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Chlorophyll fluorescence traits reveal ecological specialization of plant species through photosynthetic functional syndromes

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Photosynthesis represents a fundamental interface between plants and their environment, integrating resource acquisition, stress tolerance, and ecological adaptation. Although chlorophyll fluorescence analysis has become a widely used method for assessing photosystem II (PSII) function under environmental stress, its potential for comparative ecological studies and trait-based interpretation remains insufficiently explored. In particular, it remains unclear whether stable interspecific variation in OJIP chlorophyll fluorescence reflects long-term ecological specialisation rather than merely short-term physiological responses. This study investigated whether OJIP fluorescence parameters can be interpreted as species-level functional traits associated with ecological differentiation among vascular plants. Chlorophyll fluorescence measurements were obtained for a broad range of plant species and analysed alongside ecological indicator values describing species' preferences for light, temperature, moisture, soil pH, and nutrient availability, as well as plant ecological strategies based on Grime's CSR framework and species hemeroby. Multivariate relationships between fluorescence traits and ecological characteristics were evaluated using constrained ordination to identify environmentally structured components of PSII functional variation. The results demonstrated that interspecific differences in chlorophyll fluorescence were associated with species' ecological specialisation. OJIP parameters formed coordinated functional profiles rather than independent responses of individual variables, indicating that ecological differentiation is expressed through integrated patterns of PSII energy absorption, excitation trapping, electron transport, reaction centre density, and energy dissipation. Species associated with open, high-light habitats exhibited fluorescence characteristics consistent with enhanced photoprotective regulation and increased excitation pressure, reflecting adaptation to excessive irradiance. In contrast, species from more humid, nutrient-rich habitats displayed profiles associated with higher photochemical efficiency and greater functional capacity of PSII reaction centres, suggesting optimisation for effective utilisation of limited and heterogeneous light conditions within dense vegetation. The ecological interpretation of fluorescence traits was further supported by their relationships with disturbance affinity and plant ecological strategies. Ruderal and highly hemerobic species were characterised by fluorescence profiles reflecting dynamic regulation of excitation energy and electron transport, whereas species with contrasting stress-tolerant or competitive strategies showed distinct patterns of PSII organisation. The constrained ordination approach demonstrated that environmentally related variation in OJIP parameters represents a specific component of total fluorescence variability, separating ecologically meaningful signals from variation associated with other factors such as phenology, ontogeny, genetic differentiation, and phylogenetic history. These findings support the interpretation of OJIP chlorophyll fluorescence parameters as functional traits describing species-level differences in photosynthetic organisation. By linking PSII function with ecological specialisation, OJIP-based traits provide a physiological dimension for comparative plant ecology and expand the application of chlorophyll fluorescence from stress assessment towards the analysis of plant functional strategies across environmental gradients.

Keywords: photosystem II; functional traits; ecological specialization; plant ecological strategies; CSR strategy; ecological indicator values; comparative plant ecology; photosynthetic adaptation.

Introduction

Photosynthesis is one of the central physiological processes through which plants interact with their environment and realise their ecological strategies (Demmig-Adams et al., 2017; Kunakh et al., 2026). Differences in habitat conditions, including light availability, water supply, nutrient status, temperature regime, and disturbance intensity, directly affect the balance between energy capture, carbon assimilation, photoprotection, and stress tolerance (Sage & Stata, 2015; Ivanko et al., 2025). Ecological differentiation among plant species is expected to be reflected in both their morphological and life-history characteristics and the functional organisation of the photosynthetic apparatus (Maxwell & Johnson, 2000). In this context, chlorophyll fluorescence provides a powerful tool for linking plant physiology to ecological specialisation, as it captures how photosystem II regulates absorbed excitation energy under different environmental and adaptive constraints (Baker, 2008; Murchie & Lawson, 2013). Among the available approaches for assessing photosynthetic functioning, chlorophyll fluorescence analysis based on the OJIP transient is among the most informative and widely applied methods (Murchie & Lawson, 2013). The polyphasic rise of chlorophyll fluorescence reflects a sequence of primary photochemical events within photosystem II (PSII), allowing quantitative characterisation of excitation energy absorption, trapping, electron transport, reaction centre activity, and energy dissipation (Swoczyna et al., 2022). Because these processes are tightly interconnected, OJIP-derived parameters provide an integrated description of PSII functioning rather than isolated physiological measurements (Force et al., 2003; Alexeyeva et al., 2024). This integrative nature makes chlorophyll fluorescence particularly valuable for studying plant functional responses, as changes in environmental conditions or adaptive strategies are expected to simultaneously influence multiple components of the photosynthetic apparatus (Wright et al., 2004). Consequently, OJIP fluorescence provides a comprehensive physiological fingerprint of photosynthetic performance, linking the structural and functional organisation of PSII with the mechanisms by which plants acquire, utilise, and dissipate absorbed light energy.

The widespread application of OJIP chlorophyll fluorescence has primarily been driven by its sensitivity to environmental and physiological perturbations (Kalaji et al., 2016). OJIP chlorophyll fluores-

cence is highly sensitive to perturbations in photosystem II caused by a wide range of abiotic and biotic stressors, including drought, salinity, temperature extremes, nutrient constraint, heavy metal contamination, pollution, and pathogen infection (Swoczyna et al., 2022). Owing to this sensitivity, chlorophyll fluorescence has become a standard tool in plant ecophysiology for quantifying stress intensity, detecting functional impairment of the photosynthetic apparatus, monitoring acclimation processes, and evaluating recovery following environmental disturbance (Tsimilli-Michael, 2020; Kunakh et al., 2024). Its rapid, non-destructive, and highly reproducible measurements have also led to widespread applications in agriculture, forestry, and environmental monitoring, where chlorophyll fluorescence is routinely used to assess plant physiological status.

