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Research paper

LED streetlamps alter tree architecture, downregulate the photosynthetic process and alter the sugar metabolism of *Populus alba* L.

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ABSTRACT

The escalating issue of light pollution in urban environments poses multifaceted challenges, affecting not only the nocturnal sky but also exerting intricate influences on plant physiology. This study delves into the physiological responses of an urban ornamental tree, *Populus alba* L. clone DI-1, to varying intensities of streetlamp LED night lighting (NL), a shift from traditional High-Pressure Sodium lamps. The investigation "sheds light" on the molecular pathways underlying observed physiological regulations, aiming to offer a comprehensive understanding of the manifold effects of NL on this tree species. NL altered tree architecture, i.e. increased branch length and diameters, underscoring the dynamic response of trees to nocturnal artificial lighting conditions. Regarding tree physiology, the NL-triggered net CO_2 assimilation (P_n) during the night resulted in limitations in stomatal conductance during daylight hours. This led to a reduction in P_n, particularly during dawn, hindering the quantum yield for the reduction of end acceptors of PSI. Changes in chlorophyll a-to-b proportion and overall concentration, electron transport chain, and gene expression further highlight the intricate interplay between NL and plant metabolic regulation. Notably, the increased gene expression of sugar transporters in both NL trees suggested a responsive shift in sugar and starch metabolism. This was reflected in the absence of a starch accumulation during daylight hours in NL leaves. The study emphasizes the need for a holistic approach to urban lighting, considering its profound impact on photosynthesizing citizens. These findings highlight the pressing need for the development of innovative lighting spectra with reduced impact on plant physio-chemistry while ensuring visibility for citizen safety.

1. Introduction

In contemporary urban landscapes, there is a relentless rise in light pollution, averaging around 6 % annually due to the enhancement of human activities (Hölker et al., 2010). The artificial illumination not only shapes the nocturnal sky but also induces profound modifications in the natural world, influencing both animal behaviour and plant physiology (Bennie et al., 2016; Lo Piccolo et al., 2023; Rich and Longcore, 2006). The increasing light pollution in urban areas has been reported to alter the natural behaviours of tree (Kwak et al., 2018; Liu et al., 2021; Lockett et al., 2022) and herbaceous species (Speißer et al., 2021), affecting their growth and biomass. Although these alterations on plants have been observed for almost a century (Matzke, 1936), the comprehensive understanding of the real amplitude of this phenomenon remains elusive due to the limited number of studies dedicated to unravelling the physiochemical traits associated with night streetlamp-illuminated plants. Moreover, only a work dating back more than 20 years ago tried to provide a comprehensive table detailing the susceptibility of various woody plants to streetlamp illumination (Chaney, 2002). However, in the last years, a gradual shift to Light Emitting Diode (LED) lamps, with a completely different light spectrum (a peak in the blue and orange-red spectrum) than older High-Pressure Sodium (HPS) lamps (emitting irradiance peaks mainly in the red spectrum), poses the need to study the possible new physiological alteration on our

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'photosynthesising citizens'.

Within plant biology, the pivotal role of plant photoreceptors (phytochromes, cryptochromes, and phototropins) in orchestrating plant circadian rhythms, perceiving seasonal changes, and regulating fundamental plant metabolic functions is well-established (Kong and Zheng, 2020; Su et al., 2021; Venkat and Muneer, 2022). Against this backdrop, the artificial light pollution from streetlamps emerges as a potential disruptor of these finely tuned mechanisms (Bennie et al., 2017, 2016). These alterations may encompass pivotal metabolic processes in trees, including hormone regulation and stress response systems, ultimately shaping the morphology and altering the ecosystem services provided by urban trees.

The responses of urban trees to night streetlamp illumination are reported as multifaced and sometimes contradictory, underscoring the complexity of this phenomenon. Among others, streetlamp night illumination resulted in: i) delays in autumn leaf fall and dormancy entry in some tree species (Lo Piccolo et al., 2023; Massetti, 2018; Matzke, 1936) ii) increase in leaf chlorophyll contents (Lo Piccolo et al., 2023, 2021); iii) rapid yellowing and leaf drop (Kwak et al., 2018); iv) nighttime active net CO_2 assimilation (Kwak et al., 2018; Lo Piccolo et al., 2023); v) increase in shoot biomass (Liu et al., 2021); vi) alteration in carbohydrate contents (Czaja and Kołton, 2022; Kwak et al., 2018; Liu et al., 2021; Lo Piccolo et al., 2023). Moreover, there are species-specific tree responses to streetlamp illumination, highlighting that night streetlamp illumination remains a critical area for exploration (Lo Piccolo et al., 2021).

The present work "shed light" on the physiological, biochemical and molecular responses of an urban ornamental tree (*Populus alba* L. clone DI-1) to different nighttime light intensities provided by a commercial LED streetlamp. Our dataset offers a detailed overview of the manifold effects of streetlamp illumination on white poplar, increasing the body of knowledge that is necessary to promote the best sustainable urban planning practices, including streetlamp illumination.

2. Materials and methods

2.1. Plant material

A set of 30 individuals (three years old and 160 cm high) of white poplar trees (*Populus alba* clone DI-1), were purchased from a plant nursery (Umbraflor, Perugia, IT). The trials were conducted in 2022 (Jan-Jul) under open field conditions at the Department of Agriculture, Food and Environment, University of Pisa, Italy (43°42′N, 10°25′E). In January, each tree was transplanted into a 12-L pot filled with peat mixture substrate. During the trial, trees were adequately watered and fertilized with a slow-release fertilizer (30 g plant^{-1}). The urban LED streetlamps (Philips Iridium Gen3, Philips S.p.A, MI, Italy) were fixed at 2 m above the ground (~ 40 cm above the canopy), and starting from February, 10 trees per treatment were grown under two different night lighting (NL) intensities: one intensity was set at the photosynthetically active radiation (PAR) of ~700 μmol m^{-2} s^{-1} (NL-700), and the other one was set at ~300 μmol m^{-2} s^{-1} (NL-300) measured at the canopy level (Fig. S1). The switching on and off of the streetlamp was controlled by an astronomical timer, imitating the switching on and off times of city lighting. The other 10 individuals were designated as controls (CK) and received no further illumination after dusk. Physiological, biochemical, and molecular analyses were conducted on mature leaves in the summer (July 2022) at dawn (1 h after sunrise), midday (zenith), sunset and at night (3 h after sunset).

2.2. Tree morphometric measurements

At the end of the experiment (November 2022), tree height, stem diameter (measured at root collar level) and branch diameter (measured at the branch attachment) and length were analyzed in CK, NL-300 and NL-700 trees (n = 5). Stem and branch material (n = 5 for each treatment) was partitioned to determine the fresh weight (FW). Then, the fresh samples were dried using a laboratory electric thermostatic oven (Memmert GmbH + Co. KG Universal Oven UN30, Schwa-bach, Germany) at 105 °C until constant weight (4 dd) and the dry weight (DW) was determined.

2.3. Leaf gas exchange and chlorophyll a fluorescence and leaf pigment measurements

Leaf gas exchange parameters were measured (n = 7) using a portable infrared gas analyzer LI-6400 system (Li-Cor, Lincoln, NE, USA). All gas exchange measurements were conducted on mature leaves (July 2022). Inside the leaf chamber, the CO_2 concentration was set to 400 μmol mol^{-1} by using the CO_2 mixer, the flow rate was 500 μmol s^{-1}, and light intensity inside the chamber was set at similar levels to the previously measured ambient light. Night measurements were conducted using a 6400–08 clear chamber bottom, with the ambient light serving as the illumination source. Once the steady state was reached, net photosynthetic rate (P_n), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were recorded.

