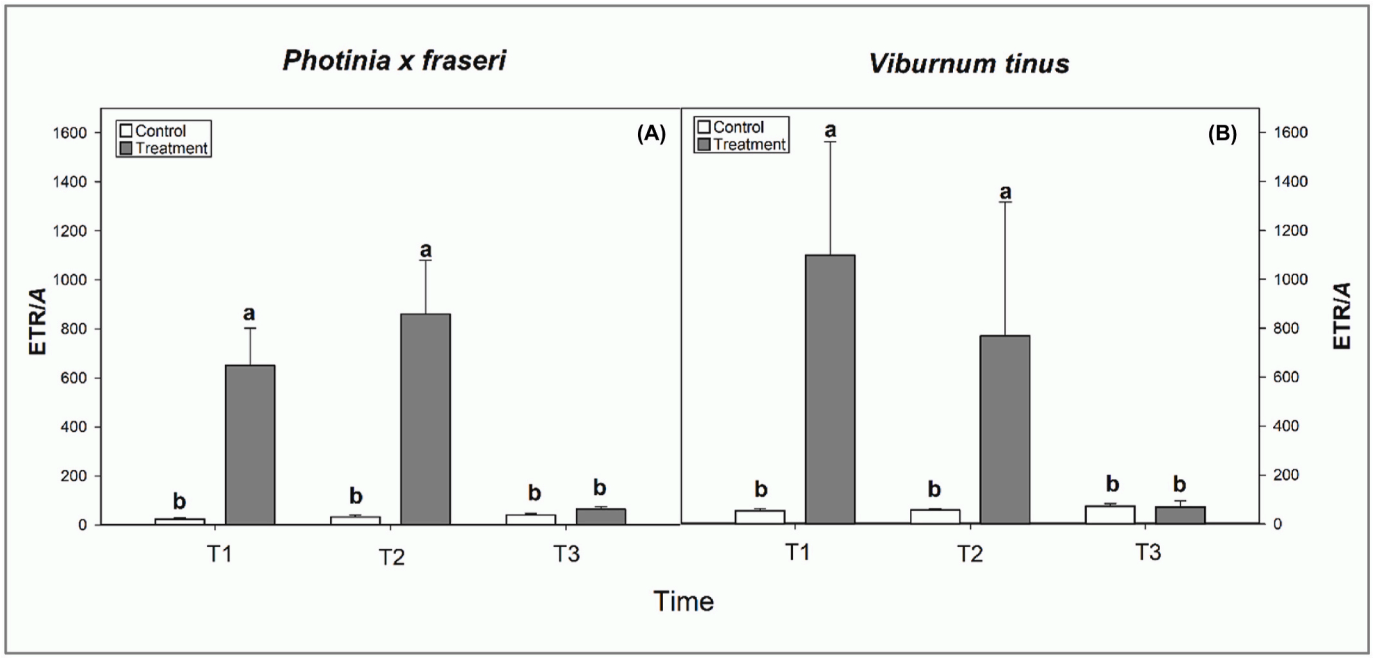

Fig. 4. Ratio of electron transport rate to net photosynthesis (ETR/*A*) in treated (dark grey bar) and control (white bar) *Photinia x fraseri* and *Viburnum tinus* (A and B, respectively) plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

In *V. tinus*, a significant decline in F_v/F_m was reported in treated plants compared to control at T2. This parameter recovered to the values of control plants at T3 (Fig. 3D). Similarly, to what was observed in F_v/F_m, treated plants showed a reduction in Φ_{PSII} values compared to controls only at T2 (−59%). However, at T3, Φ_{PSII} reached the maximum values with no statistical difference between treated and control in *V. tinus* plants (Fig. 3E). Contrary to *P. fraseri*, *V. tinus* did not show variation in Chl_i (Fig. 3F).

Interestingly, both *P. fraseri* and *V. tinus* treated plants showed a marked increment in the ratio of electron transport rate to net photosynthesis (ETR/*A*) compared to controls at T1 and T2. However, treated and control plants did not differ at T3 (Fig. 4A and B).