Despite the extensive use of chlorophyll fluorescence in plant ecophysiology, its potential for comparative ecological research remains largely unexplored. A substantial body of research has focused on physiological responses of individual species to experimentally imposed or naturally occurring environmental stress, whereas considerably less attention has been paid to stable interspecific variation in OJIP traits. Photosynthetic traits, including chlorophyll fluorescence characteristics, were incorporated into a functional trait framework for submerged macrophytes, demonstrating that these traits are associated with species' ecological functions in eutrophic lakes. This provides evidence that photosynthetic performance may represent an informative functional dimension of plant ecological strategies, although such an approach has rarely been extended to comparative analyses of terrestrial plant species (Liu et al., 2021). Comparative applications of chlorophyll fluorescence have demonstrated its value for identifying interspecific differences in photosynthetic performance under natural conditions (Cao et al., 2019). Although chlorophyll fluorescence has traditionally been applied to assess plant responses to environmental stress, several studies have demonstrated its value in ecological field investigations. For example, OJIP-derived parameters have been successfully used to compare the physiological status and vitality of trees growing under contrasting urban environmental conditions, revealing ecologically meaningful differences in photosynthetic performance (Hermans et al., 2003). An important step towards comparative applications of chlorophyll fluorescence was made by Bussotti et al. (2020), who analysed large ecological datasets to identify a reduced set of JIP-test parameters suitable for large-scale surveys using principal component analysis. Their approach demonstrated that substantial redundancy exists among OJIP parameters and that a limited number of statistically independent variables can effectively summarise photosynthetic variation across large datasets. Consequently, it remains unclear whether chlorophyll fluorescence captures only short-term physiological acclimation or also reflects long-term ecological specialisation that has developed through adaptation to contrasting environmental conditions. Comparative studies across multiple species and broad ecological gradients are still relatively uncommon, and analyses explicitly linking OJIP-derived parameters to ecological indicator values, plant strategies, or habitat preferences remain scarce. As a result, the ecological information contained in chlorophyll fluorescence has rarely been evaluated from a species-functional-differentiation perspective. This limitation has also constrained the development of a trait-based interpretation of chlorophyll fluorescence. While morphological, anatomical, and leaf economic traits are routinely used to characterise species' ecological strategies, OJIP-derived parameters have rarely been considered functional traits that describe stable differences in photosynthetic organisation among species. Whether coordinated variation in chlorophyll fluorescence represents an ecophysiological signature of long-term ecological adaptation, therefore, remains an open question.

Ecological indicator systems provide an appropriate framework for addressing this question because they summarise the long-term ecological preferences and adaptive strategies of plant species (Mucina et al., 2016). Indicator values for ecological factors like light, moisture, nutrient availability, soil pH, and temperature (Ellenberg, 1974), along with broader descriptors such as the competitive (C), stress-tolerant (S), and ruderal (R) components of Grime's CSR strategies (Grime, 1977) and hemeroby (Fanelli et al., 2006), represent independent estimates of species ecological specialisation derived from extensive field observations rather than from physiological measurements. Analysing the relationships between OJIP-derived parameters and ecological indicator values enables evaluation of whether variation in photosystem II functioning corresponds to established ecological differentiation among species.

The central hypothesis of this study was that stable interspecific variation in OJIP chlorophyll fluorescence reflects long-term ecological specialisation of plant species and represents species-level functional traits. Specifically, ecological differentiation among species was expected to be accompanied by distinct multivariate OJIP profiles reflecting coordinated variation in PSII energy absorption, excitation trapping, electron transport, reaction centre density, and energy dissipation. These photosynthetic syndromes were expected to correspond to gradients of light availability, moisture, nutrient supply, hemeroby, and CSR ecological strategies. To test this hypothesis, we analysed chlorophyll fluorescence traits of a broad set of plant species and related multivariate OJIP patterns to ecological indicator values and functional strategies using ordination analyses.

Material and methods

A total of 167 vascular plant species were sampled in Dnipropetrovsk and Zaporizhzhia regions (Ukraine) for subsequent analyses. Ecological preferences of the studied plant species were characterised using the European Ecological Indicator Values (EIVE 1.0), which provide harmonised Ellenberg-type indicator values for vascular plants across Europe. Indicator values for light (L), temperature (T), moisture (M), soil reaction (R), and nutrient availability (N) were extracted from the EIVE database (Dengler et al., 2023). Plant life strategies were characterised according to Grime's CSR framework, which distinguishes three primary ecological strategies: competitive (C), stress-tolerant (S), and ruderal (R). CSR scores for the studied species were assigned following the globally calibrated approach of Pierce et al., based on the relative contribution of C, S, and R strategy components (Pierce et al., 2017). The sensitivity of plant species to anthropogenic disturbance was characterised using hemeroby values, which reflect species' affinity for habitats with varying degrees of human transformation. Species hemeroby was assessed according to the indicator system of Frank & Klotz (1990), as subsequently adapted by Goncharenko (Goncharenko, 2017) for the forest and forest-steppe zones of Ukraine.

Chlorophyll a fluorescence induction was measured using the FLS 10s fluorometer (Voronenko, 2024). The instrument records the fast chlorophyll fluorescence induction (OJIP transient) over a 10-s measurement period, using excitation light at 460–485 nm and detecting fluorescence emission within the 600–1000 nm spectral range (Fig. 1). Each measurement consisted of 470 sampling points, providing high-resolution fluorescence induction curves for OJIP analysis. For each plant species, at least 10 chlorophyll fluorescence measurements were performed on fully developed, healthy leaves collected from randomly selected individuals. Only leaves representative of the typical morphology and physiological condition of each species were used for measurements.

Chlorophyll a fluorescence induction transients (OJIP curves) were recorded using a standard dark-adaptation protocol and analysed according to the JIP-test methodology. Measurements were performed on intact, fully expanded leaves after dark adaptation to ensure full oxidation of the photosynthetic electron transport chain.

The fluorescence transient was induced by a saturating actinic light pulse and recorded from 20 μs to 1 s, capturing the characteristic O–J–I–P phases. The OJIP curve reflects the sequential reduction of electron acceptors in photosystem II (PSII), where O corresponds to minimal fluorescence (F_0), and P corresponds to maximal fluorescence (F_m). A set of biophysical JIP-test parameters was calculated to describe PSII energy fluxes per reaction centre (RC) and per cross-section (CS_0), using standard formulations. These parameters included: ABS/CS_0 – absorption flux per excited cross section; TR_0/CS_0 – trapping flux at time zero per cross section; ET_0/CS_0 – electron transport flux per cross section; DI_0/CS_0 – dissipated energy flux per

cross section; RC/CS₀ – density of active reaction centres per cross section. Additional reaction centre–specific parameters included: ABS/RC – absorption flux per active reaction centre; TR₀/RC – trapping flux per reaction centre; ET₀/RC – electron transport flux per reaction centre; DI₀/RC – energy dissipation per reaction centre; φP₀ (FV/FM) – maximum quantum yield of primary photochemistry; ψ₀ (Psi0) – probability that a trapped exciton moves an electron into the electron transport chain; M₀ – approximated initial slope of fluorescence rise, reflecting QA reduction kinetics; VJ, VK – relative variable fluorescence at J- and K-steps, indicating PSII donor and acceptor side limitations. All parameters were normalised where appropriate to cross sections or reaction centres to allow comparison between species. A spider (radar) plot was used to visualise multivariate differences in JIP-test profiles among species.