Chlorophyll *a* fluorescence parameters were measured using a Plant Efficiency Analyzer fluorimeter (Handy PEA, Hansatech Ltd, Norfolk, UK). Measurements (n = 7) were conducted at the same time as the gas exchange analysis. Leaves were dark-adapted with leaf clips (4 mm diameter) for 20 min; then samples were lightened for one second with a saturating (up to 3000 μmol photons m^{-2} s^{-1}) red light pulse and fluorescence emission was recorded for one second. Then, the effects of the treatments were assessed by the maximum quantum yield of photosystem II (PSII) photochemistry (F_V/F_M), where $F_v = (F_M - F_0)$ is the variable fluorescence, F_0 is the basal fluorescence prior saturation pulse extrapolated from the line of best fit determined through the initial data point recorded at the onset of illumination, and F_M is the maximum chlorophyll a fluorescence after the saturating light pulse. The effects of night light treatments on trees were further considered in a second step by analysing OJIP curves. A series of other fluorescence parameters can be estimated from the analysis of OJIP curves (JIP-test; Strasser et al., 2004). Among the JIP-test parameters obtained (Fig. S2), the performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of Q_B (PI_{abs}), performance index for energy conservation from photons absorbed by PSII antenna until the reduction of PSI end acceptors (PI_{tot}), quantum yield for electron transport flux from Q_A to Q_B (φ_{Eo}), and quantum yield for the reduction of end acceptors of PSI (φ_{Ro}) were discussed in the present experiment.

For non-destructive chlorophyll analyses, 30 randomly selected mature leaves were measured at each sampling time. The chlorophyll index in leaves was determined utilising a leaf clip sensor DUALEX® (Force-A, Orsay, France). Destructive analyses of chlorophylls and carotenoids were conducted on three randomly selected mature leaves (n = 3) per treatment and time point. Leaf pigments were extracted as described by Papadakis et al. (2018). Chlorophyll and carotenoid concentrations were determined spectrophotometrically by collecting extract absorbance at 470 nm, 647 nm, and 663 nm and using the equations described by Lichtenthaler and Buschmann (2001).

2.4. Leaf starch and soluble sugar analyses

Sucrose, glucose, and fructose were extracted using the method described by Sotelo et al. (2014), with minor modifications. Around 50 mg of dried leaf samples (n = 3) were finely ground in a mortar and suspended in 10 ml of 80 % aqueous ethanol (v/v). After 30 min of exposure in an ultrasonic water bath at 60 °C, the solution was centrifuged at 10,000 g for 10 min at 10 °C. The supernatant was filtered using a high-performance liquid chromatography (HPLC) filter (pore size: 0.45 μm) to analyse soluble sugars, whereas the pellet was used for starch analysis. Quantification was performed using commercial kits (K-SUFRG and K-TSTA; Megazyme, Wicklow, Ireland) according to the

manufacturer's protocol.

2.5. RNA extraction sequencing and differential gene expression analysis

RNA-Seq analysis was conducted on the same trees in which physiological and biochemical analyses were conducted (n = 3). From each tree, we sampled one mature leaf at each time point (dawn, midday, and night), for a total of 27 samples. The leaves were immediately stored in liquid nitrogen and subsequently at −80 °C until RNA extraction post-picking. Approximately 100 mg of leaf tissue was ground in liquid nitrogen using a mortar and pestle. Total RNA isolation was carried out using the RNeasy Plant Mini kit (Qiagen, Valencia, California, USA), according to the manufacturer's protocol. The RNA samples were treated with DNase I for 30 minutes at 37 °C. Afterward, RNA samples integrity was verified through analysis on a 1 % (w/v) agarose gel, and their concentrations were measured using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Genewiz/Azenta (Leipzig, Germany) sequencing facility was engaged for cDNA library preparation (after eukaryote ribosomal RNA depletion) and sequencing on the Illumina NovaSeq platform. The samples were sequenced using a 2×150 paired-end (PE) configuration. Raw reads were deposited at NCBI under Bioproject with the accession number PRJNA1068859. The quality of raw reads was assessed using an open-source software, FastQC v0.12.1 (Andrews, 2010) and then raw reads were pre-processed to remove sequencing adapters and low-quality bases using fastp v0.23.2 (Chen et al., 2018) with a default mean quality of 20 and the automatic adapter detection. The quality of the clean reads was reassessed using FastQC. The resulting high-quality reads were aligned to the *Populus alba* reference genome (ASM523922v1) using STAR mapping through the RSEM toolkit (v.1–3-3). The raw counts of annotated genes of *Populus alba* were estimated using "rsem-calculate-expression" function of RSEM. Principal component analysis (PCA) was performed to assess the distribution of samples using the DESeq2 function plotPCA. The matrix of the read count data generated from RSEM quantification served as input to calculate Differential Expression Genes (DEGs) across samples.

We utilized the DESeq2 Bioconductor package v1.41.12 (Love et al., 2014) to normalize read counts and identify differentially expressed genes between different conditions with default parameters. The resulting p-values were adjusted (Padj) using Benjamini and Hochberg's approach for controlling the false discovery rate (FDR). We utilized the DeSeq2 "plotMA" function to generate scatter plots depicting log2 fold changes (on the y-axis) versus the mean of normalized counts (on the x-axis). The shrinkage of the log2FoldChange was performed using the DESeq2 inbuilt lfcShrink function with the apeglm method (Zhu et al., 2019). The corrected P value of 0.1 and abs |log2(Fold change)| of 0.5 was set as the threshold for significant differential expression. Gene Ontology (GO) annotation, Pfam family and InterPro domain for DEGs annotation were obtained by running InterProScan v5.25 (Quevillon et al., 2005). Gene expression heatmaps were produced with Morpheus, (https://software.broadinstitute.org/morpheus).

2.6. Statistical analysis

After checking the normality of distribution (Shapiro-Wilk test, 95 % confidence interval), data from morphological analyses were subjected to a one-way analysis of variance (ANOVA), considering treatment as the only source of variability. Physiological and biochemical data were subjected to a two-way ANOVA considering treatment and timepoint as sources of variability. Significant differences among nighttime treatments were determined by LSD Fisher post-hoc test ($P \leq 0.05$).

3. Results

3.1. Tree morphometric measurements

After the leaf fall stage, no statistical differences were observed for stem diameter at collar level, stem height, number of branches, and stem dry weight (Table 1). Whereas branch diameter, branch length, and branch dry weight data were higher in both NL trees, irrespective of the nocturnal light treatments, compared to CK trees (about +20 % in branch ø and branch length, and +58 % in branch dry weight; Table 1). The branch mass on the total tree biomass increased in both NL treatments compared to CK by about +45 % (Table 1).

3.2. Leaf gas exchange, chlorophyll a fluorescence responses and pigment contents

At dawn, both NL treatments induced reduced values in P_n compared to CK trees by about −19 %, while during midday, only NL-700 showed lower values than CK trees (Fig. 1a). No differences were observed at sunset, whereas at night, CK trees showed typical negative P_n values of trees under dark (dark respiration), while both NL trees remained photosynthetically active (Fig. 1a).

The analysis of g_s highlighted that at both dawn and midday timepoint, both NL treatments showed lower values than CK trees (−29 % and −21 % at dawn and midday, respectively; Fig. 1b). This trend was also maintained at sunset only by NL-700 trees (−31 % compared to CK trees). At night, a reverse condition was observed, and, both NL treatments showed higher values in g_s than CK trees (0.122 [average of NL treatments] *vs* 0.033 mol m^{-2} s^{-1}; Fig. 1b).

About the C_i trend, the most relevant differences were observed among treatments at night, when CK trees showed higher intercellular CO_2 concentration than NL trees (+38 %; Fig. 1c).

Both PI_{abs} and PI_{tot} were reduced in leaves from NL-300 and NL-700 plants at dawn compared to CK trees (−21 and −51 % [average of NL treatments] for PI_{abs} and PI_{tot}, respectively; Fig. 2a,b). Moreover, whereas PI_{abs} did not result in any variation during the following sampling points with respect to CK trees, PI_{tot} resulted in a consistently significant reduction in both NL treatments at midday and sunset (−61 and −31 % at midday and sunset, respectively), while during the night, only NL-700 recorded a significant value decrease compared to CK (−25 %; Fig. 2b).