3.5. Leaf polyphenol characterization

In *P. fraseri*, two main classes of compounds were detected, namely hydroxycinnamic acids and flavonoids. Overall, eleven peaks were identified as chlorogenic acid derivatives (peaks 1, 3 and 5), coumaric acid derivatives (peaks 2 and 6), caffeic acid derivatives (peaks 4, 9) myricetin derivatives (peaks 8), and quercetin derivatives (peak 7, 10 and 11) (Figure SM4), with cholorogenic acid derivatives as the most abundant (Table SM1).

The polyphenolic profile of *V. tinus* showed three main classes of compounds, namely hydroxycinnamic acids, flavonoids, and secoiridoids. Overall, thirteen peaks were identified as chlorogenic acid derivatives (peaks 1 and 2), quercetin derivatives (peaks 3–4 and 8–9), myricitrin (peak 7) and iridoids derivatives (peaks 5–6 and 10–13) (Figure SM5). In addition, rutin was the most abundant compound detected in the extracts followed by chlorogenic acid isomer 2 and peak iridoids diglycosides (Table SM2).

3.6. Leaf polyphenol quantification and $Flav_i$

Significant differences in leaf total polyphenols and $Flav_i$ between treated and control plants were observed. In *P. fraseri*, higher values of total polyphenols (~+20%) and $Flav_i$ (~+11%) were detected in treated plants compared to controls at T1 and T2 (Fig. 5A and B). However, total polyphenols was lower in treated plants rather than controls at T3, when no differences in $Flav_i$ emerged (Fig. 5A and B).

Similarly to *P. fraseri*, in *V. tinus,* treated plants showed greater total polyphenol contents (~+10%) and higher values of $Flav_i$ (~+15%) compared to control plants both at T1 and T2 (Fig. 5C and D). At T3, the lowest total polyphenols and $Flav_i$ were observed, with no significant differences between treated and control plants (Fig. 5C and D).

3.6.1. MDA

The analysis of the malondialdehyde (MDA) leaf contents revealed significant variation in lipid peroxidation among treated and control plants of both species. Indeed, in both *P. fraseri* and *V. tinus*, MDA progressively rose in treated plants passing from T1 to T2, showing an increase at T2 of around three-fold in *P. fraseri* and of almost seven-fold *V. tinus* compared to controls. At T3, treated plants showed a reduction in MDA, although their values remained significantly higher values than controls in both species (Table 2).