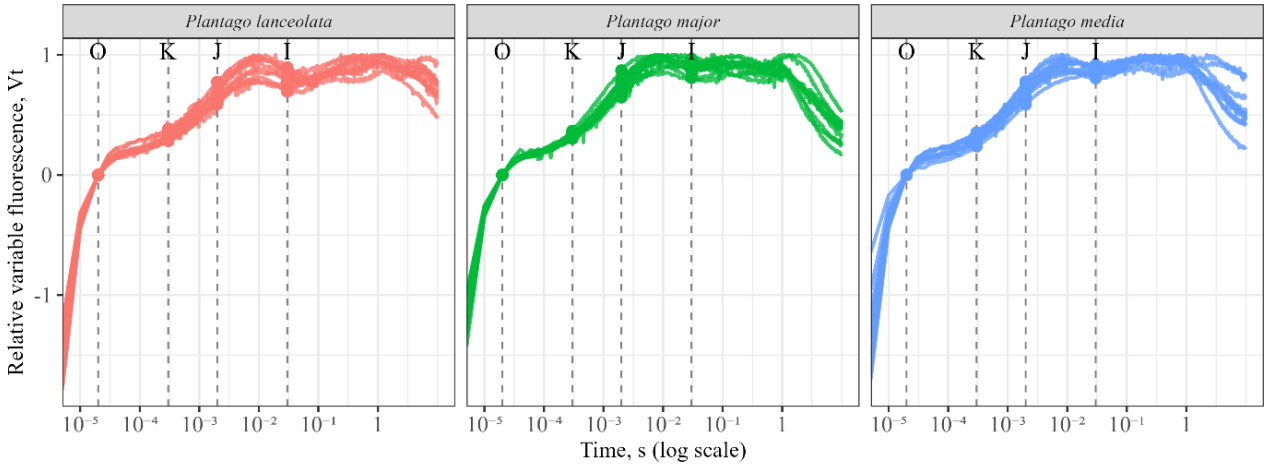

Fig. 1. Normalised chlorophyll a fluorescence induction (OJIP) curves of representative leaves from *Plantago lanceolata*, *P. major*, and *P. media*: individual curves correspond to independent measurements of different plants; vertical dashed lines indicate the characteristic OJIP phases: O (20 μs), K (300 μs), J (2 ms), and I (30 ms); fluorescence intensity is expressed as relative variable fluorescence (Vt), and time is shown on a logarithmic scale

Within the JIP-test framework, ABS/CS₀ (absorption flux per excited cross section) is a derived parameter obtained from chlorophyll a fluorescence induction (OJIP transient). It is not directly measured but calculated from energy fluxes through photosystem II (PSII).

ABS/CS₀ is computed as:

$$ABS/CS_0 = \frac{TR_0/CS_0}{\varphi P_0},$$

where: TR₀/CS₀ is the trapped energy flux per cross section at time zero; φP₀ = FV/FM represents the maximum quantum yield of primary photochemistry; F₀ and F_m are minimal and maximal fluorescence values, respectively.

The trapped energy flux per cross section is further defined as:

$$TR_0/CS_0 = (RC/CS_0) \cdot (TR_0/RC),$$

where: RC/CS₀ is the density of active PSII reaction centres per cross-section, TR₀/RC is the trapping flux per active reaction centre.

ABS/CS₀ represents the apparent absorption flux of excitation energy per excited photosynthetic cross-section of the leaf. It integrates the effective light-harvesting capacity of the PSII antenna system and reflects the amount of absorbed light energy that becomes available for photochemical processes.

TR₀/CS₀ is computed as:

$$TR_0/CS_0 = (RC/CS_0) \cdot (TR_0/RC),$$

where: RC/CS₀ is the density of active PSII reaction centres per excited cross section, and TR₀/RC is the trapping flux per active reaction centre at time zero.

The trapping flux per reaction centre (TR₀/RC) reflects the efficiency with which open PSII reaction centres trap absorbed excitation energy to drive primary photochemistry. TR₀/CS₀ therefore represents the total flux of excitation energy trapped by PSII reaction centres per excited cross section of the leaf at the onset of illumination. It integrates both the structural component of the photosynthetic apparatus (the number of active reaction centres) and the functional component (the efficiency of energy trapping). It is commonly used to describe the initial photochemical capacity of PSII.

ET₀/CS₀ is computed as:

$$ET_0/CS_0 = (RC/CS_0) \cdot (ET_0/RC),$$

where: RC/CS₀ is the density of active PSII reaction centres per excited cross section, and ET₀/RC is the electron transport flux per active reaction centre at time zero.

The electron transport flux per reaction centre (ET₀/RC) represents the rate at which electrons are transferred from the primary quinone acceptor QA^- further into the electron transport chain beyond PSII. ET₀/CS₀ therefore, reflects the total flux of electrons transported beyond PSII per excited cross-section of the leaf. This parameter integrates the structural status of PSII (reaction centre density) and the functional efficiency of downstream electron transport processes. It is widely used as an indicator of the operational capacity of the photosynthetic electron transport chain.

DI₀/CS₀ is computed as:

$$DI_0/CS_0 = ABS/CS_0 - TR_0/CS_0,$$

where: ABS/CS₀ is the apparent absorption flux per excited cross section, and TR₀/CS₀ is the trapped energy flux per cross section at time zero.

The dissipated energy flux per cross section (DI₀/CS₀) represents the portion of absorbed excitation energy that is not used for photochemical charge separation in PSII and is instead dissipated as heat and/or re-emitted as chlorophyll fluorescence. This parameter reflects the balance between energy harvesting and non-photochemical energy dissipation processes. It is widely used as an indicator of photoprotective energy loss and stress-induced downregulation of PSII efficiency.

RC/CS₀ is computed as:

$$RC/CS_0 = \frac{V_J - V_0}{M_0} \cdot \frac{ABS}{CS_0},$$

or in the simplified JIP-test formulation it is derived as a function of initial fluorescence kinetics and expressed as:

$$RC/CS_0 \propto \frac{1 - V_J}{M_0},$$

where: VJ is the relative variable fluorescence at the J-step, V₀ is minimal fluorescence (F₀), and M₀ is the initial slope of the OJIP fluorescence rise, reflecting the rate of QA reduction.