No statistical differences in the F_V/F_M (i.e., φ_{Po}) parameter were observed among treatments but only among time (Fig. 2c). The highest F_V/F_M values were recorded at dawn and during the night, while a significant reduction of PSII maximum quantum yield was observed at midday (Fig. 2c).

Table 1
Tree morphological traits (stem diameter at the root collar level [$Ø_{collar}$], stem height, branch diameters at the branch attachment [$Ø_{attachment}$], branch length, branch number, stem and branch dry weights and % of branch weight on total tree biomass) of trees (*Populus alba* L. Clone DI-1), measured in November. Parameters were analysed in controls (CK) and trees grown under streetlamp night lighting (NL) with a PAR of 300 (NL-300) and 700 (NL-700) µmol m^{-2} s^{-1} (n = 5). Means ± SD were subjected to one-way ANOVA with treatment as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$).

		Treatments		
Parameters		**CK**	**NL-300**	**NL-700**
Stem $Ø_{collar}$	mm	22.33 ± 1.18	21.00 ± 1.27	20.90 ± 0.89
Stem height	cm	274.80 ± 10.57	271.00 ± 15.10	279.20 ± 5.63
Branch number	-	8.00 ± 1.41	7.60 ± 1.51	7.20 ± 2.77
Branch $Ø_{attachment}$	mm	3.36 ± 0.37^b	3.88 ± 0.17^a	4.19 ± 0.50^a
Branch length	cm	55.23 ± 7.71^b	67.44 ± 7.97^a	65.75 ± 6.73^a
Stem dry weight	g	149.05 ± 13.84	150.32 ± 14.42	156.67 ± 15.82
Branch dry weight	g	19.64 ± 3.37^b	30.93 ± 3.45^a	31.09 ± 8.29^a
Branch mass/Total biomass	%	11.60 ± 1.25^b	17.12 ± 1.95^a	16.51 ± 3.78^a

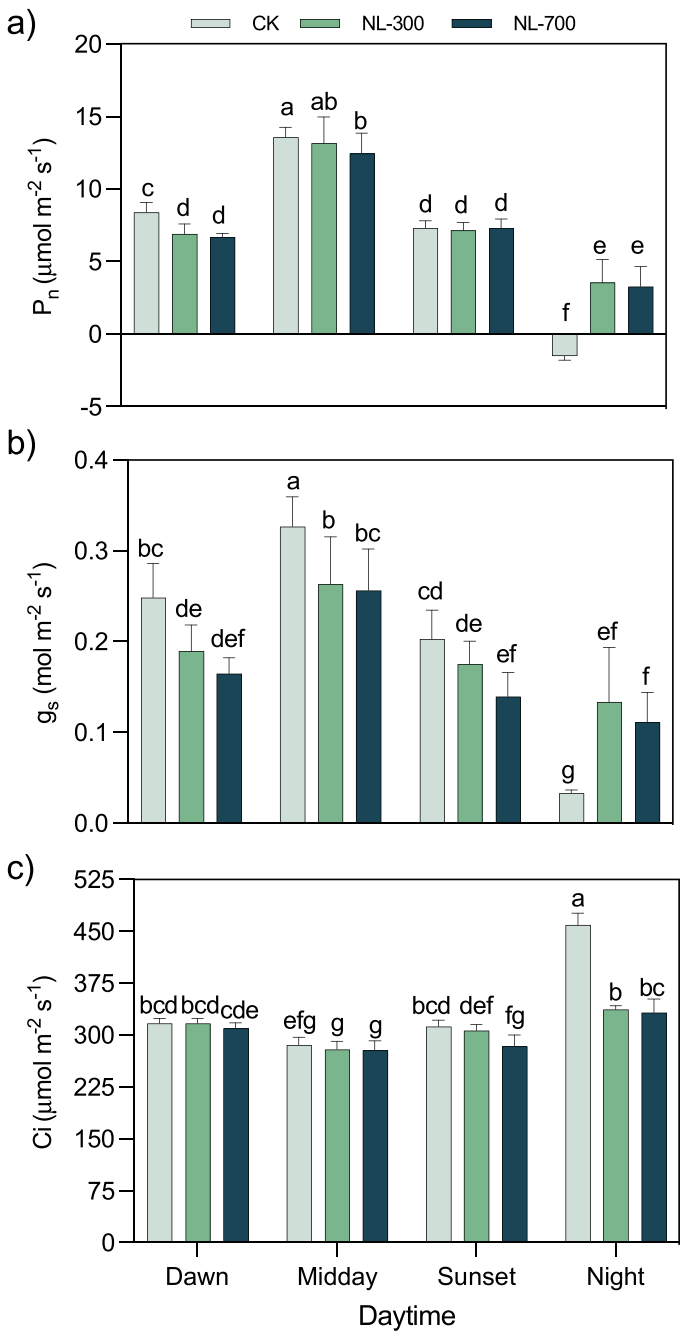

Fig. 1. Net CO_2 assimilation (P_n; a), stomatal conductance (g_s; b) and intercellular CO_2 concentration (C_i; c), in white poplar trees (*Populus alba* L. clone DI-1), measured at dawn, midday, sunset and night in leaves of controls (CK) and trees grown under streetlamp night lighting (NL) with a PAR of 300 (NL-300) and 700 (NL-700) µmol m^{-2} s^{-1} (n = 7). Means ± SD were subjected to two-way ANOVA with treatment and timepoint as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$).

The quantum yield of the electron transport flux from Q_A to Q_B (φ_{Eo}) was reduced at dawn only in NL-700 compared to CK trees (−15 %), while a significant increase in NL-300 was recorded at midday and at sunset compared to CK trees (+24 an +15 % at midday and sunset, respectively; Fig. 2d). At night, no differences were observed for this parameter among treatments (Fig. 2d). Concerning the quantum yield of the electron transport flux until the PSI electron acceptors (φ_{Ro}), it resulted reduced at any timepoint in both the treated NL leaves compared to CK (−16, −23, −21 and −16 % at dawn, midday, sunset and night, in NL-300 respectively; −28, −35, −25 and −19 % at dawn, midday, sunset and night, in NL-700 respectively; Fig. 2e).

The non-destructive analysis of total leaf chlorophylls Chl_{dualex} showed higher values in both NL treatments compared to CK trees (+7 %; Fig. 3a), and its daily trend showed lower values in all treatments at midday compared to both dawn and nighttime data (−9 %; Fig. 3a). Destructive leaf total chlorophyll (Chl_{tot}) analyses confirmed the non-destructive analyses showing higher values in Chl_{tot} in NL treatments compared to CK trees (+12 %; Fig. 3b). The analysis of leaf chlorophyll *a* (Chl_a) and chlorophyll *b* (Chl_b) confirmed same previous results showing a +11 % for Chl_a and +16 % for Chl_b for both NL treatments compared to CK trees (Fig. 3c,d). The leaf chlorophyll *a*:*b* ratio ($Chl_{a/b}$) decreased in both NL treatments compared to CK trees by 5 % (Fig. 3e). Its daily trend in all treatments showed lower values at midday compared to both dawn and nighttime (−5 %; Fig. 3e). The leaf total carotenoid (Car_{tot}) analysis showed statistical differences only among sampling time, with the lowest values at midday and highest at dawn (Fig. 3f).