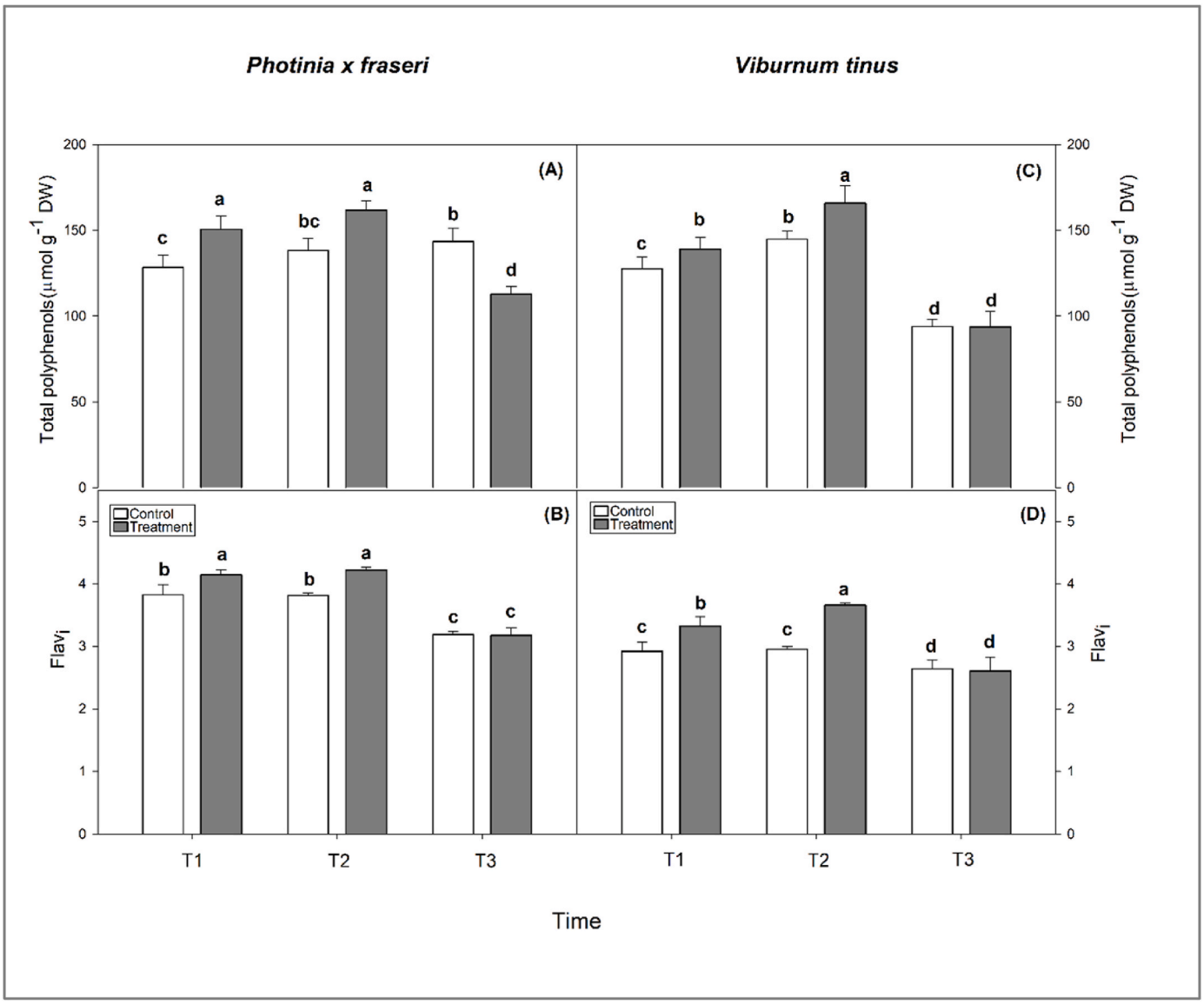

Fig. 5. Total polyphenols contents (panels A and C) and Flavonoind index ($Flav_i$) (panels Band D in treated (dark grey bar) and control (white bar) *Photinia x fraseri* and *Viburnum tinus* plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

Table 2
Analysis of Malondialdehyde leaf content (MDA) in treated and control *Photinia x fraseri* and *Viburnum tinus* plants at different sampling times. Specifically, treated plants were under a progressive water stress treatment at T1 and T2, and under rewatering at T3. Data are means ± sd ($n = 4$). Different letters indicate significant differences among treated and control plants at the different sampling times ($p \leq 0.05$). Detailed results of two-way ANOVA are reported in Tables SM3.

Time	Malondialdehyde analysis (MDA)			
	Photinia x fraseri		Viburum tinus	
	control	treated	control	treated
T1-WS1	2.98 ± 0.25 d	8.30 ± 0.90 b	2.08 ± 0.97 d	11.58 ± 1.00 b
T2-WS2	2.86 ± 0.33 d	12.26 ± 0.80 a	1.87 ± 0.76 d	14.74 ± 1.11 a
T3-RW	2.99 ± 0.25 d	5.16 ± 0.72 c	1.90 ± 0.71 d	5.60 ± 0.75 c

4. Discussion

The importance of water management in the urban environment is indisputable and crucial for plant growth and development and, consequently, to ensure the provisioning of related ecosystem services (Wattenhofer et al., 2021; Franceschi et al., 2023). Indeed, drought stress has a detrimental impact on plant growth through the modification of physiological aspects such as plant water status and CO_2 assimilation, as well as biochemical features such as photosynthetic pigments and secondary metabolites contents (Flexas et al., 2002; Toscano et al., 2019). During water stress progression, *P. fraseri* and *V. tinus* showed a strong reduction in the leaf RWC and water and osmotic potentials. The reduction in these plant-water relation parameters is a common phenomenon also observed in previous studies on these drought-stressed plants (Toscano et al., 2016). In particular, stressed *P. fraseri* plants showed a lower reduction at T1 with respect to its relative controls in the leaf RWC parameter than *V. tinus* species, underlining a higher sensitivity for *V. tinus* plants to leaf dehydration when subjected to drought (Toscano et al., 2016). At T2, the decrease in the RWC parameter was

