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Response of the photosynthetic apparatus of turfgrass varieties of perennial ryegrass and red fescue to naphthalene and fluoranthene contamination

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Abstract

The accumulation of polycyclic aromatic hydrocarbons (PAH) in urban soils poses a serious threat to plants. In this study, we compared the effects of naphthalene and fluoranthene on the photosynthetic apparatus of two lawn grass species: *Lolium perenne* L. (vars. Nira and Roadrunner) and *Festuca rubra* L. (var. Nimba). Compounds were applied to the substrate at concentrations of 20 and 40 mg kg⁻¹(DM). Plant gas exchange and chlorophyll *a* fluorescence parameters were measured at 0, 240, and 480 h after their application. All treatments caused a progressive, time-dependent decline in the photosynthetic performance of the plants: net photosynthesis, transpiration rates, and stomatal conductance decreased. On the other hand, intercellular CO₂ concentration (C_i) increased, indicating a shift from stomatal to nonstomatal limitations of photosynthesis. The performance index was shown to be a sensitive indicator of PAH phytotoxicity. Distinct patterns of photosystem damage were observed among the varieties, including antenna dissociation in Nimba, reaction centre destruction in Nira, and overall degradation of the photosynthetic apparatus in Roadrunner. Among the tested cultivars, Nimba (*Festuca rubra*) demonstrated the greatest overall tolerance to PAH-induced photosynthetic stress and is therefore recommended for use in contaminated urban landscapes.

Keywords: nonstomatal limitation; performance index; phytotoxic stress; plant gas exchange; polycyclic aromatic hydrocarbons.

Introduction

Transport and industry are the primary sources of polycyclic aromatic hydrocarbon (PAH) emissions (Ali-Taleshi *et al.* 2021). The accumulation of PAHs in urban and industrial soil systems represents a critical environmental challenge, directly affecting the physiological stability of urban greenery. Among these pollutants, naphthalene –

a bicyclic, relatively volatile compound, and fluoranthene – a tetracyclic, persistent hydrocarbon are frequently utilised as model pollutants due to their contrasting chemical behaviours. As demonstrated by Smolewska *et al.* (2022), urban traffic corridors serve as primary sources of these contaminants. Naphthalene exhibits high mobility and direct root uptake, whereas the more lipophilic fluoranthene tends to accumulate in the soil matrix and

Highlights

- Polycyclic aromatic hydrocarbons impair photosynthesis in lawn grasses
- Photosynthesis efficiency declines under naphthalene and fluoranthene stress
- Grass varieties show different damage patterns in the photosynthetic system

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Abbreviations: ChlF – chlorophyll *a* fluorescence; C_i – intercellular CO₂ concentration; CSI – Composite Stress Index; DM – dry mass; *E* – transpiration rates; g_s – stomatal conductance; LHCII – light-harvesting complex of PSII; OEC – oxygen-evolving complex; PAH – polycyclic aromatic hydrocarbons; PCA – Principal component analysis; PI_{abs} – performance index; P_N – net photosynthetic rate; ROS – reactive oxygen species; Q_A – primary quinone acceptor of PSII.

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on leaf surfaces through dry deposition. Consequently, evaluating the photosynthetic apparatus response in specific turfgrass cultivars to these distinct pollutants is essential for advancing urban phytoremediation strategies.

Turfgrasses, particularly perennial ryegrass (*Lolium perenne* L.) and red fescue (*Festuca rubra* L.), are recognised for their high resilience and extensive root systems, which facilitate rhizoremediation – the microbial degradation of hydrocarbons stimulated by root exudates. According to Gawryluk and Krzyszczak (2024), while these species exhibit higher germination energy in PAH-contaminated substrates compared to other lawn grasses, their long-term vitality is significantly compromised by chronic chemical stress. Zeng *et al.* (2025) further highlighted that although these grasses can enhance PAH removal by up to 80% through rhizosphere interactions, the bioenergetic cost of maintaining this metabolic activity often leads to a decline in photosynthetic efficiency. At the cellular level, both naphthalene and fluoranthene exert phytotoxic effects through partially distinct but overlapping mechanisms. The primary cellular target of both compounds is the lipid bilayer of biological membranes. Owing to their lipophilicity, PAH molecules intercalate into the hydrophobic core of the plasma membrane and organellar membranes, increasing membrane fluidity, disrupting the ordered arrangement of phospholipids, and ultimately compromising membrane integrity and selective permeability (Sikkema *et al.* 1995). These membrane perturbations impair ion transport, proton motive force maintenance, and the activity of membrane-associated protein complexes, including those of the photosynthetic apparatus. Naphthalene, due to its relatively higher water solubility and volatility, is absorbed rapidly *via* roots and translocated to aerial tissues, where it rapidly partitions into plasma and chloroplast envelope membranes. In contrast, fluoranthene has lower aqueous solubility. Because of that, it accumulates more slowly but persistently in the lipid phase of thylakoid membranes, reaching higher local concentrations over time (Fismes *et al.* 2002). A key downstream consequence of membrane disruption by both compounds is the induction of oxidative stress (Molina and Segura 2021). The resulting oxidative burst triggers lipid peroxidation of thylakoid (Kummerová *et al.* 2006, Kreslavski *et al.* 2014). Simultaneously, ROS accumulation impairs the oxygen-evolving complex (OEC) on the donor side of PSII, interfering with water oxidation and electron donation to the reaction centre.

The photosynthetic apparatus, specifically PSII, serves as a highly sensitive sensor for chemical stress. During recent years, chlorophyll *a* fluorescence has emerged as a rapid and sensitive tool for diagnosing changes in PSII structure and function caused by environmental pollutants (Dąbrowski *et al.* 2016). The selection of specific fluorescence parameters, as analysed by the JIP-test, enables a precise evaluation of photosynthetic efficiency in large-scale ecological studies (Bussotti *et al.* 2020). Research conducted by Kalaji *et al.* (2017), Dąbrowski *et al.* (2024), and Małuszyński *et al.* (2026) emphasized that chlorophyll fluorescence measurements allow the identification of specific bottlenecks in

the electron transport chain, including alterations in electron flow associated with both the donor and acceptor sides of PSII (Habibi and Vaziri 2017). In species such as *Lolium perenne*, JIP-test measurements have demonstrated that combined abiotic and chemical stresses affect overall photosynthetic performance (Digrado *et al.* 2017).

The JIP-test, a multiparametric analysis of the fast chlorophyll *a* fluorescence transient (OJIP), has proven to be a rapid and noninvasive tool for quantifying these sublethal changes. Unlike simple calculated parameters such as F_v/F_m , which are often too insensitive to detect early-stage stress, JIP-test parameters, particularly the performance index on an absorption basis (PI_{abs}), provide a more comprehensive view of plant vitality.