The parameter RC/CS₀ represents the density of active PSII reaction centres per excited cross-section of the leaf. It reflects the number of functional reaction centres capable of photochemical charge separation at the onset of illumination. This parameter integrates the structural integrity of PSII (number of active reaction centres) and their functional connectivity within the photosynthetic electron transport chain. It is widely used as an indicator of PSII reaction-centre activity and of stress-induced inactivation or degradation.

ABS/RC is computed as:

$$ABS/RC = \frac{ABS/CS_0}{RC/CS_0},$$

where: ABS/CS₀ is the absorption flux per excited cross section, and RC/CS₀ is the density of active PSII reaction centres per excited cross section.

The parameter ABS/RC represents the average light energy absorbed by a single active PSII reaction centre. It reflects the functional antenna size of each active reaction centre and indicates the amount of excitation energy available per reaction centre at the onset of illumination. An increase in ABS/RC typically indicates a reduction in the number of active reaction centres (RC inactivation) or an increase in the antenna size per remaining active centre. In contrast, a decrease suggests higher RC density or reduced antenna connectivity. This parameter integrates structural (reaction centre density) and functional (light-harvesting efficiency) aspects of PSII organisation. It is widely used as a sensitive indicator of photoinhibitory damage or acclimation of the photosynthetic apparatus.

TR₀/RC is computed as:

$$TR_0/RC = \frac{TR_0/CS_0}{RC/CS_0},$$

where: TR₀/CS₀ is the trapping flux per excited cross section, and RC/CS₀ is the density of active PSII reaction centres per excited cross section.

The parameter TR₀/RC represents the average amount of excitation energy trapped by a single active PSII reaction centre at the onset of illumination. It reflects the functional trapping efficiency of PSII reaction centres and indicates how effectively absorbed light energy is converted into photochemical charge separation. An increase in TR₀/RC may indicate enlargement of effective antenna size per reaction centre or compensatory energy redistribution due to partial inactivation of reaction centres. In contrast, a decrease suggests reduced excitation pressure per reaction centre or a more even distribution of excitation energy across a larger number of active centres. This parameter integrates functional properties of PSII antenna–reaction centre units and is widely used as an indicator of excitation pressure and photochemical performance under environmental stress.

ET₀/RC is computed as:

$$ET_0/RC = \frac{ET_0/CS_0}{RC/CS_0},$$

where: ET₀/CS₀ is the electron transport flux per excited cross section, and RC/CS₀ is the density of active PSII reaction centres per excited cross section.

The parameter ET₀/RC represents the average electron transport rate beyond photosystem II per active reaction centre at the onset of illumination. It reflects the efficiency with which electrons generated after primary charge separation at PSII are transferred to the downstream electron transport chain. This parameter integrates the functional performance of the PSII acceptor side and the connectivity between reaction centres and the plastoquinone pool. Higher values of ET₀/RC indicate more efficient electron flow per reaction centre. In contrast, lower values may reflect a limitation at the acceptor side of PSII or reduced electron transport capacity under stress conditions.

DI₀/RC is computed as:

$$DI_0/RC = \frac{DI_0/CS_0}{RC/CS_0},$$

where: DI₀/CS₀ is the dissipated energy flux per excited cross section, and RC/CS₀ is the density of active PSII reaction centres per excited cross section.

The parameter DI₀/RC represents the average amount of excitation energy dissipated per active PSII reaction centre at the onset of illumination. It reflects non-photochemical energy losses in PSII, including thermal dissipation and chlorophyll fluorescence, which occur when absorbed energy is not utilised for photochemical charge separation. An increase in DI₀/RC typically indicates enhanced energy dissipation due to stress-induced photoprotective mechanisms or partial inactivation of reaction centres. In contrast, lower values suggest more efficient utilisation of absorbed energy for photochemistry and electron transport. This parameter integrates structural (reaction-centre density) and functional (energy-dissipation capacity) aspects of PSII. It is widely used as an indicator of photoinhibition and stress responses in the photosynthetic apparatus.

φP₀ (FV/FM) is defined as:

$$\varphi P_0 = \frac{F_V}{F_M} = \frac{F_M - F_0}{F_M},$$

where: F₀ is the minimal fluorescence yield (all PSII reaction centres open), and F_m is the maximal fluorescence yield (all PSII reaction centres closed under saturating light). The parameter φP₀ (FV/FM) represents the maximum quantum yield of primary photochemistry in photosystem II (PSII). It reflects the intrinsic efficiency with which absorbed excitation energy is converted into stable photochemical charge separation at the level of PSII reaction centres. This parameter is widely used as a sensitive indicator of PSII photochemical performance and the physiological status of the photosynthetic apparatus. Under optimal conditions, FV/FM values are typically close to 0.83 in most C_3 plants, whereas reductions indicate photoinhibition, environmental stress, or structural/functional impairment of PSII.

ψ₀ (Psi0) is defined as:

$$\psi_0 = 1 - V_J,$$

where: VJ is the relative variable fluorescence at the J-step of the OJIP transient, calculated as:

$$V_J = \frac{F_J - F_0}{F_M - F_0}.$$

The parameter ψ₀ (Psi0) represents the probability that an electron trapped by the PSII reaction centre moves further beyond the primary quinone electron acceptor QA^- into the electron transport chain. It reflects the efficiency of electron transport beyond PSII and is closely related to the redox state and functional openness of the PSII acceptor side. Higher values of ψ₀ indicate more efficient electron transfer from QA^- to downstream electron carriers, whereas lower values suggest limitations at the acceptor side of PSII or restricted electron transport capacity. This parameter is widely used in JIP-test analysis to describe the functional connectivity between PSII reaction centres and the plastoquinone pool under different environmental or stress conditions.

M₀ is defined as:

$$M_0 = \frac{4 \cdot (F_{300\mu s} - F_0)}{F_M - F_0},$$

where: F₀ is the minimal fluorescence yield, F_m is the maximal fluorescence yield, and $F_{(300\mu s)}$ is the fluorescence intensity measured at 300 μs (K-step region of the OJIP transient). The parameter M₀ represents the initial slope of the relative variable fluorescence rise at the onset of illumination. It reflects the rate of primary photochemical events, specifically the reduction kinetics of the primary quinone electron acceptor (QA) in PSII. M₀ is commonly interpreted as an indicator of the initial excitation pressure on PSII reaction centres and the speed of closure of reaction centres under saturating light. Higher values of M₀ indicate faster QA reduction and higher excitation pressure, often associated with stress conditions or reduced PSII connectivity. In contrast, lower values reflect slower excitation pressure build-up and more stable PSII photochemistry.