paralleled with a strong and progressive reduction of both water and osmotic potentials in both species, underlining an intensification of the stress. Lowering values of water and osmotic potentials in leaves under severe drought treatment is a plant defensive strategy to facilitate the water transport from the soil to the transpiring leaves to maintain leaf cell tissue integrity until the rehydration (i.e., irrigation time) (Ugolini et al., 2012). Indeed, both *P. fraseri* and *V. tinus* showed to be tolerant species to drought, as treated plants promptly recovered plant water relation parameters to control values after the rewatering period (Toscano et al., 2014).

Plant hydraulic alterations significantly affected gas exchange parameters in both *P. fraseri* and *V. tinus*. Both species showed a reduction in the net CO_2 assimilation rate, mainly due to stomatal limitations as reduction in g_s occurred relatively soon after the imposition of water stress. Reductions in the stomatal conductance represent an early response of plants to drought (Flexas et al., 2014). Indeed, although stomatal closure limited CO_2 assimilation, it effectively protected leaves from excessive water loss and dehydration (Flexas et al., 2002). However, during prolonged water limitations, also, non-stomatal factors (e.g., biochemical and/or phochemical limitations) could rise, limiting leaf photosynthetic rates (Flexas et al., 2004). Indeed, the high values of C_i coupled with low and g_s suggested a metabolic impairment of the photosynthetic activity that could be linked to impairment of Rubisco regeneration under water stress in both species analysed (Grassi and Magnani, 2005). Moreover, drought stress also induced different leaf photochemical responses according to the species analysed. In particular, *Photinia* x *fraseri* did not experience reductions in the F_v/F_m values during all the drought stress period, which underlined that water treatment did not affect the PSII photochemical efficiency (i.e., no PSII photoinhibition) (Rai et al., 2008). The avoidance of photoinhibition in *P. fraseri* could be attributed to the degradation of chlorophylls, which, in turn, led to a reduction in the Φ_{PSII} (Kong et al., 2022; Wang et al., 2022). Indeed, previous authors suggested that the degradation of chlorophylls, by reducing excessive absorption of sunlight, may serve as a photoprotection mechanism (Flexas et al., 2002; Gori et al., 2019).

On the other hand, stressed *V. tinus* plants showed downregulation of F_v/F_m at T2, without any observable adjustments in chlorophyll content, a species response documented in prior studies (Toscano et al., 2014; Tribulato et al., 2019). However, this effect was swiftly reversed upon rewatering, suggesting that PSII functionality was restored quickly upon relief from drought (Guidi and Calatayud, 2014).

With the decrease in CO_2 assimilation rates, accompanied by a simultaneous reduction in NADPH consumption due to diminished sinking capacity, a feedback limitation occurred in the linear electron transport flow. This led to an imbalance between the generation and utilization of reducing power, as evidenced by the significant increase in ETR/A observed in both drought-stressed *P. fraseri* and *V. tinus* plants (Sebastiani et al., 2019). These imbalances can increase the production of reactive oxygen species (ROS), leading to lipid membrane damage (Ozkur et al., 2009). In accordance, MDA levels increased in both species under drought compared to their relative controls, followed by a decrease during the rewatering period. The observed decreases in MDA values at rewatering may be associated with plant tolerance to water stress (Toscano et al., 2016). Indeed, plants can effectively dispose of the excess of reducing power thanks to the biosynthesis of secondary metabolites, which may improve photoinhibition tolerance and offer protection from oxidative damage to photosynthetic organelles (Brunetti et al., 2015). Based on our knowledge, a characterization of polyphenols in *P. fraseri* leaves has not been carried out yet. Our results showed that both species have mainly derivatives of hydroxycinnamic acid and flavonoids (Figure SM4). In addition, *V. tinus* extracts also displayed the presence of secoiridoids, as previously reported by Sharifi-Rad et al. (2021) (Figure SM5). These last compounds could increase the leaf lifespan of the plants exposed to an excess of light and drought stresses (Tattini et al., 2004). Concerning quantitative differences, water stress induced a significant increase in total polyphenolic compound concentrations in both species (Fig. 5A–C). Among secondary metabolites, polyphenols perform different functions depending on their location in the leaf acting principally as antioxidants. Flavonoids accumulate in a range of tissues and subcellular compartments and can potentially help to reduce photo-oxidative damage under prolonged water stress conditions (Agati et al., 2012). Moreover, both species showed an increase in epidermal flavonoids index under water stress (Fig. 5B–D), which, together with an increment in hydroxycinnamic acids reduces the excessive UV-B radiation to chloroplasts, which usually contributes to stressing plants in conditions of water stress (Alipieva et al., 2014).