Despite these insights, a critical research gap remains regarding the comparative biophysical responses of specific cultivars of *Lolium perenne* and *Festuca rubra* to different concentration gradients of naphthalene and fluoranthene. Most existing studies have focused on total petroleum hydrocarbons or generalised species-level responses, often overlooking cultivar-specific sensitivity. Therefore, the present study aimed to address this gap by using JIP-test analysis to evaluate how these cultivars adapt their photosynthetic machinery to organic contamination. By identifying specific bottlenecks in energy flow, this research sought to establish early-warning physiological markers and support the selection of the most suitable cultivars for the sustainable management of contaminated urban landscapes.

Materials and methods

Plants, growth conditions, and experimental design:

Two turf-type varieties of *Lolium perenne* L. (Roadrunner – Turf Seed Inc., USA; Nira – Małopolska Hodowla Roślin, Poland) and one turf-type variety of *Festuca rubra* ssp. *commutata* (Nimba – Małopolska Hodowla Roślin, Poland) were used in the experiment.

The experiment was conducted in a greenhouse at the Warsaw University of Life Sciences (SGGW) under controlled conditions with a 16-h light/8-h dark photoperiod. The average day and night temperatures were 24 and 16°C, respectively. Seeds of all varieties were sown on 29 May 2015 in pots (13 × 13 × 13 cm) filled with a substrate composed of loose sand (70%) and light clay (30%). A single dose of a multi-component fertiliser (Substral 100) was applied. Each pot was sown with 0.42 g of *Lolium perenne* seeds or 0.20 g of *Festuca rubra* seeds, depending on the species.

The stress factors (naphthalene and fluoranthene from Sigma-Aldrich Inc., Saint Louis, MO, USA) were applied 8 weeks after sowing by introducing a solution of the tested compounds into the substrate. To obtain the required concentrations of both compounds [20 mg kg⁻¹(DM) and 40 mg kg⁻¹(DM)], 20 mg or 40 mg of each compound was weighed and transferred into 100-mL volumetric flasks. Subsequently, 1 mL of acetone was added to dissolve the compounds. The flasks were sealed and shaken until the substances dissolved completely. The solution was applied once at a volume of 50 mL per

pot. Measurements were taken before stress application and at 240 and 480 h after treatment.

Physiological parameter measurements: Measurements were performed on six randomly selected flag leaves. Chlorophyll fluorescence (ChlF) was measured after 30 min of dark adaptation using the *M-PEA Chlorophyll Fluorescence Measurement System* (Hansatech Instruments Ltd., UK). The following measurement protocol was applied: measurement time – 1.0 s; actinic light intensity – $3,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \cdot \text{s}^{-1}$; wavelength – $635 \pm 10 \text{ nm}$.

Plant gas-exchange parameters, including net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i), were measured using a portable gas analyser *LCpro+* (ADC BioScientific Ltd., UK). A narrow leaf chamber (aperture: $1.0 \text{ cm} \times 5.8 \text{ cm}$; gasket area: 5.8 cm^2) was used, as it is specifically designed for elongated, narrow leaves such as those of turfgrass species. The open gas-exchange system operated in differential mode at an ambient air flow rate of $150 \mu\text{mol s}^{-1}$ during measurements. Air temperature during measurements was 22°C , saturating irradiance was approximately $1,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \cdot \text{s}^{-1}$, and relative air humidity was 60%. Measurements were performed on the same leaves used for ChlF analysis, always after stabilisation of chamber conditions. After enclosing a leaf in the chamber, a minimum stabilisation period of 3–5 min was allowed before recording, ensuring stable CO_2 and H_2O differential readings. Since the width of individual grass blades of *Lolium perenne* (2.5–4.0 mm) and *Festuca rubra* (1.5–2.5 mm) was narrower than the chamber aperture width (10 mm), the actual leaf area enclosed was smaller than the nominal gasket area (5.8 cm^2). To correct for this, the actual leaf area enclosed within the chamber was determined for each measurement by scanning the gasket impression of each leaf blade immediately after measurement using a flatbed scanner with the *WinRhizo 2009* program (Regent Instrument Ltd., Canada) and calculating the true leaf area, following the approach described by Savvides and Fotopoulos (2018). All gas-exchange parameters were subsequently recalculated using the measured value, replacing the default gasket

area in the *LCpro+* software equations. Descriptions of ChlF and plant gas-exchange parameters are presented in the table below.

Composite Stress Index: The Composite Stress Index (CSI) integrates multiple physiological indicators into a single, unitless metric (0–1 scale) to quantify overall photosynthetic impairment. This approach follows the multiparametric stress assessment framework proposed by Lichtenthaler (1987, 1996) and the Performance Index concept of Strasser and Tsimilli-Michael (2001).

Statistical analysis: All statistical analyses and visualisations were performed in *R version 4.4.x* (R Core Team 2024) using the *RStudio IDE* (Posit Team 2024). Three-way analysis of variance (*ANOVA* – Type III) was conducted using the *car* package with contrast sums, followed by Tukey's HSD post-hoc comparisons via *emmeans*. Principal component analysis was performed using *FactoMineR* (Lê et al. 2008) on standardised variables, with biplots generated via *factoextra* (Kassambara and Mundt 2020). All figures were produced using *ggplot2* (Wickham 2016).

Relationships between ChlF signals and plant gas-exchange parameters were assessed using Pearson's correlation coefficient. A statistically significant main effect ($p < 0.05$) indicates that a given factor significantly influences the response variable (Cohen 1988). While p -values indicate whether an effect is likely to be real rather than due to chance, effect sizes quantify the magnitude or practical importance of the effect (Nakagawa and Cuthill 2007). In this study, eta-squared (η^2) was used as a measure of effect size to estimate the proportion of total variance in the response variable attributable to each factor or interaction (Richardson 2011). Unlike the coefficient of determination (R^2) used in regression analysis, η^2 quantifies the relative contribution of each factor within the *ANOVA*.

Results

PCA analysis: PCA was conducted on all 12 standardised photosynthetic parameters to identify the dataset's latent structure and visualise multivariate treatment effects (Fig. 1). The first principal component (PC1) explained

Definition of terms used by the JIP-test and plant gas-exchange parameters (*LCpro+ manual 2007*, Tsimilli-Michael 2020)

t_{FM}	time (in ms) to reach the maximal fluorescence
Area	Total complementary area between the fluorescence induction curve and $F = F_p$
F_0	Minimal fluorescence
F_m	Maximal fluorescence
F_v	Maximal variable fluorescence
F_v/F_m	Maximum quantum yield of PSII photochemistry
F_v/F_0	Maximum primary photochemical efficiency of PSII (or potential activity of PSII)
PI_{abs}	Performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors
P_N	Net photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]
E	H_2O transpiration rate [$\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$]
g_s	Stomatal conductance [$\text{mol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$]
C_i	Substomatal CO_2 concentration [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}(\text{air})$]

56.6% of the total variance. At the same time, PC2 accounted for an additional 11.4%, and PC3 for 8.9%. PC1 was characterised by strong positive loadings from F_v/F_0 (0.357), F_v/F_m (0.348), P_N (0.326), F_v (0.314), E (0.299), and PI_{abs} (0.294), and strong negative loadings from F_0 (-0.299) and C_i (-0.298). This configuration identifies PC1 as a comprehensive “photosynthetic health” axis: high PC1 scores correspond to vigorous photosynthetic function (efficient PSII, high carbon fixation, low basal fluorescence), while low PC1 scores reflect generalised photosynthetic impairment. PC2 was primarily loaded by F_m (0.616) and F_v (0.485), indicating variation in maximal fluorescence capacity independent of photochemical efficiency. This component may capture structural differences in the abundance of chloroplast pigment-protein complexes or thylakoid membrane integrity.