3.3. Leaf starch and soluble sugars

Leaf starch content in CK increased during the daytime, whilst both NL treatments showed consistent values at every sampling point (Fig. 4a). Leaf sucrose contents increased at midday in all the treatments, but NL trees showed lower contents compared to CK trees (−18 %; Fig. 4b) without differences between the two light treatments and a similar pattern was observed at night. In all the treatments, leaf glucose and fructose showed lower values at night compared to those observed at dawn and midday (−35 %; Fig. 4c,d). Moreover, both leaf glucose and fructose contents were higher in NL treatments compared to CK trees (+22 and +41 % for glucose and fructose, respectively; Fig. 4c,d). No differences were observed in total soluble sugars among treatments but only among times, showing the highest values at midday (Fig. 4e).

3.4. Analysis of differentially expressed genes (DEGs)

RNA-Seq was used to identify differentially expressed genes associated with the response of white poplar to two distinct night lighting intensities. To identify transcripts of interest, 27 cDNA libraries were created from leaf RNA samples and underwent RNA-Seq analysis on the Illumina NovaSeq platform. A total of 884,927,802 short reads were generated, comprising approximately 20–44 million raw reads (~ 150-bp length) from each mRNA-seq library, with an average of 33 million reads. After quality assessment and raw data filtering, such as removing adaptor sequences and discarding low quality reads, 881,958,981 high–quality clean reads with a base quality score of 20 % were selected for further analysis. High quality reads were then mapped to the reference sequence, revealing that around 12–26 million trimmed paired-end reads from each RNA-Seq library were uniquely mapped to the reference genome (an average of 62 %).

Principal component analysis (PCA) was performed to assess the consistency of sample grouping with the experimental assumptions, elucidating the relatedness of the biological replicates (Fig. 5A). The PCA plot revealed that the samples primarily clustered based on the three sampling time points, highlighting the similarity of gene expression among the biological replicates for each sample. Gene expression patterns of trees treated with different NL intensities compared to CK were analysed using the DESeq2 pipeline (Fig. 5B)

In the pairwise comparisons (Table S1), a total of 1424 genes were significantly differentially expressed, with 726 genes up-regulated and 698 genes down-regulated. Among the significantly expressed genes, the highest number of DEGs was observed at midday in the NL-700 trees compared to CK (547), while NL-300 trees exhibited no significant differences in gene expressions. At dawn, only 58 differentially expressed genes were identified in the NL-300 trees, whereas NL-700 showed a higher number of DEGs, specifically 119. Furthermore, more DEGs were discovered at night in trees treated with NL-300 (525) compared to

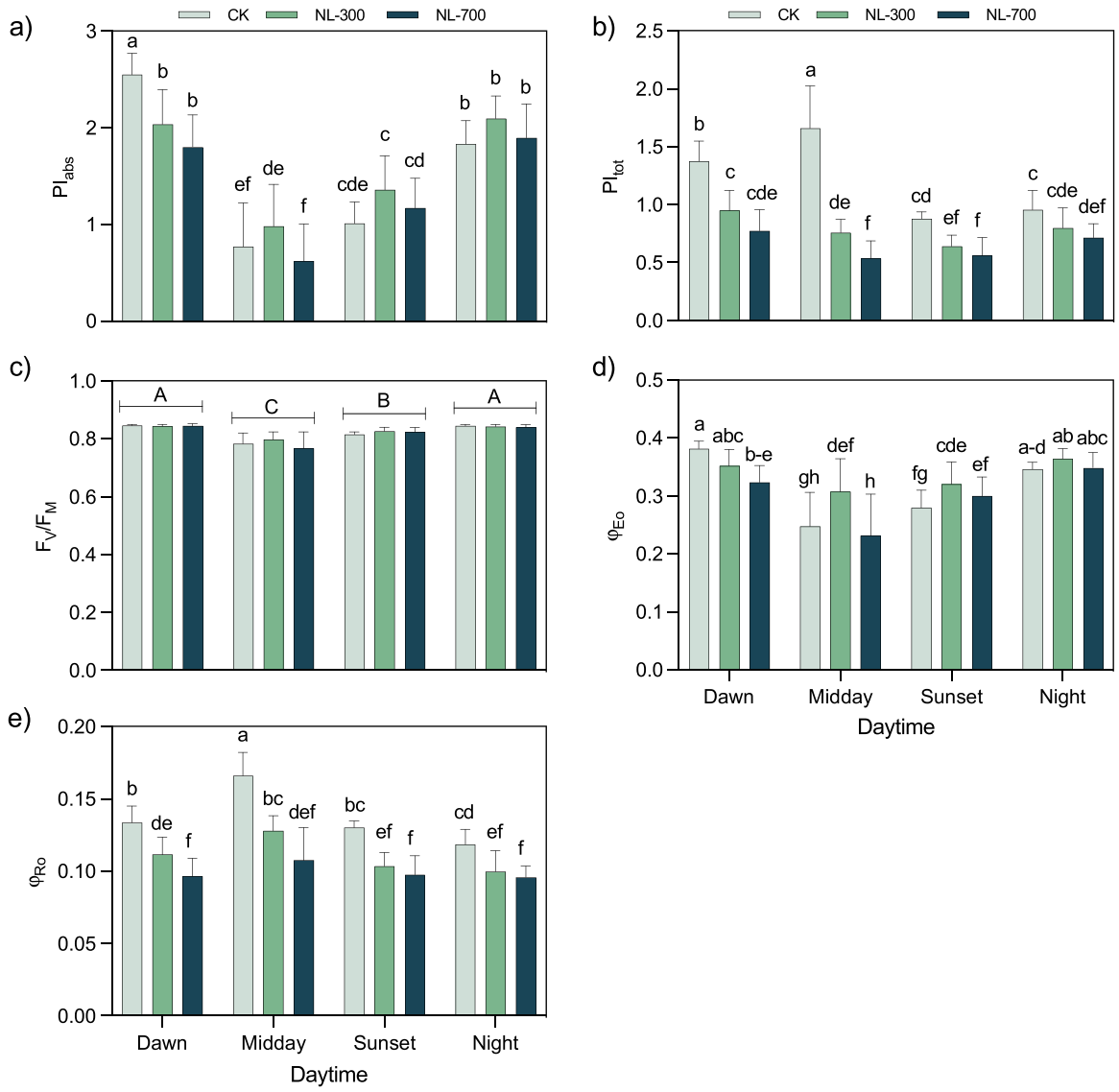

Fig. 2. Performance index on absorption basis (PI_{abs}; a), total performance index measuring the performance up to the PSI end electron acceptors (PI_{tot}; b), PSII maximum quantum yield (F_V/F_M or φ_{Po}; c); quantum yield for electron transport at t = 0 (φ_{Eo}; d), quantum yield for the reduction of end acceptors of PSI (φ_{Ro}; e), in white poplar trees (*Populus alba* L. clone DI-1), measured at dawn, midday, sunset and night in leaves of controls (CK) and trees grown under streetlamp night lighting (NL) with a PAR of 300 (NL-300) and 700 (NL-700) μmol m^{-2} s^{-1} (n = 7). Means ± SD were subjected to two-way ANOVA with treatment and timepoint as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$). When the F ratio of the interaction between the variability factors is not significant, capital letters indicate statistically significant differences between means over time.

those subjected to the higher light intensity NL-700 (179). At dawn, the majority of DEGs were up-regulated in both NL treatments (72 and 61 % for NL-300 and NL-700, respectively), while at other time-points, the number of up- and down-regulated genes are comparable (about 50 %).

We analyzed the biological function of the identified DEGs based on gene annotation. The identified DEGs are involved in a wide variety of biological functions, including not only stress signaling and hormone regulation pathways, but also the maintenance of the delicate balance of redox systems, the regulation of transcription and ion transport (Table S1). To explore the correlation between molecular pathways and observed physiological regulations, we focused on annotated genes associated with circadian rhythms, night stomatal aperture, and the production of starch and soluble sugars in the leaf that were significantly differentially expressed (Fig. 6).

At dawn, in both NL treatments, we observed the up-regulation of the gene (D5086_0000256040) encoding the Light-regulated (Lir1) protein. At nighttime, DEGs encoding matrix metalloproteinases (MMPs) and ATP-binding cassette (ABC) transporters were highly up-regulated both in NL-300 and NL-700 trees (Fig. 6).