In this study, the dehydration level measured by LWM sensors in *P. fraseri* and *V. tinus* showed a clear rising trend during the water stress progression and a reduction after rewatering, confirming the reliability and suitability of such sensors in plant water status monitoring, even in outdoor conditions. Brunetti et al. (2022) highlighted the great potential of the LWM sensors as easy instrument technology to be utilized non-invasively in species with different leaf characteristics to monitor leaf water status. Thus, the significant and negative correlation found between the dehydration level measured by the sensor and the periodic measurements of stomatal conductance performed during the experiment in both species showed a further validation of the application of LWM sensors to monitor the physiological status of plants in outdoor contexts.

In the urban context, the application of such sensors may improve plant management practices aimed to guarantee the optimal water requirements based on plant performances. In particular, we suggest that LWM application would be beneficial in monitoring plant water status, especially in the delicate establishment period, thus allowing a prompt watering intervention to reduce plant mortality after transplanting in urban conditions (Wattenhofer et al., 2021).

5. Conclusion

In conclusion, our study sheds new light on the complex responses of *P. fraseri* and *V. tinus* species to drought stress and subsequent recovery. Indeed, we observed significant changes in plant water relations, physiology, and biochemistry, showcasing plant adaptive strategies to cope with water scarcity, including stomatal closure and biochemical adjustments. Both species exhibited tolerance to drought stress, swiftly recovering plant performances after rewatering, confirming our first hypothesis. In particular, our research underscores the role of secondary metabolites, particularly polyphenols, in mitigating oxidative damage and enhancing drought tolerance, in line with the second hypothesis. The increase in polyphenolic compounds under water stress suggests their vital role as antioxidants, while epidermal flavonoids may act as UV-B screening agents. Moreover, the third hypothesis was confirmed supporting the reliability of LWM sensors for detecting drought stress in out-door conditions as shown by the significant correlation between DL and stomatal conductance. This technology holds promise for real-time monitoring of plant water status in urban settings, potentially revolutionizing urban plant management practices. Future studies could explore the use of LWM sensors to optimize watering regimes and enhance plant establishment in urban landscapes.

CRediT authorship contribution statement

Cassandra Detti: Investigation, Data curation, Validation, Writing - original draft, Formal analysis. **Antonella Gori:** Investigation, Conceptualization, Methodology, Supervision, Writing - review & editing. **Lapo Azzini:** Investigation, Data curation, Formal analysis. Writing - original draft. **Francesco Paolo Nicese:** Writing - review & editing. **Francesca Alderotti:** Data curation, Writing - review & editing. **Ermes Lo Piccolo:** Data curation, Writing - review & editing. **Carlo Stella:** Methodology, Software, Data curation. **Francesco Ferrini:** Supervision, Writing - review & editing. **Cecilia Brunetti:** Investigation, Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.109208.

Data availability

Data will be made available on request.

References

Agati, G., Azzarello, E., Pollastri, S., Tattini, M., 2012. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. J. 196, 67–76.

Agati, G., Tuccio, L., Kusznierewicz, B., Chmiel, T., Bartoszek, A., Kowalski, A., Kaniszewski, S., 2016. Nondestructive optical sensing of flavonols and chlorophyll in white head cabbage (*Brassica oleracea* L. var. capitata subvar. alba) grown under different nitrogen regimens. J. Agric. Food Chem. 64, 85–94.