In the PC1–PC2 biplot, the ordination space revealed a clear separation of samples by PAH treatment along

the PC1 axis. Control samples clustered at high PC1 values across all three time points, while PAH-treated samples progressively shifted toward lower (more negative) PC1 scores with increasing exposure duration. After 480 h of stress exposure, the application of naphthalene and fluoranthene at 40 mg (N40 and F40 treatments) resulted in these groups occupying the extreme negative end of PC, spatially well separated from both controls and lower-dose treatments. Variety-based separation was primarily visible along PC2, with Roadrunner samples showing distinctly different positioning compared to Nira and Nimba. This is consistent with the relatively lower F_m values and baseline PI_{abs} observed in Roadrunner.

The temporal dynamics of PC1 scores, tracked separately for each variety, further illustrated the differential stress trajectories. In all three varieties, control PC1 scores remained relatively stable, while PAH-treated groups showed a monotonic decline. The rate of PC1 decline differed among varieties. Nimba and Nira showed

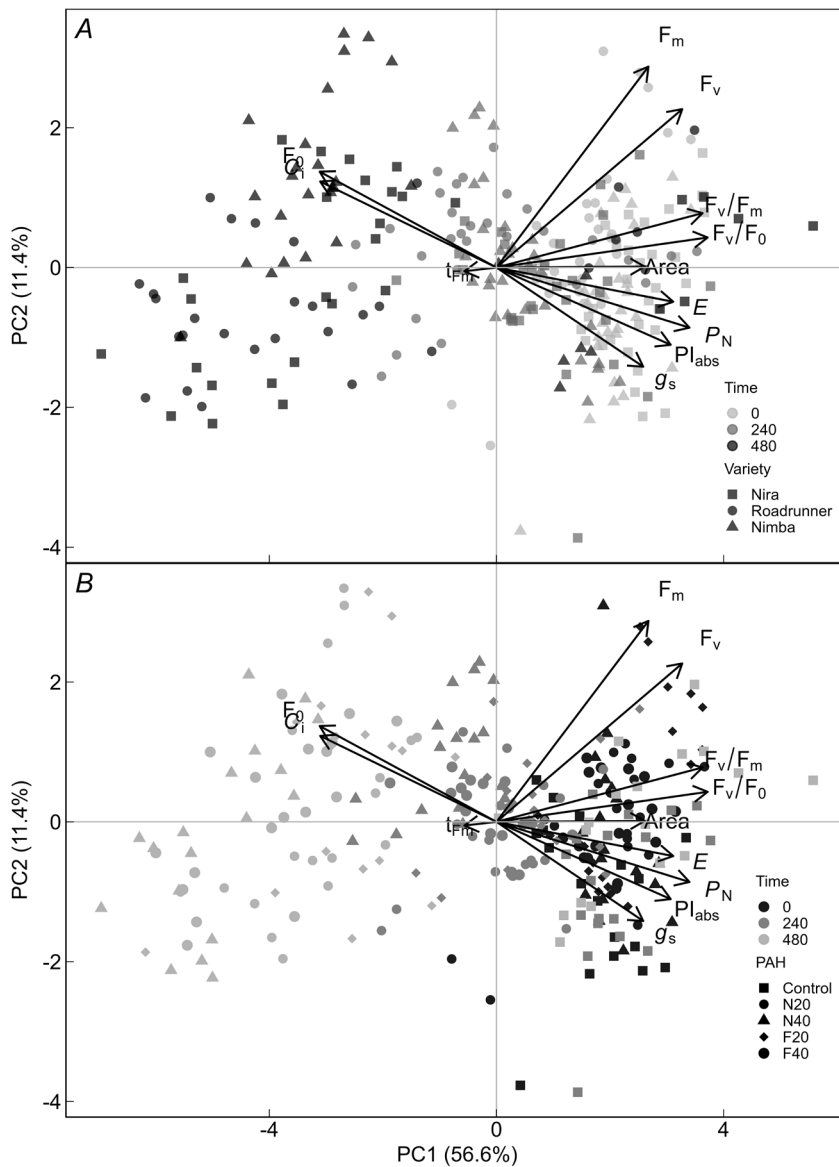


Fig. 1. Principal component analysis of naphthalene and fluoranthene stress effect on the twelve parameters related to photosynthesis of turf grass varieties (*Festuca rubra* L. var. Nimba; *Lolium perenne* L. Nira and Roadrunner). Biplot graph showing the relative “contribution” of each input variable to the formation of the principal components by time and variety (A), and by time and polycyclic aromatic hydrocarbons (PAH) (B). Area – total complementary area between the fluorescence induction curve and $F = F_P$; C_i – substomatal CO_2 concentration; E – H_2O transpiration rate; F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – maximal variable fluorescence; F_v/F_0 – maximum primary photochemical efficiency of PSII (or potential activity of PSII); F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; PI_{abs} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; P_N – net photosynthetic rate; t_{FM} – time (in ms) to reach the maximal fluorescence.

steeper declines under naphthalene in both doses (N20 and N40) between 240 and 480 h after application. On the other hand, Roadrunner exhibited an earlier onset of stress response but a shallower late-stage trajectory.

ANOVA analysis: A three-way factorial analysis of variance (ANOVA) (Type III sums of squares; Quinn and Keough 2002) was performed for each of the twelve photosynthetic parameters, with time, variety, and PAH treatment as fixed factors (Table 1). All three main effects were statistically significant for the majority of measured variables. Time exerted the strongest overall influence, yielding the largest partial eta-squared values across most plant gas-exchange and fluorescence parameters. These results reflect the progressive intensification of PAH-induced phytotoxicity over the 480-h experimental period. PAH treatment was universally significant across all twelve variables ($p < 0.001$ in all cases). The largest effect sizes for PAH treatment were observed for PI_{abs} , P_N , E , t_{FM} , and F_v/F_0 . The variety main effect was significant for ten of twelve dependent variables ($p < 0.05$). Notable exceptions were g_s and F_m , suggesting that stomatal conductance and maximal fluorescence were comparatively uniform across varieties when averaged over all treatments and time points. Conversely, the variety explained a substantial proportion of the variance in t_{FM} , PI_{abs} , E , and F_0 .