Moreover, we found 2 genes (D5086_0000254070, D5086_0000073630) down-regulated that encode for a Nitrite/Sulfite reductase ferredoxin-like domain in treated trees at both NL intensities during the nighttime.

At night, we identified a transcript (D5086_0000289410) encoding a starch synthase catalytic domain that was up-regulated (LFC = 2.5), specifically in NL-300 plants. Moreover, a down-regulated transcript (D5086_0000109120) encoding for a 6-phosphofructo-2-kinase was observed. This protein is involved in the synthesis and degradation of fructose 2,6-bisphosphate, regulating carbon partitioning between sucrose and starch during the diurnal cycle. Regarding sucrose metabolism at night, a sucrose-6 F-phosphate phosphohydrolase (D5086_0000260310) (involved in the final step of sucrose biosynthesis) was found to be down-regulated in NL-300 plants, while in NL-700 plants, a down-regulation was observed in a gene (D5086_0000053870) encoding sucrose synthase. For both NL treatments compared to CK, we identified several genes encoding sugar

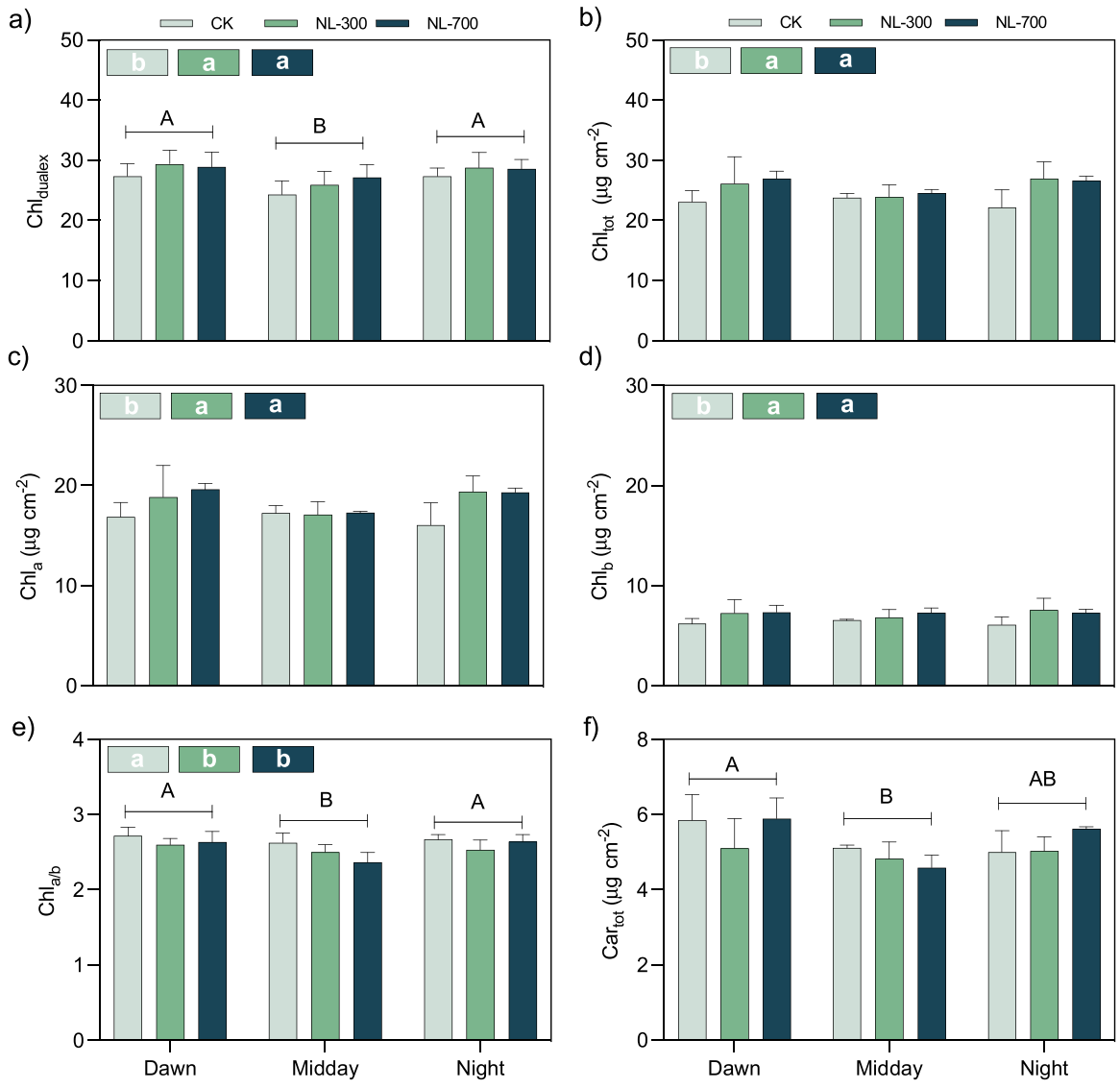

Fig. 3. Dualex chlorophyll index (Chl_{dualex}; a), total chlorophylls (Chl_{tot}; b), chlorophyll *a* (Chl_a; c), chlorophyll *b* (Chl_b; d), chlorophyll *a*:*b* ratio ($Chl_{a/b}$; e) and total carotenoids (Car_{tot}; f) in white poplar trees (*Populus alba* L. clone DI-1), measured at dawn, midday and night in leaves of controls (CK) and trees grown under streetlamp night lighting (NL) with a PAR of 300 (NL-300) and 700 (NL-700) µmol m^{-2} s^{-1} (n = 30 for Chl_{dualex}; n = 3 for destructive measurements). Means ± SD were subjected to two-way ANOVA with treatment and timepoint as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$). When the F ratio of the interaction between the variability factors is not significant, capital letters indicate statistically significant differences between means over time and lower-case letters in boxes over treatments.

transporters that exhibited up-regulation at all three time points. The most substantial increase was observed in NL-300 trees at nighttime, where one transcript (D5086_0000267690) reached a LFC of 2.4.

4. Discussion

The primary purpose of streetlamp installation in urban areas is to improve safety and visibility. Streetlamps emit light across the visible spectra, disturbing the natural light-dark cycle crucial for tree adaptation (Bennie et al., 2016). This disturbance can affect the intricate circadian rhythms governing various physiological activities, potentially altering tree architecture (Kwak et al., 2018; Liu et al., 2021).

In our experiment, the evidence suggesting an alteration in the circadian rhythm of NL trees is evidenced by the up-regulation of Lir1. Indeed, studies have shown that Lir1 mRNA accumulates in response to light and reaches its maximum level at the end of the light period, decreasing to a minimum at the end of the dark period (Dhandapani et al., 2015; Hayama et al., 2002; Reimmann and Dudler, 1993). In a previous work conducted on plants grown under continuous light, it was observed that circadian pattern of Lir1 was lost, maintaining constant levels throughout the 24 hours (Reimmann and Dudler, 1993), proving that its expression is regulated by both light and the circadian clock. The up-regulation of the Lir1 gene during the timepoint in NL compared to the CK trees highlights the alteration of circadian rhythms of NL poplar trees, although its role remains unelucidated. Moreover, our results show that streetlamp lighting altered, irrespectively of the light intensity used, the tree architecture by increasing the branch length and diameters, suggesting a higher C allocation on branches compared to CK trees (no statistical differences between treatments regarding stem biomass). To the best of our knowledge, although some studies have investigated total above-ground biomass or tree height finding in some cases a negative impact of streetlamps (Kwak et al., 2018), none of them has explored the influence of streetlamp lighting on above-ground tree architecture (branch/stem relationships). In our work, the larger size of branches found in both NL trees can be the consequence of the altered photoperiodism (Singhal et al., 2019). Indeed, both NL trees showed