Alderotti, F., Brunetti, C., Marino, G., Centritto, M., Ferrini, F., Giordano, C., Gori, A., 2020. Coordination of morpho-physiological and metabolic traits of *Cistus incanus* L. to overcome heatwave-associated summer drought: a two-year on-site field study. Front. Ecol. Evol. 8, 576296.

Alipieva, K., Korkina, L., Orhan, I.E., Georgiev, M.I., 2014. Verbascoside—a review of its occurrence, (bio) synthesis and pharmacological significance. Biotechnol. Adv. 32, 1065–1076.

Brunetti, C., Guidi, L., Sebastiani, F., Tattini, M., 2015. Isoprenoids and phenylpropanoids are key components of the antioxidant defense system of plants facing severe excess light stress. Environ. Exp. Bot. 119, 54–62.

Brunetti, C., Loreto, F., Ferrini, F., Gori, A., Guidi, L., Remorini, D., Tattini, M., 2018. Metabolic plasticity in the hygrophyte *Moringa oleifera* exposed to water stress. Tree Physiol. 38, 1640–1654.

Brunetti, C., Alderotti, C., Pasquni, D., Stella, C., Gori, A., Ferrini, F., Centritto, M., 2022. On-line monitoring of plant water status: validation of a novel sensor based on photon attenuation of radiation through the leaf. Sci. Total Environ. 817, 152881.

Bussotti, F., Ferrini, F., Pollastrini, M., Fini, A., 2014. The challenge of Mediterranean sclerophyllous vegetation under climate change: from acclimation to adaptation. Environ. Exp. Bot. 103, 80–98.

Bwambale, E., Abagale, F.K., Anornu, G.K., 2022. Smart irrigation monitoring and control strategies for improving water use efficiency in precision agriculture: a review. Agric. Water Manag. 260, 107324.

Callister, A.N., Arndt, S.K., Adams, M.A., 2006. Comparison of four methods for measuring osmotic potential of tree leaves. Physiol. Plantarum 127, 383–392.

Flexas, J., Bota, J., Escalona, J.M., Sampol, B., Medrano, H., 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29 (4), 461–471.

Flexas, J., Bota, J., Cifre, J., Mariano Escalona, J., Galmés, J., Gulías, J., Medrano, H., 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. Ann. Appl. Biol. 144 (3), 273–283.

Flexas, J., Díaz-Espejo, A., Gago, J., Gallé, A., Galmés, J., Gulías, J., Medrano, H., 2014. Photosynthetic limitations in Mediterranean plants: a review. Environ. Exp. Bot. 103, 12–23.

Franceschi, E., Moser-Reischl, A., Honold, M., Rahman, M.A., Pretzsch, H., Pauleit, S., Rötzer, T., 2023. Urban environment, drought events and climate change strongly affect the growth of common urban tree species in a temperate city. Urban For. Urban Green. 88, 128083.

Fuentes, S., Tongson, E., Gonzalez Viejo, C., 2021. Urban green infrastructure monitoring using remote sensing from integrated visible and thermal infrared cameras mounted on a moving vehicle. Sensors 21 (1), 295.

Gori, A., Tattini, M., Centritto, M., Ferrini, F., Marino, G., Mori, J., Guidi, L., Brunetti, C., 2019. Seasonal and daily variations in primary and secondary metabolism of three maquis shrubs unveil different adaptive responses to Mediterranean climate. Cons. Physiol. 7 (1), coz070.

Grassi, G., Magnani, F., 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ. 28 (7), 834–849.

Guidi, L., Calatayud, A., 2014. Non-invasive tools to estimate stress-induced changes in photosynthetic performance in plants inhabiting Mediterranean areas. Environ. Exp. Bot. 103, 42–52.

Kong, Q., Zhang, J., Chen, S., Zhang, J., Ren, Y., Jin, X., Chen, J., 2022. Effects of periodic drought with severe exhaust exposure on particle retention capacity and physiological responses of *Photinia× fraseri* Dress. Ecotoxicol. Environ. Saf. 241, 113807.