VJ is defined as:

$$V_J = \frac{F_J - F_0}{F_M - F_0},$$

where: F₀ is the minimal fluorescence yield, FJ is the fluorescence intensity at the J-step (≈2 ms), and F_m is the maximal fluorescence yield.

The parameter VJ represents the relative variable fluorescence at the J-step of the OJIP transient. It reflects the reduction state of the primary quinone electron acceptor (QA) and provides information about the early bottleneck in electron transport at the PSII acceptor side. Higher VJ values indicate stronger accumulation of QA^- and reduced electron transfer efficiency beyond PSII, whereas lower values suggest more efficient electron transport and faster re-oxidation of QA.

VK is defined as:

$$V_K = \frac{F_K - F_0}{F_M - F_0},$$

where: F₀ is the minimal fluorescence yield, FK is the fluorescence intensity at the K-step (≈300 μs), and F_m is the maximal fluorescence yield.

The parameter VK represents the relative variable fluorescence at the K-step of the OJIP transient. It is associated with the donor side of PSII, particularly the oxygen-evolving complex (OEC), and reflects the functional integrity of water-splitting activity. An increase in VK indicates impairment or inactivation of the oxygen-evolving complex, often associated with photoinhibition or environmental stress. At the

same time, lower values suggest stable donor-side function and intact PSII water-splitting capacity.

Species-level chlorophyll fluorescence parameters were averaged across all measured individuals for each species using arithmetic means of all numeric variables. Ecological indicator values and species traits were extracted from a reference dataset (Tarasov database) and matched to fluorescence data based on binomial species names. Before merging, species names were standardised by retaining only genus and species epithets, and duplicate records were resolved by retaining the first occurrence per species.

After merging datasets, a combined matrix was constructed containing two groups of variables: (i) chlorophyll fluorescence (OJIP-derived) parameters and (ii) ecological indicator variables describing species habitat preferences.

The Performance Index on absorption basis (PIabs) was calculated according to the JIP-test formulation as a composite indicator of PSII functionality: $PIabs = (RC/ABS) \times [\varphi P_0/(1 - \varphi P_0)] \times [\psi_0/(1 - \psi_0)]$, where RC/ABS represents the density of active reaction centres per absorbed energy flux, φP_0 is the maximum quantum yield of PSII (FV/FM), and ψ_0 is the probability that a trapped exciton moves an electron beyond QA^-. PIabs integrates structural and functional components of PSII and reflects overall photosynthetic performance and stress status.

Ecological predictors included light regime, temperature regime, continentality, humidity, soil acidity, nutrient availability, salinity, hemeroby, and CSR strategy components (C, S, R). Functional photophysiological descriptors included OJIP-derived parameters (e.g., Fv/Fm, φP_0, ψ_0, φE_0, ABS/CS_0, TR_0/CS_0, ET_0/CS_0, DI_0/CS_0, RC/CS_0, and PIabs). Variables with zero variance were excluded before analysis. Only complete cases across both ecological and physiological datasets were retained to ensure consistency of multivariate ordination. All variables were standardised (z-score transformation) before analysis to ensure comparability across different measurement scales and units.

The relationships between chlorophyll fluorescence traits and ecological indicators were examined using redundancy analysis (RDA). Two models were fitted. A full model including all ecological variables: light regime, temperature, continentality, humidity, acidity, nutrient availability, salinity, hemeroby, and CSR strategy components. A partial RDA model, where the main climatic–edaphic gradient was conditioned: ecological structure was partitioned using Condition() to isolate the effects of hemeroby and CSR strategies independent of climatic and soil-related variables. Statistical significance of the models, constrained axes, and individual terms was assessed using permutation tests (999 permutations). Multicollinearity among explanatory variables was evaluated using variance inflation factors (VIF). RDA ordination results were visualised using type II scaling. Species scores (OJIP parameters) and environmental biplot scores (ecological indicators) were extracted and plotted in a shared ordination space. Only key fluorescence parameters were retained for visualisation to improve interpretability. The first two canonical axes were used for graphical representation, with explained variance expressed as a percentage of constrained inertia.

Results

The dataset comprised 167 vascular plant species spanning a broad range of ecological preferences and functional strategies. According to Ellenberg indicator values, the species were predominantly associated with moderately high light availability (Light Regime: 6.95 ± 1.19), temperate conditions (Temperatures: 4.82 ± 0.57), and moderately continental climates (Continentality: 9.38 ± 2.57). The flora encompassed habitats ranging from dry to wet (Humidity: 1.96–8.11, mean 4.01 ± 1.11), acidic to moderately basic soils (Acidity: 4.02–8.26, mean 6.61 ± 0.76), and nutrient-poor to nutrient-rich environments (Nutrient Availability: 1.37–9.73, mean 5.56 ± 1.78). Salinity preferences also varied considerably (0.42–6.05, mean 1.68 ± 0.76), although most species were associated with non-saline habitats. Hemeroby values covered almost the entire possible gradient (1–100, mean 50.8 ± 24.4), indicating that the dataset included both species characteristic of natural ecosystems and species associated with highly transformed habitats. Similarly, Grime's CSR strategy scores exhibited wide variation, with competitive (C), stress-tolerant (S), and ruderal (R) components ranging from nearly absent to strongly expressed (C: 0.4–81.9; S: 0–94.4; R: 0–88.4), demonstrating that the analysed flora encompassed a broad spectrum of plant ecological strategies.