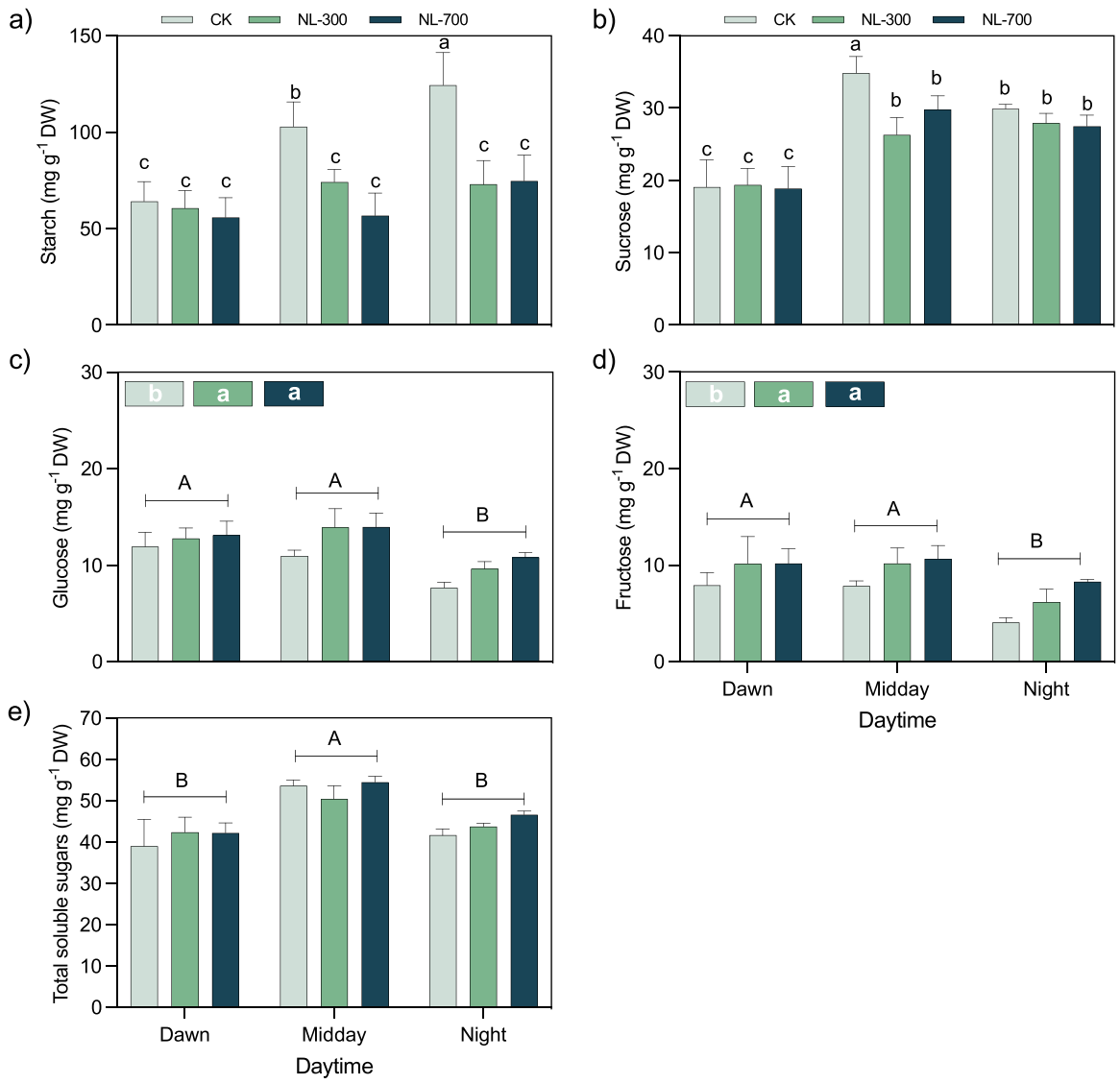

Fig. 4. Leaf starch (a), sucrose (b), glucose (c), fructose (d) and total soluble sugars (e) contents in white poplar trees (*Populus alba* L. clone DI-1), measured at dawn midday and night in leaves of controls (CK) and trees grown under streetlamp night lighting (NL) with a PAR of 300 (NL-300) and 700 (NL-700) μmol m^{-2} s^{-1} (n = 3). Means ± SD were subjected to two-way ANOVA with treatment and timepoint as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$). When the F ratio of the interaction between the variability factors is not significant, capital letters indicate statistically significant differences between means over time and lower-case letters in boxes over treatments.

positive net CO_2 assimilation and high stomatal opening compared to CK trees during the night, likely providing the C necessary for increased stimulation in the growth of sink organs, i.e., lateral branches.

At the genetic level, the upregulation of MMPs and ABC transporter genes in NL-treated plants, particularly during nighttime, might be associated with the sustained night stomatal opening and with the increased branch growth of both NL trees. MMPs are a group of zinc-dependent endopeptidases whose precise biological role in higher plants remains unclear (Liu et al., 2018). It has been suggested that plant MMPs participate in the remodelling of the extracellular matrix (ECM) in conjunction with plant growth and development. Previous studies (Flinn, 2008; Jill M. Maidment et al., 1999; Ratnaparkhe et al., 2009) have already proposed this association. The overexpression of Gm1-MMP (Liu et al., 2017) and Gm2-MMP (Liu et al., 2018) in *Arabidopsis* has been shown to impact leaf growth and leaf development transgenic, showing that *Arabidopsis* lines exhibited more robust growth, and their leaves displayed alterations in stomatal length and aperture.

In plants, ABC transporters play a variety of crucial roles, including nutrient transport, heavy metal uptake, detoxification of toxic compounds, hormone transport, and regulation of lipid transport (Do et al., 2018). Among these functions, it is worth noting the role played by two ABC transporters in *Arabidopsis thaliana* in stomatal opening, namely AtMRP4 (Klein et al., 2004) and AtABCB14 (Lee et al., 2008). Although the relevant genes in *Populus alba* are poorly characterized, considering this information collectively, it suggests their potential role in the nocturnal response of stomata and the growth of lateral branches of illuminated plants.

The observed increase in branch biomass leads to suppose a favourable impact of streetlamp lighting on tree physiology. However, trees illuminated at night showed lower net CO_2 assimilation rates at dawn and NL-700 also at midday but without significant difference with NL-300 individuals. This condition was mainly dependent on stomatal limitations to photosynthesis, as lower stomatal conductance, maintained almost throughout the day, was observed in both treatments compared to controls. Lower stomatal conductance values due to the loss of circadian rhythms in night-lighted trees were also observed by Kwak et al. (2017), (2018). Kwak et al. (2017) suggested that the alteration in stomatal movement in night-lighted trees is attributable to