All three two-way interactions were significant for most parameters, underscoring the complexity of the photosynthetic stress response. The time $\times$ PAH treatment interaction was significant for all twelve variables ($p < 0.001$ in each case), with consistently large effect sizes: F_v/F_0 , F_v/F_m , P_N , F_v , PI_{abs} , F_0 , and t_{FM} . This interaction indicates that the deleterious effects of both naphthalene and fluoranthene on the photosynthetic apparatus were

strongly time-dependent, intensifying markedly between 240 and 480 h of exposure. While at $t = 0$ h, PAH-treated plants showed only marginal deviations from controls, by $t = 480$ h, declines in PSII efficiency and plant gas exchange were evident across all PAH treatments. The time $\times$ variety interaction was significant for all parameters except g_s . This interaction was particularly pronounced for t_{FM} and PI_{abs} , indicating that varieties diverged substantially in their temporal stress trajectories. The variety $\times$ PAH treatment interaction was significant for 10 of 12 variables ($p < 0.05$), except for g_s and C_i . This result indicates that the three grass varieties exhibited differential sensitivity to the specific PAH compounds and their concentrations. Notably, t_{FM} , F_m , and F_v showed particularly strong varietal $\times$ treatment interactions. The three-way interaction (time $\times$ variety $\times$ PAH treatment) was statistically significant for ten of the twelve parameters. The only exceptions were Area and g_s . The significance of the three-way interaction for most parameters, including key indicators of photosystem function such as F_v/F_m , F_v/F_0 , PI_{abs} , F_0 , and F_m , provides unequivocal evidence that the temporal dynamics of PAH phytotoxicity are modulated by both the grass variety and the specific PAH compound and dose administered. In other words, varieties differ not merely in their overall stress tolerance but in the specific way they respond to different PAH compounds over time.

Correlations: Fig. 2 presents a correlation matrix showing the strength and direction of relationships between gas-exchange parameters and chlorophyll fluorescence indices. The results indicate strong integration of photochemical processes with CO_2 and water–gas exchange, alongside an inverse relationship between photosynthetic efficiency and intercellular CO_2 concentration. Strong positive

Table 1. A three-way factorial ANOVA (Type III sums of squares; Quinn and Keough 2002) for each of the twelve photosynthetic parameters of turf grass varieties (*Festuca rubra* L. var. Nimba; *Lolium perenne* L. Nira and Roadrunner) under naphthalene and fluoranthene stress in different times (0, 240, and 480 h after stress application) ($n = 6$). PAH – polycyclic aromatic hydrocarbons. Area – total complementary area between the fluorescence induction curve and $F = F_p$; C_i – substomatal CO_2 concentration; E – H_2O transpiration rate; F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – maximal variable fluorescence; F_v/F_0 – maximum primary photochemical efficiency of PSII (or potential activity of PSII); F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; PI_{abs} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; P_N – net photosynthetic rate; t_{FM} – time (in ms) to reach the maximal fluorescence. Asterisks *, **, *** refer to different significance levels represented by p -values lower or equal to 0.05, 0.01, and 0.001, respectively.

Variable	Time (T)	Variety (V)	PAH	T $\times$ V	T $\times$ PAH	V $\times$ PAH	T $\times$ V $\times$ PAH
P_N	*** ($\eta^2 = 0.72$)	*** (0.13)	*** (0.62)	* (0.06)	*** (0.45)	* (0.08)	* (0.11)
E	*** (0.75)	*** (0.48)	*** (0.53)	*** (0.14)	*** (0.32)	** (0.10)	* (0.12)
C_i	*** (0.57)	*** (0.13)	*** (0.28)	*** (0.08)	*** (0.26)	* (0.07)	* (0.12)
g_s	*** (0.31)	ns	*** (0.41)	** (0.07)	*** (0.12)	ns	ns
t_{FM}	*** (0.31)	*** (0.55)	*** (0.55)	*** (0.79)	*** (0.65)	*** (0.72)	*** (0.80)
Area	*** (0.31)	** (0.06)	*** (0.21)	*** (0.20)	*** (0.33)	*** (0.11)	ns
F_0	*** (0.62)	*** (0.39)	*** (0.45)	** (0.06)	*** (0.36)	*** (0.13)	*** (0.19)
F_m	*** (0.30)	ns	*** (0.08)	*** (0.10)	*** (0.32)	*** (0.24)	*** (0.24)
F_v	*** (0.49)	* (0.04)	*** (0.17)	*** (0.10)	*** (0.42)	*** (0.22)	*** (0.23)
F_v/F_m	*** (0.70)	*** (0.29)	*** (0.43)	*** (0.15)	*** (0.50)	*** (0.14)	*** (0.20)
F_v/F_0	*** (0.69)	*** (0.32)	*** (0.49)	** (0.06)	*** (0.56)	*** (0.12)	*** (0.19)
PI_{abs}	*** (0.99)	*** (0.95)	*** (0.94)	*** (0.95)	*** (0.93)	*** (0.86)	*** (0.94)

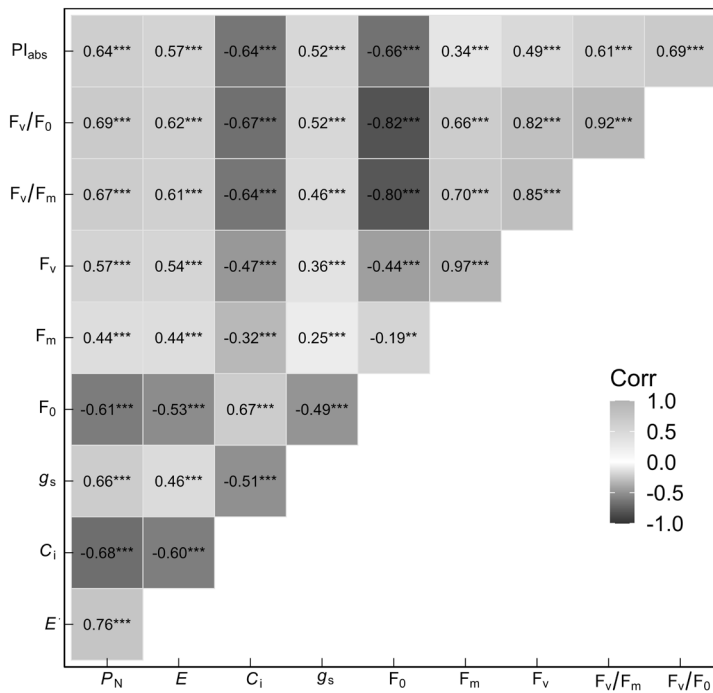


Fig. 2. Correlation coefficient between the twelve photosynthetic parameters of turf grass varieties (*Festuca rubra* L. var. Nimba; *Lolium perenne* L. Nira and Roadrunner) under naphthalene and fluoranthene stress. C_i – substomatal CO_2 concentration; E – H_2O transpiration rate; F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – maximal variable fluorescence; F_v/F_0 – maximum primary photochemical efficiency of PSII (or potential activity of PSII); F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; P_{i_0} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; P_N – net photosynthetic rate.