The redundancy analysis showed a statistically significant relationship between the set of OJIP chlorophyll fluorescence parameters and the ecological characteristics of plant species. The full model, including Ellenberg indicator values, Hemeroby, and Grime's CSR strategy components, explained 14.5% of the total variation in fluorescence parameters, with an adjusted explained variation of 8.5%. The permutation test confirmed that this constrained component was highly significant ($F = 2.4$, $P = 0.002$). Thus, although most variation in OJIP parameters remained species-specific or related to factors not included in the model, ecological characteristics accounted for a non-random and interpretable part of the interspecific variation in PSII functioning. This indicates that chlorophyll fluorescence traits reflect not only the instantaneous physiological state of plants, but also broader species-level ecological differentiation. After accounting for the effects of Hemeroby and Grime's CSR strategy components, Ellenberg ecological indicator values retained a significant independent relationship with interspecific variation in chlorophyll fluorescence traits. The ecological predictors explained 8.9% of the total variation in OJIP parameters (adjusted $R^2 = 5.2\%$; permutation test: $F = 2.3$, $P = 0.002$). The constrained variation was strongly dominated by the first canonical axis, which accounted for 86.8% of the constrained inertia (11.9% of the total variation), suggesting that a single ecological gradient largely determined the relationship between species' ecological preferences and PSII functional characteristics. In contrast, Hemeroby showed no significant independent effect after controlling for Ellenberg indicators and CSR strategies ($R^2 = 0.56\%$, $P = 0.34$). Grime's CSR strategy components explained only a small proportion of the variation, independent of Ellenberg indicators and Hemeroby ($R^2 = 3.8\%$, $P = 0.03$). After accounting for the major climatic and edaphic gradients represented by the Ellenberg indicator values, Hemeroby, together with Grime's CSR strategy components, explained an additional but relatively small proportion of the variation in chlorophyll fluorescence traits ($R^2 = 4.3\%$, adjusted $R^2 = 2.2\%$). Although this independent contribution was modest, it remained statistically significant (permutation test: $P = 0.021$), indicating that disturbance affinity and plant ecological strategies exert effects on PSII functioning beyond those associated with environmental preferences.

The first canonical axis (RDA1), explaining 54.6% of the constrained variation, represented the principal ecological gradient of adaptation to light availability (Fig. 2). Species associated with high-light, warm, and more continental habitats were characterized by increased energy dissipation (DI_0/ABS and DI_0/RC), higher antenna-related energy fluxes (ABS/CS_0), and increased Performance Index (PIabs), indicating a photosynthetic strategy optimized for coping with excess irradiance. In contrast, species occupying comparatively more humid and nutrient-rich habitats exhibited higher photochemical efficiency, as reflected in increased RC/CS_0 and TR_0/ABS, along with reduced energy dissipation. This pattern indicates that the dominant interspecific variation in OJIP fluorescence reflects long-term adaptation of PSII functioning to contrasting light environments rather than short-term physiological stress. The second and third canonical axes (RDA2 and RDA3), jointly explaining 33.5% of the constrained variation, primarily described differences among Grime's ecological strategies after accounting for the main climatic and edaphic gradients. The ruderal (R) component and Hemeroby were closely aligned, indicating that both variables captured a common disturbance-related ecological syndrome. Species characterised by ruderal strategies showed higher VJ, VI, M_0, and ABS/CS_0 values, indicating rapid excitation build-up, faster QA reduction kinetics, and greater excitation pressure on PSII, consistent with rapid resource acquisition in disturbed environments. Conversely, stress-tolerant (S) species were associated with higher ET_0/RC and TR_0/RC values and lower donor-

and acceptor-side limitations, indicating more conservative and efficient electron transport through PSII. Competitive (C) species occupied an intermediate position and were primarily associated with increased RC/CS$_0$ and efficient utilisation of absorbed excitation energy, consistent with optimisation for productive, densely vegetated habitats. The fourth canonical axis (RDA4), accounting for the remaining 6.7% of the constrained variation, separated highly hemerobic stress-tolerant species from naturally occurring ruderal species. This residual gradient suggests that the ruderal component of the CSR framework alone cannot fully explain anthropogenic disturbance. Instead, it reflects an alternative ecological pathway in which species adapted to chronically disturbed habitats combine stress tolerance with distinct modifications of PSII energy allocation and electron transport.

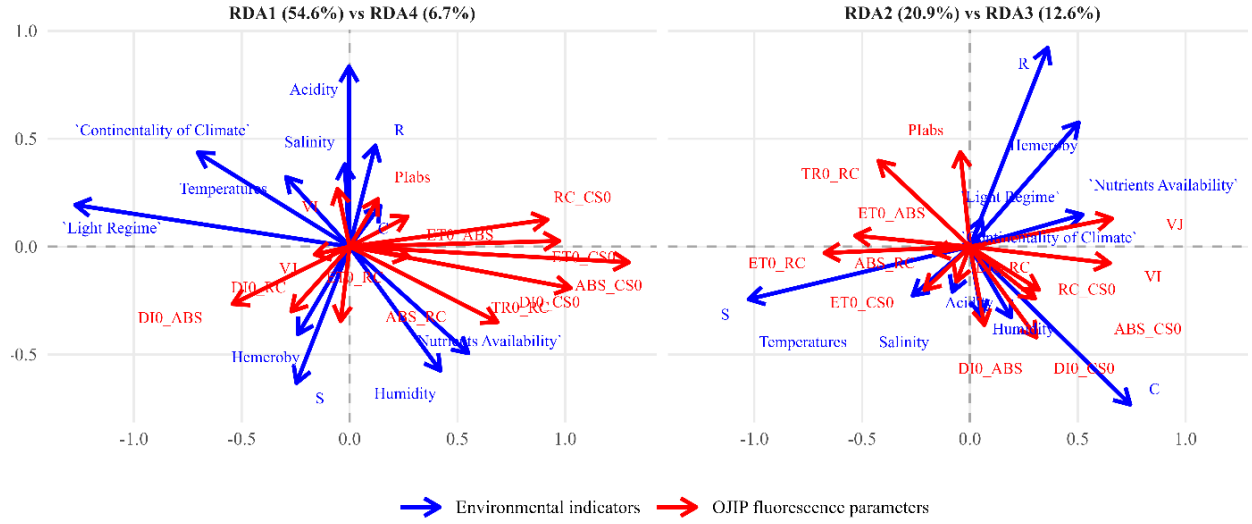

Fig. 2. Redundancy analysis (RDA) ordination showing relationships between OJIP chlorophyll fluorescence parameters and species ecological characteristics: blue vectors represent ecological predictors, including Ellenberg indicator values (Light Regime, Temperatures, Continentality of Climate, Humidity, Acidity, Nutrients Availability, and Salinity), hemeroby, and Grime's CSR strategy components (C, S, and R). Red vectors represent chlorophyll fluorescence (OJIP) parameters; the direction and length of vectors indicate the strength and direction of their relationships within the constrained ordination space; the first two canonical axes explained 71.2% and 13.6% of the constrained variation, respectively

The spider plots illustrate multivariate profiles of JIP-test chlorophyll fluorescence parameters for species positioned at the extremes of the RDA ordination space (Fig. 3). Species exhibiting the highest positive scores along the first ordination axis (RDA1), represented by *Impatiens parviflora*, *Acer negundo*, and *Glechoma hederacea*, showed a consistent shift in their chlorophyll fluorescence profiles toward a high-flux, fast-turnover photochemical phenotype.