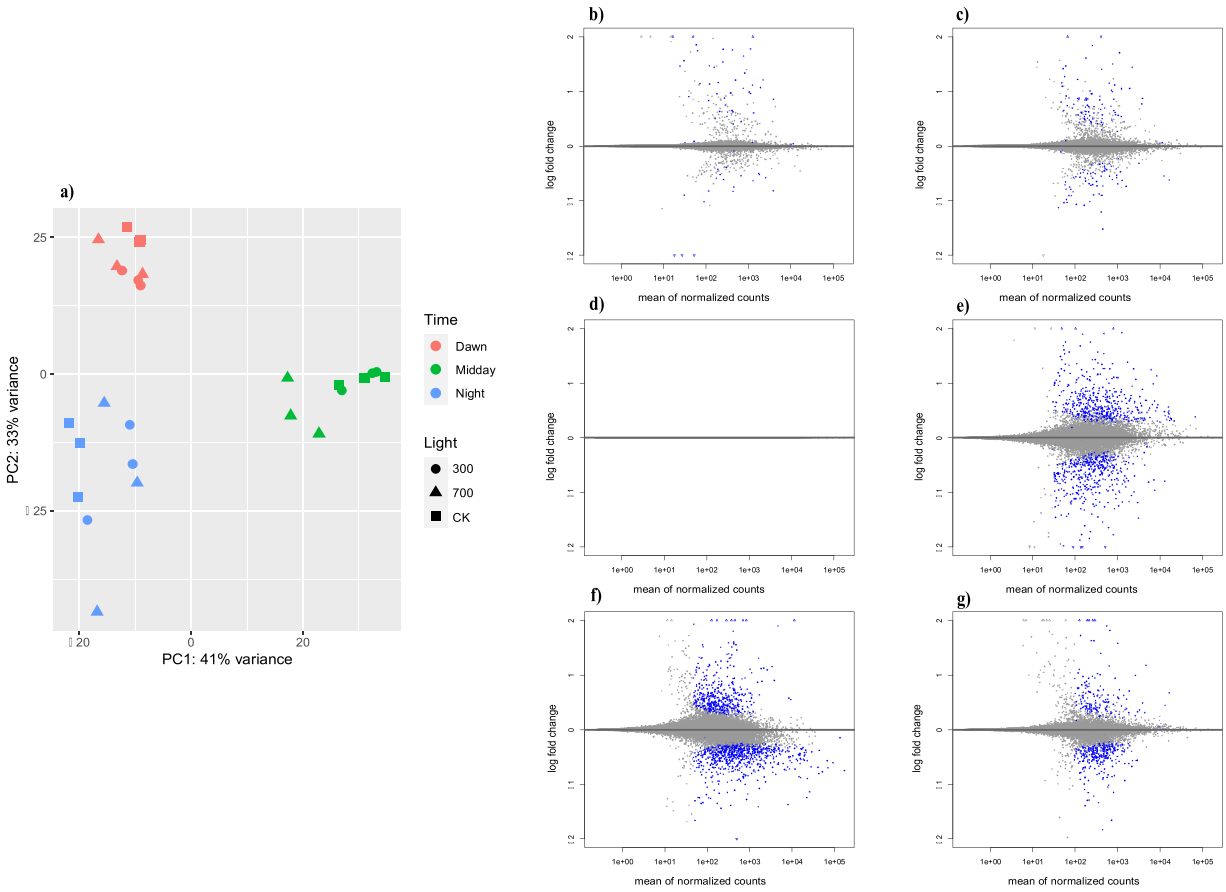

Fig. 5. a) Principal component analysis (PCA) reveals a clustering pattern in RNA-Seq samples. PC1, explaining 41 % of the total variance, distinguishes midday samples from dawn and night samples, while PC2, explaining 33 % of the total variance, separates night and dawn samples. b-g) MA plots show the differential expression of control and treated samples. Each dot represents a gene, with grey dots indicating non-significant differential expression between conditions and blue dots indicating significant differential expression (b) NL300 vs. CK at dawn, c) NL700 vs. CK at dawn, d) NL300 vs. CK at midday, e) NL700 vs. CK at midday, f) NL300 vs. CK at night, g) NL700 vs. CK at night).

dysfunctional movement and reduced guard cell turgor linked to incomplete synthesis and degradation of starch and slow degradation of ABA at dawn. Indeed, when dawn occurs and plants experience the first sunlight, generally the starch stored in guard cells is rapidly converted into sugars which increase the osmotic potential on the one hand, and are used for malate synthesis on the other, providing a counterion for K^+ ions supporting stomatal opening (Flütsch et al., 2020; Santelia and Lunn, 2017). In our work, leaves of NL trees maintained low and unvarying starch contents throughout the daylight with respect to CK, and no degradation was observed overnight. Therefore, it is conceivable that the lower stomatal opening response during daylight was likely induced by a low starch content in NL leaf cells, including guard cells, due to the altered synthesis and degradation of leaf starch contents and/or to leaf metabolic alterations to sustain stomatal opening during the preceding night.

In this framework, multiple parameters from the JIP test led to also identifying the potential alterations in leaves at the photochemical level induced by NL treatment. The electron transport chain was impacted by the NL treatment, as also observed in previous works by Kumar et al. (2022) in *Saraca asoca*, *Terminalia catappa*, *Bauhinia variegata* and *Holoptelea integrifolia* and by Kwak et al. (2018) in *Liriodendron tulipifera* trees. However, according to our results, the degree of damage to the PSII centre was relatively low under NL treatment. Our study revealed that the quantum yields of reactions after Q_B were more susceptible to NL exposure (i.e., fewer statistical differences among treatments in PI_{abs} with respect to the PI_{tot} parameter). Specifically, φ_{Ro} demonstrated heightened sensitivity to NL compared to F_V/F_M (i.e., φ_{Po}) and the φ_{Eo} parameter. The decrease in φ_{Ro} highlights that NL lighting hindered the electron transfer process, leading to a reduction at the PSI end acceptor side. Under non-stressed conditions, at the end of the linear electron transfer chain, the ferredoxin transfers electrons to ferredoxin $NADP^+$ reductase, which can then reduce $NADP^+$ to NADPH to support CO_2 assimilation (Rochaix, 2011). In our study, stomatal limitations caused a decrease in net CO_2 assimilation rates, especially at dawn. Therefore, it is possible that the concurrent reduction in NADPH consumption, caused by the decrease in sinking capacity, resulted in a feedback-limitation of the linear electron transport flow (Campos et al., 2014; Li et al., 2021). This, in turn, reduced the requirement for the reduction of end electron acceptors. Moreover, at the gene expression level, the downregulation of two ferredoxin nitrite/sulphite reductase domains in NL trees could be interpreted as another sign of this impaired electron transport at the PSI acceptor side.

PSII, PSI, light-harvesting antenna complexes, and minor antennae are composed of chlorophylls and carotenoids, though their proportion varies among these systems (Voitsekhovskaja and Tyutereva, 2015). Our results highlight that the trees exposed to NL treatment increased both chlorophyll *a* and *b* contents, even though the increments in chlorophyll *b* were higher than those observed in chlorophyll *a*, thus lowering the chlorophyll *a*:*b* ratio. From the literature, leaf chlorophyll content alterations due to NL treatment are specie-specific since contrasting results were observed (Kwak et al., 2018; Liu et al., 2021; Lo Piccolo et al., 2023; Sarala et al., 2013; Wei et al., 2023). Lo Piccolo et al. (2023) argued that the higher chlorophyll content found in *Tilia platyphyllos* and *Platanus* × *acerifolia* was due to the light quantity/quality provided by