correlations were demonstrated among parameters describing the efficiency of the photosynthetic apparatus, particularly between F_m and F_v ($r \approx 0.97$) and between F_v/F_0 and F_v/F_m ($r \approx 0.92$). Similarly, high correlation coefficients were found between F_v and the photochemical efficiency indices (F_v/F_m and F_v/F_0), confirming their close physiological association and shared functional basis. The strong positive correlations among fluorescence parameters suggest coordinated changes in PSII function. Plant gas-exchange parameters also showed positive relationships with one another. Net photosynthetic rate (P_N) was positively correlated with stomatal conductance (g_s) and transpiration rate (E), indicating the interdependence of processes regulating plant gas exchange and plant water balance. These relationships were moderate to strong in magnitude. A different pattern of relationships was observed for intercellular CO_2 concentration (C_i), which exhibited negative correlations with P_N and g_s . This may indicate that increased photosynthetic activity and stomatal opening are associated with enhanced CO_2 consumption, leading to a reduction in its concentration within the leaf. The C_i parameter also showed negative relationships with selected fluorescence parameters, suggesting a link between photochemical efficiency and net photosynthetic rate. The P_{i_0} parameter was positively correlated with fluorescence parameters, confirming its usefulness as a synthetic indicator of photosynthetic apparatus performance. At the same time, its negative relationship with C_i is consistent with the overall observed pattern linking photosynthetic efficiency and CO_2 concentration in plant tissues.

Plant gas-exchange parameters: PAH treatments progressively inhibited plant gas-exchange parameters in all three tested lawn grass varieties over the 480-h exposure period. P_N declined significantly across all

treatments, reaching 66–84% below control values (Fig. 3). The most pronounced reduction in the P_N parameter was observed in Nira under the N20 treatment (84.1% compared with the control) and in Roadrunner and Nimba under the F40 treatment (both 82.0%). Transpiration rate (E) followed a similar pattern, with reductions of up to 93.0% in Roadrunner and 88.8% in Nira under N40 treatment. Stomatal conductance (g_s) was among the earliest-responding parameters, decreasing by 57–64%. Notably, intercellular CO_2 concentration (C_i) increased consistently with exposure to stress treatments, in contrast to the declining trends observed in P_N , E , and g_s . This parameter increased by 35.7–105.2% relative to controls, with the largest increases recorded in Nimba under N20 treatment.

Chlorophyll *a* fluorescence parameters: The fluorescence response revealed a clear dissociation between the behaviour of the F_0 parameter (minimal fluorescence) and that of the photochemical efficiency parameters (Fig. 4). F_0 increased progressively with exposure duration, rising by 38–84% at 480 h across treatments, indicating an inactivation of PSII reaction centres and/or dissociation of light-harvesting complexes from PSII cores. In contrast, F_m showed variable, often declining trends, particularly in Nira and Roadrunner, whereas Nimba maintained relatively stable F_m values. A key finding of this study is that the combined F_0 – F_m response pattern differed qualitatively not only among species but also among varieties, revealing genotype-specific PSII-damage mechanisms. Nimba exhibited high F_0 increases combined with relatively maintained F_m . Nira showed both increased F_0 and severely decreased F_m (the most pronounced changes were observed under N40 treatment), indicating direct damage to the PSII reaction centres themselves, likely involving D1 protein

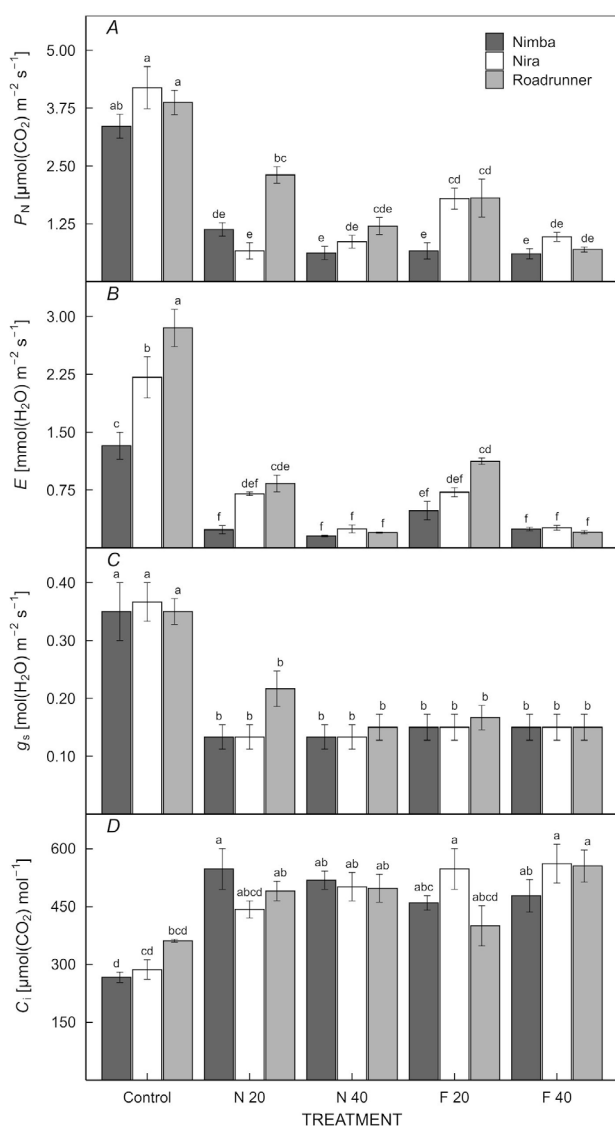


Fig. 3. The dependence of plant gas-exchange parameters of the turf grass varieties (*Festuca rubra* L. var. Nimba; *Lolium perenne* L. Nira and Roadrunner) after different times (0, 240, and 480 h) of the application of naphthalene and fluoranthene (N20, N40, F20, and F40). P_N : net photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$] (A); E : transpiration rate [$\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$] (B); g_s : stomatal conductance [$\text{mol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$] (C); and C_i : substomatal CO_2 concentration [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$] (D). The means marked by the same letter do not differ significantly ($p < 0.05$, $n = 6$).

degradation and impairment of the oxygen-evolving complex. Roadrunner showed simultaneous declines in F_m and increases in F_0 .

The maximum quantum yield of PSII (F_v/F_m) showed relatively moderate reductions compared to other parameters at early time points (before and 240 h after stress application), but declined substantially at 480 h after stress application. The ratio F_v/F_0 proved considerably more sensitive, with reductions of 45–83% at 480 h. The most severe decline in F_v/F_0 was recorded in Nira under N40 at 480 h.