Species positioned at the positive end of the first ordination axis (RDA1) showed increased energy absorption per cross-section (ABS/CS$_0$) and enhanced electron transport per cross-section (ET$_0$/CS$_0$), indicating a higher ability to process excitation energy within PSII. This was accompanied by a greater density of reaction centres (RC/CS$_0$) and increased absorption and trapping fluxes per reaction centre (ABS/RC and TR$_0$/RC), reflecting coordinated structural and functional improvements in the photosynthetic system. Additionally, higher values of the early OJIP parameters VJ and M$_0$ indicated faster QA reduction kinetics and more dynamic regulation of the PSII acceptor side under high excitation pressure. In contrast, species at the negative end of RDA1 displayed very similar chlorophyll fluorescence profiles, showing strong convergence in PSII functional organisation. Their profiles consistently had comparable values of DI$_0$/ABS, DI$_0$/RC, and VI, with only slight quantitative differences among species. These parameters indicate a conserved balance between non-photochemical energy dissipation and electron transport, reflecting stable control of excess excitation energy at both the reaction centre and acceptor side. Overall, RDA1 represents a coordinated reorganisation of PSII energy partitioning rather than a simple gradient of photochemical efficiency. The positive side of the axis is associated with increased structural capacity and higher energy throughput, as indicated by elevated RC/CS$_0$, ABS/CS$_0$, ET$_0$/CS$_0$, ABS/RC, and TR$_0$/RC. Conversely, the negative side is characterised by higher DI$_0$/ABS, DI$_0$/RC, and VI, indicating a shift toward greater energy dissipation and regulation of electron transport on the acceptor side. Therefore, the first ordination axis describes a shift from a high-capacity photochemical state to a more tightly regulated PSII state, in which a larger portion of absorbed excitation energy is directed into dissipative pathways.

The species *Poa angustifolia*, *Tulipa sylvestris*, and *Secale sylvestre*, associated with the positive pole of RDA4, share a coherent chlorophyll fluorescence signature characterised by elevated PIabs and VJ. This combination reflects a functionally integrated PSII state with high overall photochemical performance and a consistent pattern of early electron transport dynamics at the QA reduction level. Despite minor quantitative variation among taxa, both variables exhibit parallel shifts, indicating that the observed pattern is driven by coordinated changes in PSII efficiency rather than species-specific divergence in trait structure. The remaining JIP-test parameters remain broadly similar across species, without pronounced deviations that would indicate alternative functional configurations. This group is defined by a stable, homogeneous PSII response, in which enhanced photochemical efficiency (PIabs) is coupled with consistent regulation of early acceptor-side kinetics (VJ). Across the species associated with the negative pole of the fourth ordination axis (RDA4−), a highly consistent chlorophyll fluorescence pattern is observed, characterised by elevated values of RC-normalised energy fluxes, specifically ABS/RC, TR$_0$/RC, and ET$_0$/RC. Despite moderate quantitative differences among species, these variables show a coherent and parallel increase, indicating a shared functional configuration of PSII reaction centres. This common pattern reflects a coordinated RC-level organisation of energy processing, in which absorbed energy is efficiently channelled into trapping and subsequent electron transport at the level of individual reaction centres. The similarity of ABS/RC, TR$_0$/RC, and ET$_0$/RC across species suggests a conserved functional syndrome characterised by intensified reaction-centre–oriented energy flux regulation, without major divergence in the structure of the photochemical apparatus. Overall, species positioned at RDA4− share a convergent PSII functional profile defined by enhanced RC-based energy throughput and tightly coupled trapping–transport dynamics at the reaction centre level. RDA4 represents a mechanistic gradient from a system-level PSII configuration characterised by high overall performance (PIabs) and altered early QA redox dynamics (VJ) toward a reaction-centre–oriented state in which energy fluxes are redistributed across absorption, trapping, and electron transport processes (ABS/RC, TR$_0$/RC, ET$_0$/RC).