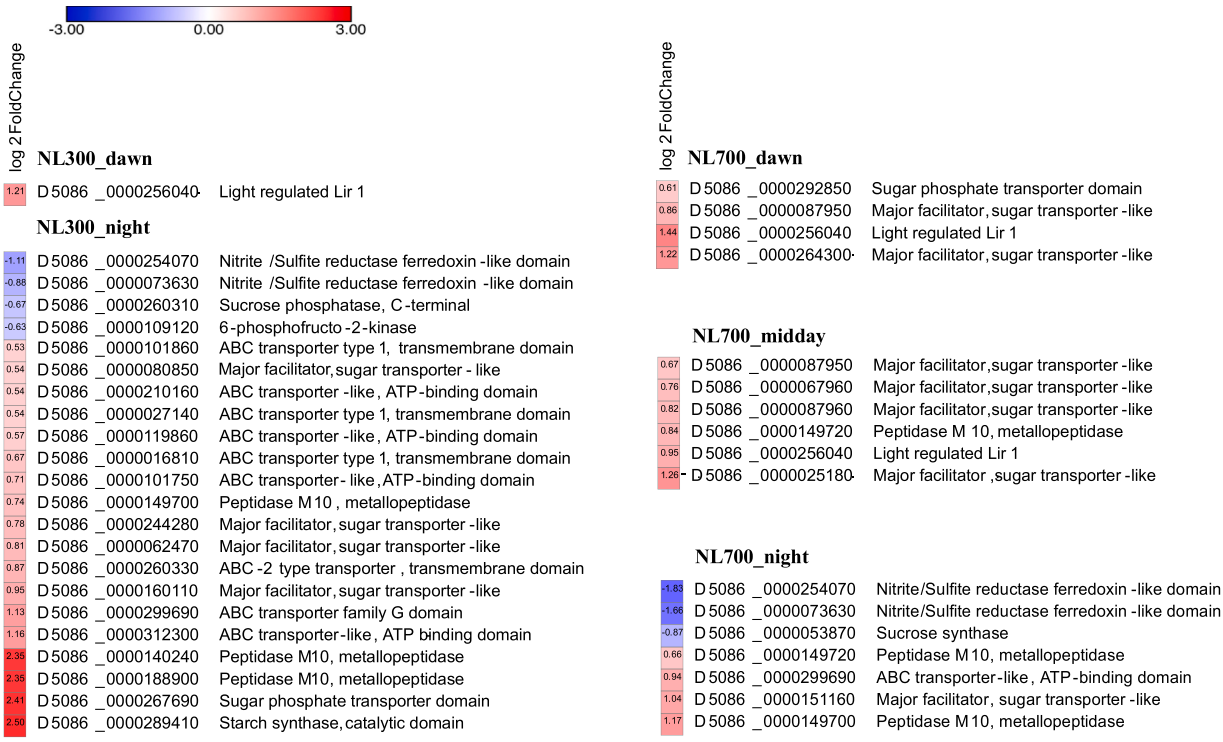

Fig. 6. Heatmap illustrating the expression levels of selected differentially expressed genes (DEGs) discussed in the text. The data presented represents the log2 fold change in transcript abundance between control and treated samples. The labels NL300_dawn, NL300_night, NL700_dawn, NL700_midday, and NL700_night correspond to the specific comparisons between NL300 or NL700 samples versus the control (CK) at dawn, night, dawn, midday, and night, respectively (n = 3). The comprehensive dataset of all DEGs can be found in Supplementary Table S1.

streetlamps, which hampered the natural decrease in chlorophyll biosynthesis during the night in NL trees. An intriguing aspect was the higher concentration of chlorophyll *b* in both NL leaves than in controls. Chlorophyll *b* is a pigment only present in antenna complexes, and changes in chlorophyll *a*-to-*b* proportion are important for understanding the mechanism of adaptation of leaves in trees grown under altered light environments (Kume et al., 2018; Tanaka et al., 2001). It was reported that chlorophyll *b* biosynthesis controls antenna size (large antenna is linked to low chlorophyll *a*:*b* ratio, whereas small antenna with a high chlorophyll *a*:*b* ratio; Tanaka et al., 2001) and affects not only light harvesting and thermal dissipation energy but also the stability of pigment-protein complex in the antennae, becoming an important factor in the regulation of leaf ontogenesis (Voitsekhovskaja and Tyutereva, 2015). Therefore, the observed increase in chlorophyll *b* contents provided to improve the functions of light-harvesting complexes and/or to the dissipation of excitation energy (statistically significative higher dissipation per active reaction center [DI_O/RC] was observed in both NL trees at dawn compared to controls; Fig. S2; Kwak et al., 2018; Voitsekhovskaja and Tyutereva, 2015). The aspect related to the interactions between chlorophyll *b* and the regulation of leaf ontogenesis is intriguing and needs to be investigated with further dedicated studies since the portion of trees exposed to streetlamp NL usually has a slower onset of leaf senescence (Lo Piccolo et al., 2023; Massetti, 2018; Matzke, 1936; Škvareninová et al., 2017).

All the physiological changes observed suggest that significant variations occurred in NL trees. However, how do we explain the lack of starch accumulation in NL leaves during the day despite sustained rates of photosynthesis? And how, then, is possible to explain a greater accumulation of biomass at the branch level in NL trees compared to controls? Starch is an overflow product and represents an alternative carbon sink when the CO_2 assimilation rate exceeds the rate of sugar biosynthesis (Osorio et al., 2014; Stitt and Zeeman, 2012). Moreover, in a recent review conducted by Yoon et al. (2021), it was reported that sucrose levels, but not other sugars, act as regulators for starch biosynthesis and metabolism in many higher plant species, influencing the expression of genes connected to starch synthesis (Wang et al., 2001). Indeed, NL leaves of both treatments showed lower sucrose contents at midday, even if the content in total soluble sugars was not different among treatments. Several genes related to sugar metabolism were differentially expressed in light-treated trees at night, indicating altered transcriptional activity in response to NL. However, these changes did not influence the leaf starch and soluble sugar contents obtained through biochemical analyses. A key finding is that genetic analyses unveil the up-regulation of several genes encoding sugar transporters in treated trees, a phenomenon consistently observed at all three-time points. An intricate interplay, and not yet fully elucidated, between light signalling and sugar transporters exists in plants. The light-mediated signals exert profound effects on the expression and function of sugar transporters, ensuring tight coupling between sugar metabolism and the light conditions (Haydon et al., 2011; Henry et al., 2011; Ji et al., 2020). The research conducted by Haydon et al. (2011) underscores that transported photosynthates, including sugars, might serve as a feedback mechanism directly regulating circadian clocks. Moreover, Ji et al. (2020) and Henry et al. (2011) identified a pattern of up-regulation of several transcripts encoding for sugar transporters in response to light radiation in their respective studies. The genetic analyses carried out in these studies not only corroborate but also fortify the consistent observation of this phenomenon across diverse experimental setups. Therefore, we suggest that the faster transport of sugars to sink organs such as branch tissues (larger branches at the end of the growing season) or branch terminal apex (longer branches at the end of the growing season) hampered the natural accumulation of primary starch on leaves of NL trees. The altered patterns of sugar metabolism and transport observed in NL trees may contribute to the altered physiological responses observed, highlighting the complex interplay between light signalling, sugar transport, and physiological processes in plants under nighttime streetlamp lighting conditions.

5. Conclusion

In conclusion, this article provides a comprehensive overview of the manifold effects of streetlamp illumination on urban trees, incorporating the latest findings and shedding light on the gaps in our current understanding. The observed alterations in tree architecture, including increased branch length and diameters, provide a tangible indication of the impact on biomass distribution, suggesting a dynamic response to artificial lighting conditions. The implications of these changes extend beyond mere aesthetics, raising questions about the long-term effects on tree health, structural integrity, and overall ecosystem dynamics within urban environments.

Examining the photosynthetic processes, our findings uncover a complex scenario. While night-lighted trees exhibited positive net CO_2 assimilation during the night, the study also revealed limitations in stomatal conductance during daylight hours, especially at dawn impairing the electron transport chain. The observed changes in gene expression underscore the complex interplay between night lighting and molecular regulation. Notably, the up-regulation of genes associated with sugar transporters indicates a dynamic response in sugar and starch metabolism. The understanding of these alterations on trees provides valuable information to improve tree management in our cities. Moreover, this study paves the way to further research lines, such as the specific mechanisms underlying gene expression changes and physiological responses in night-illuminated trees. By addressing these knowledge gaps, we can enhance our ability to predict and mitigate the ecological consequences of urban light pollution.

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CRediT authorship contribution statement

Lucia Guidi: Writing – review & editing. **Concetta De Quattro:** Writing – review & editing, Data curation. **Federico Sebastiani:** Writing – review & editing, Data curation. **Sara Torre:** Writing – original draft, Formal analysis, Data curation. **Giulia Lauria:** Writing – review & editing, Formal analysis, Data curation. **Ermes Lo Piccolo:** Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Marco Landi:** Writing – review & editing, Supervision, Methodology. **Damiano Remorini:** Writing – review & editing. **Rossano Massai:** 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2024.105861.

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