The performance index (PI_{abs}) emerged as the most responsive and earliest-responding fluorescence parameter throughout the experiment. A critical comparison of the temporal dynamics of PI_{abs} vs. F_v/F_m revealed a striking dissociation. After 240 h of stress application, this parameter already showed substantial reductions of 18.7–76.4%, while F_v/F_m remained relatively stable with changes of only 1–9% at the same time point. By 480 h, PI_{abs} declined by 33.8–91.5% depending on the treatment and variety. The strongest decline occurred in Roadrunner under N40, followed by Nimba under N40.

Composite Stress Index: The Composite Stress Index (CSI), integrating all measured parameters, confirmed that stress severity increased markedly with exposure time and PAH concentration (Fig. 5). Before stress application (0 h), CSI values remained low across all treatments and varieties (near zero). By 240 h, moderate stress was evident, with Roadrunner variety showing the highest CSI values, particularly under N40 and F40 treatments. At 480 h, CSI values reached their maxima, with N40 consistently producing the highest stress scores across all three varieties. The dose-response pattern was clear: N40 > F40 > N20 $\approx$ F20 in terms of overall photosynthetic impairment.

Discussion

The present study, to the best of our knowledge, provides the first detailed comparative assessment of the effects of naphthalene and fluoranthene on plant gas exchange and photosynthetic efficiency of lawn grass varieties over an extended time course. While the majority of studies about the influence of PAH on photosynthesis have focused on model species such as pea (*Pisum sativum*; Kummerová *et al.* 2006, Váňová *et al.* 2009, Kreslavski *et al.* 2014), wheat (*Triticum aestivum*; Tomar and Jajoo 2013, 2014), or *Arabidopsis* (Jajoo *et al.* 2014), research on lawn grasses, which are important in urban areas, under PAH contamination remains scarce. Our findings revealed three principal outcomes: (1) based on changes in individual parameters, the response of PSII to PAH contamination differed not only between species, but also among cultivars, (2) PI_{abs} functions as a significantly more sensitive early indicator of PAH phytotoxicity than F_v/F_m , and (3) significant differences were found in the negative effects of the two PAH compounds studied.

Before discussing PAH-induced changes, we should note that the control P_N values recorded in the present study are at the lower end of the range reported for both species in the literature. This is fully consistent with the biological characteristics of the studied material and experimental conditions. Turf-type cultivars of *L. perenne* and *F. rubra* are well known to exhibit inherently lower net photosynthetic rates than forage-type counterparts, owing to their smaller leaf area, higher tiller density, and the allocation of resources towards lateral growth rather than leaf-level carbon gain. Van Huylbroeck *et al.* (1999), who evaluated photosynthetic characteristics specifically of turf-type cultivars of *L. perenne* and

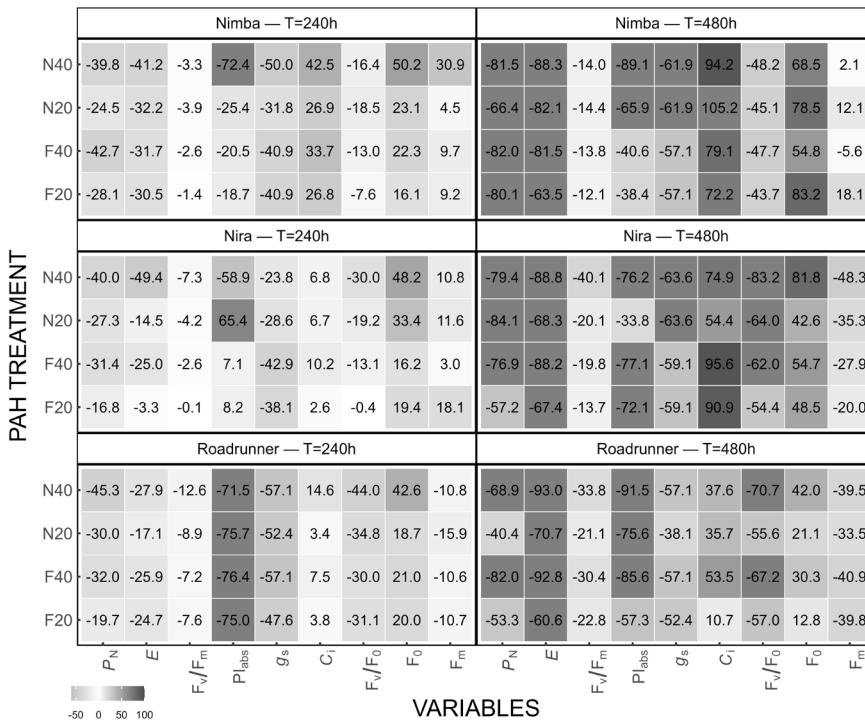


Fig. 4. Most important photosynthetic parameters under naphthalene and fluoranthene stress (N20, N40, F20, and F40) expressed as relative decrease [%] compared to the values measured in non-stressed control plants of turf grass varieties (*Festuca rubra* L. var. Nimba; *Lolium perenne* L. Nira and Roadrunner) at 240 and 480 h after stress application ($n = 6$). C_i – substomatal CO_2 concentration; E – H_2O transpiration rate; F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v/F_0 – maximum primary photochemical efficiency of PSII (or potential activity of PSII); F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; PI_{abs} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; P_N – net photosynthetic rate.

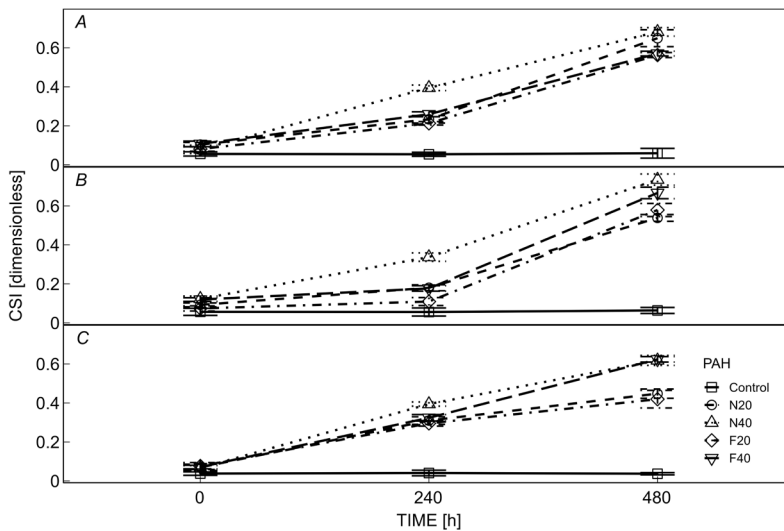


Fig. 5. The Composite Stress Index (CSI) of turf grass varieties (*Festuca rubra* L. var. Nimba – A; *Lolium perenne* L. Nira – B and Roadrunner – C) under naphthalene and fluoranthene stress (N20, N40, F20, and F40) at different times (0, 240, and 480 h after stress application).