Larraburu, E.E., Apóstolo, N.M., Llorente, B.E., 2010. Anatomy and morphology of photinia (*Photinia× fraseri* Dress) in vitro plants inoculated with rhizobacteria. Trees (Berl.) 24, 635–642.

McPherson, E.G., Berry, A.M., Van Doorn, N.S., 2018. Performance testing to identify climate-ready trees. Urban For. Urban Green. 29, 28–39.

Ozkur, O., Ozdemir, F., Bor, M., Turkan, I., 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environ. Exp. Bot. 66 (3), 487–492.

Pincetl, S., Gillespie, T., Pataki, D.E., Saatchi, S., Saphores, J.D., 2013. Urban tree planting programs, function or fashion? Los Angeles and urban tree planting campaigns. Geojournal 78, 475–493.

Percival, G.C., 2023. Heat tolerance of urban tree species-A review. Urban For. Urban Green. 128021.

Rai, R., Agrawal, M., 2008. Evaluation of physiological and biochemical responses of two rice (*Oryza sativa* L.) cultivars to ambient air pollution using open top chambers at a rural site in India. Sci. Total Environ. 407 (1), 679–691.

Sebastiani, F., Torre, S., Gori, A., Brunetti, C., Centritto, M., Ferrini, F., Tattini, M., 2019. Dissecting adaptation mechanisms to contrasting solar irradiance in the mediterranean shrub *Cistus incanus*. Int. J. Mol. Sci. 20 (14), 3599.

Shao, J.H., Chen, J., Xu, X.Q., Zhao, C.C., Dong, Z.L., Liu, W.Y., Shen, J., 2019. Chemical constituents and biological activities of *Viburnum macrocephalum* f. keteleeri. Nat. Prod. Res. 33, 1612–1616.

Sharifi-Rad, J., Quispe, C., Vergara, C.V., Kitic, D., Kostic, M., Armstrong, L., Cho, W.C., 2021. Genus *Viburnum:* therapeutic potentialities and agro-food-pharma applications: oxid. Med. Cell Longev.

Smith, I.A., Dearborn, V.K., Hutyra, L.R., 2019. Live fast, die young: Accelerated growth, mortality, and turnover in street trees. PLoS One 14 (5), e0215846.

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G., 2004. Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. New Phytol. 163 (3), 547–561.

Toscano, S., Scuderi, D., Giuffrida, F., Romano, D., 2014. Responses of Mediterranean ornamental shrubs to drought stress and recovery. Sci. Hortic. 178, 145–153.

Toscano, S., Farieri, E., Ferrante, A., Romano, D., 2016. Physiological and biochemical responses in two ornamental shrubs to drought stress. Front. Plant Sci. 7, 186872.

Toscano, S., Ferrante, A., Romano, D., 2019. Response of Mediterranean ornamental plants to drought stress. Horticulturae 5 (1), 6.

Toscano, S., Cavallaro, V., Ferrante, A., Romano, D., Patané, C., 2021. Effects of different light spectra on final biomass production and nutritional quality of two microgreens. Plants 10 (8), 1584.

Tribulato, A., Toscano, S., Di Lorenzo, V., Romano, D., 2019. Effects of water stress on gas exchange, water relations and leaf structure in two ornamental shrubs in the Mediterranean area. Agronomy 9 (7), 381.

Ugolini, F., Bussotti, F., 2012. Effects of deficit irrigation on leaf gas exchange, water potential and biomass of two ornamental species: *photinia× fraseri'Red* Robin'and *Viburnum opulus* L. In: VII International Symposium on Irrigation of Horticultural Crops 1038, pp. 463–470.

Velikova, V., Yordanov, I., Edreva, A.J.P.S., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151 (1), 59–66.

Wang, G., Zeng, F., Song, P., Sun, B., Wang, Q., Wang, J., 2022. Effects of reduced chlorophyll content on photosystem functions and photosynthetic electron transport rate in rice leaves. J. Plant Physiol. 272, 153669.

Wattenhofer, D.J., Johnson, G.R., 2021. Understanding why young urban trees die can improve future success. Urban For. Urban Green. 64, 127247.