F. rubra, reported net photosynthesis values in a similar low range, confirming that this is a characteristic feature of turf-type varieties.

The early and pronounced decline in g_s indicates that stomatal closure is an initial plant response to PAH exposure. However, the simultaneous and progressive increase in C_i requires careful interpretation. Under stress conditions that induce strong or spatially heterogeneous stomatal closure, stomata do not always close uniformly across the leaf surface. This phenomenon, known as stomatal patchiness, has been demonstrated in a wide range of species and stress scenarios (Terashima *et al.* 1988, Terashima 1992, Marengo *et al.* 2006, Peak *et al.* 2023) and can lead to systematic overestimation of the calculated C_i value, because the standard gas-exchange equations assume spatially uniform stomatal conductance

over the entire leaf area. When patchiness occurs, regions with closed stomata contribute little to net photosynthetic rate but still influence the bulk g_s signal, causing calculated C_i to rise artifactually (Terashima *et al.* 1988). This caveat is particularly relevant when interpreting increases in C_i under stress, as the apparent rise may partly reflect a measurement artefact rather than a true increase in substomatal CO_2 (Márquez *et al.* 2025). We therefore acknowledge that the observed C_i increases in PAH-treated plants cannot be attributed solely to nonstomatal limitation of photosynthesis without the caveat that stomatal patchiness may have contributed to overestimation of C_i , particularly at 480 h when g_s had declined most severely.

Nevertheless, several lines of evidence in the present study support the conclusion that genuine nonstomatal limitation was operative alongside, and progressively

dominating over, any patchiness-related artefact. According to the classical work of Farquhar and Sharkey (1982), if stomatal closure were the sole limitation on P_N , C_i would be expected to decrease as CO_2 supply to the mesophyll is curtailed. The observed increase in C_i is therefore at least partly indicative of impaired CO_2 fixation capacity rather than reduced supply alone. Second, the parallel and pronounced decline in all chlorophyll fluorescence parameters – particularly the early collapse of PI_{abs} at 240 h, when g_s had declined only moderately – provides independent, stomata-independent evidence for direct impairment of the photochemical apparatus. Third, PAH accumulation in thylakoid membrane lipids (Sikkema *et al.* 1995) and the generation of ROS (Kreslavski *et al.* 2014) are well-established mechanisms of direct mesophyll damage that operate independently of stomatal aperture. Beyond the classical stomatal limitation framework, the observed C_i increase may also reflect impaired mesophyll conductance (g_m), as PAH accumulation in the plasma and chloroplast envelope membranes would be expected to reduce CO_2 diffusion from the substomatal cavity to the chloroplast stroma independently of stomatal aperture (Flexas *et al.* 2008, Joffe *et al.* 2022). Taken together, the most parsimonious interpretation of the data is that PAH stress induced a progressive shift from initial stomatal limitation, potentially compounded by stomatal patchiness artefacts in C_i calculation, toward genuine nonstomatal limitation of photosynthesis, driven by direct damage to the mesophyll and the photosynthetic machinery.

Although direct evidence for PAH-specific g_m suppression remains limited, analogous membrane-disrupting phytotoxic pollutants, such as ozone, have been shown to reduce mesophyll conductance independently of stomatal aperture, constituting the primary limitation on net photosynthetic rate under oxidative stress conditions (Joffe *et al.* 2022).

PAH accumulation in the plasma and chloroplast envelope membranes (Sikkema *et al.* 1995) would be expected to reduce g_m independently of stomatal aperture, further explaining why C_i rose despite reduced g_s . The observed increase in C_i therefore implies substantial impairment of the mesophyll conductance to CO_2 and/or the carboxylation capacity of Rubisco and Calvin cycle enzymes. Tomar and Jajoo (2014) demonstrated that fluoranthene inhibits dark reactions of photosynthesis more severely than light reactions in wheat, supporting this interpretation. Notably, the magnitude of C_i increase varied considerably among varieties, suggesting variety-dependent differences in the susceptibility of the carbon fixation apparatus to PAH-induced damage.

The central novel finding of this study is the identification of qualitatively different PSII damage patterns among the three lawn varieties, discernible through the combined analysis of F_0 and F_m dynamics. This approach extends beyond the conventional reliance on F_v/F_m alone and reveals the underlying damage mechanism. In Nimba, the pronounced increase in F_0 with maintained F_m indicates that the total chlorophyll pool and functional reaction centres (RC) remained largely intact, while

the energy transfer from LHCII to the PSII core was disrupted. This is consistent with membrane-level damage caused by PAH accumulation in the lipid bilayer (Sikkema *et al.* 1995), which would preferentially affect the organisation of pigment–protein complexes within the thylakoid membrane without necessarily destroying them. Bussotti *et al.* (2020) demonstrated that F_0 and F_v/F_m cluster together in PCA of large-scale fluorescence datasets, confirming that antenna-level disruption (elevated F_0) and reduced photochemical yield are mechanistically linked. In Nira, the concurrent increase in F_0 and severe decrease in F_m indicates direct destruction of PSII reaction centres, likely involving degradation of the D1 protein and impairment of the oxygen-evolving complex (OEC). Kreslavski *et al.* (2014) demonstrated that PAH exposure induces H_2O_2 generation in a biphasic pattern, first associated with plasma membrane damage and subsequently with organellar membrane disruption. The reactive oxygen species thus generated would promote D1 protein turnover and, under severe stress, overwhelm the PSII repair cycle (Aro *et al.* 1993). Nira's vulnerability to this pathway may reflect lower intrinsic capacity for reactive oxygen species (ROS) scavenging or D1 protein repair compared to the other varieties. Roadrunner's pattern, characterised by early decreases in F_m with moderate F_0 increases, suggests a more generalised degradation of the entire photosynthetic apparatus, affecting both antenna and RC components simultaneously. The rapid and synchronous decline of both F_m and F_v in this variety at 240 h indicates that once protective mechanisms are overwhelmed, this variety undergoes particularly rapid deterioration.

The markedly earlier response of PI_{abs} compared to F_v/F_m constitutes an important finding for practical applications in environmental monitoring and crop phenotyping. At 240 h, PI_{abs} had already declined by 19–76% across treatments and varieties, while F_v/F_m showed changes of only 1–9%. The greater sensitivity of PI_{abs} arises from its mathematical structure. As defined by Strasser *et al.* (2004), this parameter integrates three independent functional steps of photosynthesis: the density of active reaction centres per chlorophyll (RC/ABS), the quantum yield of primary photochemistry (F_v/F_m), and the efficiency of electron transport beyond plastoquinone A (Q_A). A perturbation affecting any of these three components will reduce PI_{abs} , whereas F_v/F_m reflects only the second component. Our data suggest that PAH stress initially impairs RC density and electron transport efficiency before significantly affecting the overall quantum yield, which explains why PI_{abs} detects damage well before F_v/F_m responds. Živčák *et al.* (2008) showed that this parameter declined from the onset of drought, while F_v/F_m remained unaffected until severe dehydration. Rastogi *et al.* (2020) demonstrated that PI_{abs} could discriminate salinity-tolerant from sensitive sweet sorghum genotypes. More recently, Rodríguez *et al.* (2023) confirmed that multiparameter fluorescence indices outperform single-ratio metrics in discriminating stress severity across genotypes under field conditions, further supporting the use of PI_{abs} in large-scale screening. The present study adds PAH phytotoxicity to the list of

stresses for which PI_{abs} provides earlier and more sensitive detection than F_v/F_m and suggests that screening based on PI_{abs} could be incorporated into field monitoring protocols for PAH-contaminated sites.

Both PAH types produced qualitatively similar effects on photosynthetic parameters, consistent with the findings of Jajoo *et al.* (2014), who reported that the inhibitory effects of PAHs on photosynthesis are not strictly related to aromaticity. Nevertheless, quantitative differences were observed. Naphthalene (2-ring, higher water solubility) tended to cause more rapid initial effects on E and g_s , consistent with faster cellular uptake from the aqueous phase. Kreslavski *et al.* (2014) reported that naphthalene exhibited the strongest short-term effects on PSII among the three tested PAHs, attributing this to its higher water solubility, which enables faster partitioning into the thylakoid membrane. Fluoranthene (4-ring, lower water solubility) showed somewhat stronger effects on F_v/F_m and F_v/F_0 at later time points, particularly in Nira. Tomar and Jajoo (2013, 2017) demonstrated that fluoranthene significantly alters PSII heterogeneity in wheat. The lower water solubility of fluoranthene may lead to its gradual accumulation in the lipid phase of the thylakoid membrane over time, resulting in higher local concentrations within the membrane than with naphthalene. This differential accumulation kinetics could explain why fluoranthene's effects on PSII become relatively more severe than naphthalene's at the 480-h time point. The dose-dependent response was consistent for both PAH types, with higher concentrations (N40, F40) consistently producing greater damage than lower concentrations (N20, F20). The average P_N reduction at 480 h was 71% across all treatments, confirming that PAH contamination at these concentrations poses a severe threat to photosynthetic productivity in the tested lawn varieties. Overall, naphthalene at 40 mg kg⁻¹ (N40) exerted the greatest phytotoxic effect across both species and all time points. At equivalent concentrations, naphthalene caused faster early-stage impairment of stomatal conductance and transpiration, consistent with its higher water solubility and faster cellular uptake. Fluoranthene, by contrast, produced relatively stronger late-stage inhibition of PSII photochemical efficiency (F_v/F_m , F_v/F_0) at 480 h, consistent with its gradual accumulation in the lipid phase of the thylakoid membrane due to its higher log Kow (Fismes *et al.* 2002).

The PCA and CSI analyses provided complementary perspectives on the multidimensional stress response. The PCA biplot confirmed the strong positive correlations among P_N , E , g_s , F_v/F_m , F_v/F_0 , and PI_{abs} (all on the same side of PC1), reflecting the coordinated shutdown of photosynthetic function under PAH stress. The negative correlation of C_i and F_0 with these parameters on PC1 confirms that PAH stress simultaneously impairs carbon fixation capacity while increasing the fraction of inactive PSII centres. Similar correlation structures have been reported by Bussotti *et al.* (2020) in large-scale ecological studies using JIP-test parameters and by Kalaji *et al.* (2016) in abiotic stress reviews. The separation of varieties along PC2, driven primarily by F_m and F_v loading, confirms

that the genotype-specific damage fingerprints described above represent an independent dimension of variation not captured by overall stress severity (PC1). This finding has methodological implications: future studies assessing PAH tolerance in breeding material should include both PC1-type parameters (stress severity) and PC2-type parameters (damage mechanism) to fully characterise variety responses. The CSI provided a single integrated metric that effectively ranked both treatment severity ($N40 > F40 > N20 \approx F20$) and temporal progression of damage. This composite approach is particularly valuable when multiple physiological parameters respond in different directions (*e.g.*, C_i increasing while P_N decreases), as it captures the overall magnitude of deviation from control conditions regardless of direction.

The CSI proved particularly valuable when individual physiological parameters responded in opposing directions. For example, C_i increased while P_N and g_s declined simultaneously under PAH stress. In such cases, relying on any single parameter would produce a misleading picture of overall plant condition. By integrating deviations across all twelve parameters, the CSI provides a single, direction-independent metric that enables unambiguous ranking of treatment severity and temporal progression of damage. This approach may be especially useful in field monitoring scenarios where rapid triage of contamination severity across multiple cultivars or sites is required.

Conclusions: This study set out to (1) characterise and compare the photosynthetic responses of three turfgrass cultivars (*Festuca rubra* var. Nimba; *Lolium perenne* var. Nira and Roadrunner) to naphthalene and fluoranthene contamination over an extended 480-h time course, (2) identify the most sensitive early biomarker of PAH phytotoxicity among the measured parameters, and (3) distinguish cultivar-specific damage mechanisms to inform the selection of cultivars for use in PAH-contaminated urban landscapes. PAH-induced PSII damage was confirmed to follow qualitatively different pathways depending on cultivar. The combined analysis of F_0 and F_m dynamics revealed antenna dissociation (impaired connectivity between antenna complexes and PSII reaction centres) in Nimba, reaction centre destruction/inactivation (loss of functional PSII reaction centres) in Nira, and generalised apparatus degradation (broad impairment of energy absorption, trapping, and electron transport) in Roadrunner. This “damage fingerprint” approach provides a mechanistic framework for characterising stress tolerance in breeding programs and environmental monitoring. PI_{abs} was confirmed as a significantly more sensitive early biomarker of PAH phytotoxicity than F_v/F_m . At 240 h, PI_{abs} had already declined by 19–76% across treatments, while F_v/F_m changed by only 1–9%. This finding extends the utility of JIP-test-derived parameters beyond drought and salinity applications to organic pollutant stress detection. Based on the integrated analysis of plant gas exchange, chlorophyll *a* fluorescence, and the Composite Stress Index, Nimba (*Festuca rubra*) was identified as the most tolerant cultivar, maintaining relatively stable F_m values and showing the most limited degradation of

the overall photosynthetic apparatus. This cultivar could therefore be recommended as the preferred choice for deployment in PAH-contaminated urban green spaces. Naphthalene, particularly at 40 mg kg⁻¹ (N40), was confirmed as the more phytotoxic compound compared to fluoranthene at equivalent concentrations.

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