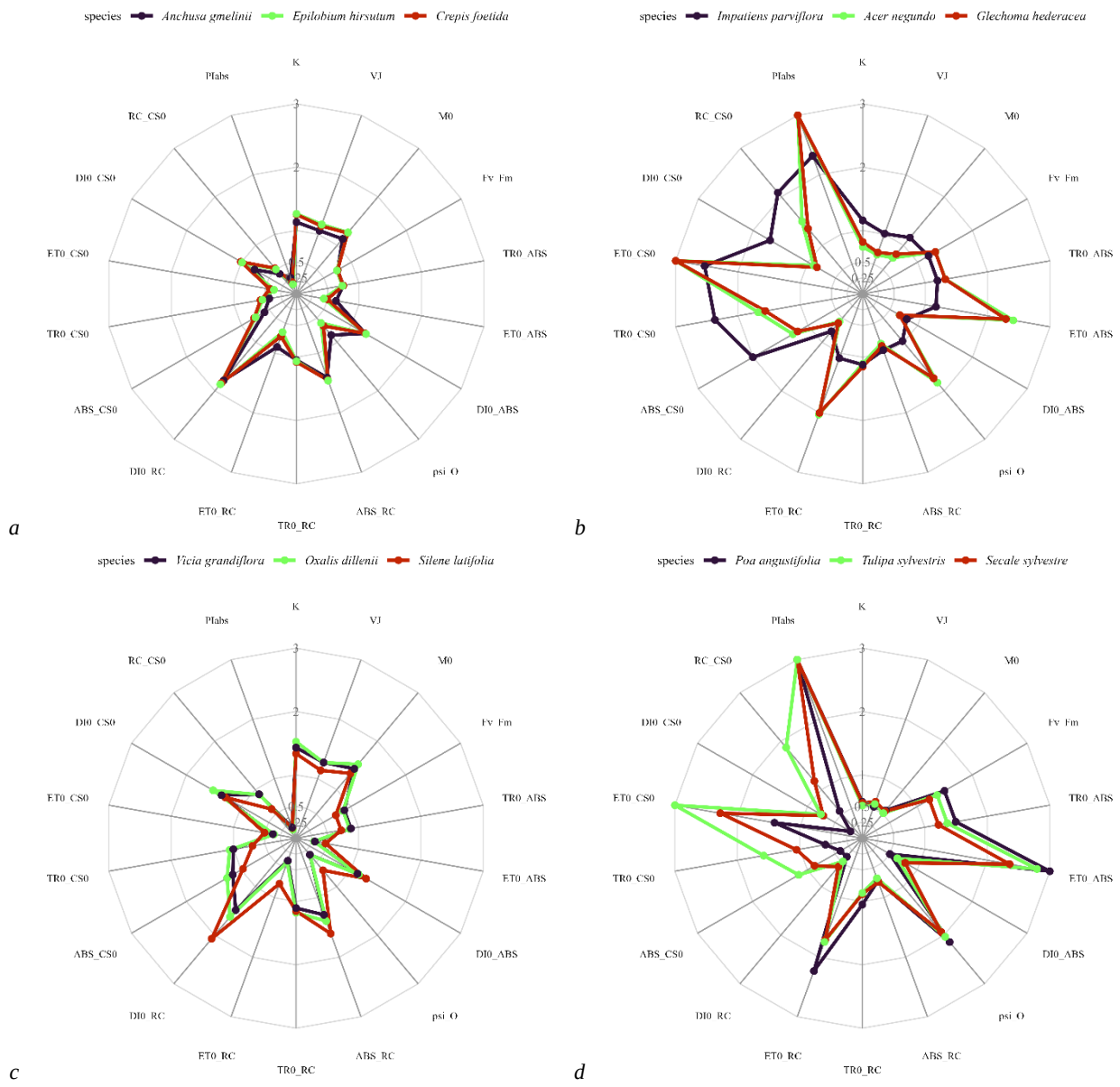

Fig. 3. Spider plots of JIP-test parameters for species exhibiting extreme scores along RDA axes. Spider (radar) plots showing standardised JIP-test chlorophyll fluorescence parameters for the three species with the highest absolute RDA scores along each canonical axis of redundancy analysis (RDA): panels correspond to: (*a*) species with the most negative scores on RDA1; (*b*) species with the most positive scores on RDA1; (*c*) species with the most negative scores on RDA4; (*d*) species with the most positive scores on RDA4; values are normalised using a global scaling approach across all species (0–3 scale), allowing direct comparison of multivariate photosynthetic trait profiles; the plotted variables include key parameters of PSII photochemistry and energy fluxes derived from the JIP-test: K – relative variable fluorescence at 300 μs (donor side limitation of PSII); VJ and VI – relative variable fluorescence at J- and I-steps (electron transport bottlenecks at QA and PQ pool level); M_0 – initial slope of fluorescence rise (rate of QA reduction); Fv/Fm – maximum quantum yield of PSII photochemistry; TR_0/ABS – trapping efficiency per absorbed energy; ET_0/ABS – electron transport efficiency; DI_0/ABS – energy dissipation per absorbed energy; ABS_RC, TR_0_RC, ET_0_RC, DI_0_RC – energy fluxes per active reaction centre; ABS_CS_0, TR_0_CS_0, ET_0_CS_0, DI_0_CS_0 – energy fluxes per cross-section; RC_CS_0 – density of active PSII reaction centres; and PIabs – performance index based on absorption

Across the species at the positive end of the second ordination axis, a clear common functional pattern emerges in chlorophyll fluorescence traits (Fig. 4). These species consistently exhibit elevated VJ and VI, with both parameters showing a coherent, comparable increase across species. This indicates a shared state of PSII acceptor-side functioning, characterised by similar dynamics of QA reduction and constraints on electron transport during the early phases of the OJIP transient. In addition, all three species show relatively higher ABS/CS_0 values than other components of the fluorescence profile, suggesting a convergent tendency toward higher light absorption per unit leaf cross-section.

Despite minor quantitative differences among species, this trait remains consistently elevated within the RDA2+ group, reinforcing its role as a shared marker of this ordination pole. Species associated with the positive side of RDA2 are characterised by a consistent combination of elevated VJ, VI, and ABS/CS_0, reflecting a common photosynthetic signature in which increased energy absorption is coupled with coordinated modifications in early electron transport dynamics at the PSII acceptor side. At the same time, the overall multivariate fluorescence structure remains broadly conserved among species. Across the species positioned at the negative end of the second ordination axis (RDA2−), namely *Poa angustifolia*, *Artemisia vulgaris*, and *Achillea millefolium*, a highly consistent chlorophyll fluorescence (OJIP-derived) pattern is observed, indicating strong convergence in PSII functional organisation. In particular, these species exhibit elevated RC-normalised energy fluxes, including ABS/RC, TR_0/RC, and ET_0/RC, as well as increased ET_0/CS_0. This coordinated pattern reflects a common functional configuration characterised by enhanced energy-processing efficiency at the level of individual reaction centres and sustained electron-transport capacity

per unit leaf area. Despite taxonomic differences, the overall shape of the fluorescence profiles is highly similar among species, with strong overlap across all key JIP-test parameters and no evidence of major structural divergence in PSII trait organisation. The consistency of elevated RC-based and electron-transport–related parameters suggests a shared functional regime in which excitation energy is efficiently channelled through reaction centres, thereby maintaining effective downstream electron transport. Species associated with the negative side of RDA2 are characterised by a convergent PSII functional syndrome, dominated by increased RC-specific energy utilisation and coordinated electron transport dynamics, resulting in a stable and functionally coherent photosynthetic profile across species. RDA2 represents a mechanistic gradient of PSII energy allocation, balancing the incoming excitation load against the capacity of reaction-centre–mediated energy processing and electron transport. The positive side of the axis is associated with a high-energy-input, acceptor-side-constrained state, characterised by elevated ABS/CS$_0$, along with increased VJ and VI, indicating intensified light harvesting combined with restrictions in early electron transport beyond QA. In contrast, the negative side of the axis reflects a reaction-centre–coordinated configuration with enhanced energy processing capacity, where higher values of TR$_0$/RC, ET$_0$/RC, ET$_0$/CS$_0$, and ABS/RC indicate more efficient energy trapping and electron transport through PSII reaction centres. Thus, RDA2 describes a functional transition from a system dominated by excitation pressure and acceptor-side limitation toward a more efficiently regulated PSII state in which energy absorption is more effectively coupled to trapping and downstream electron transport.

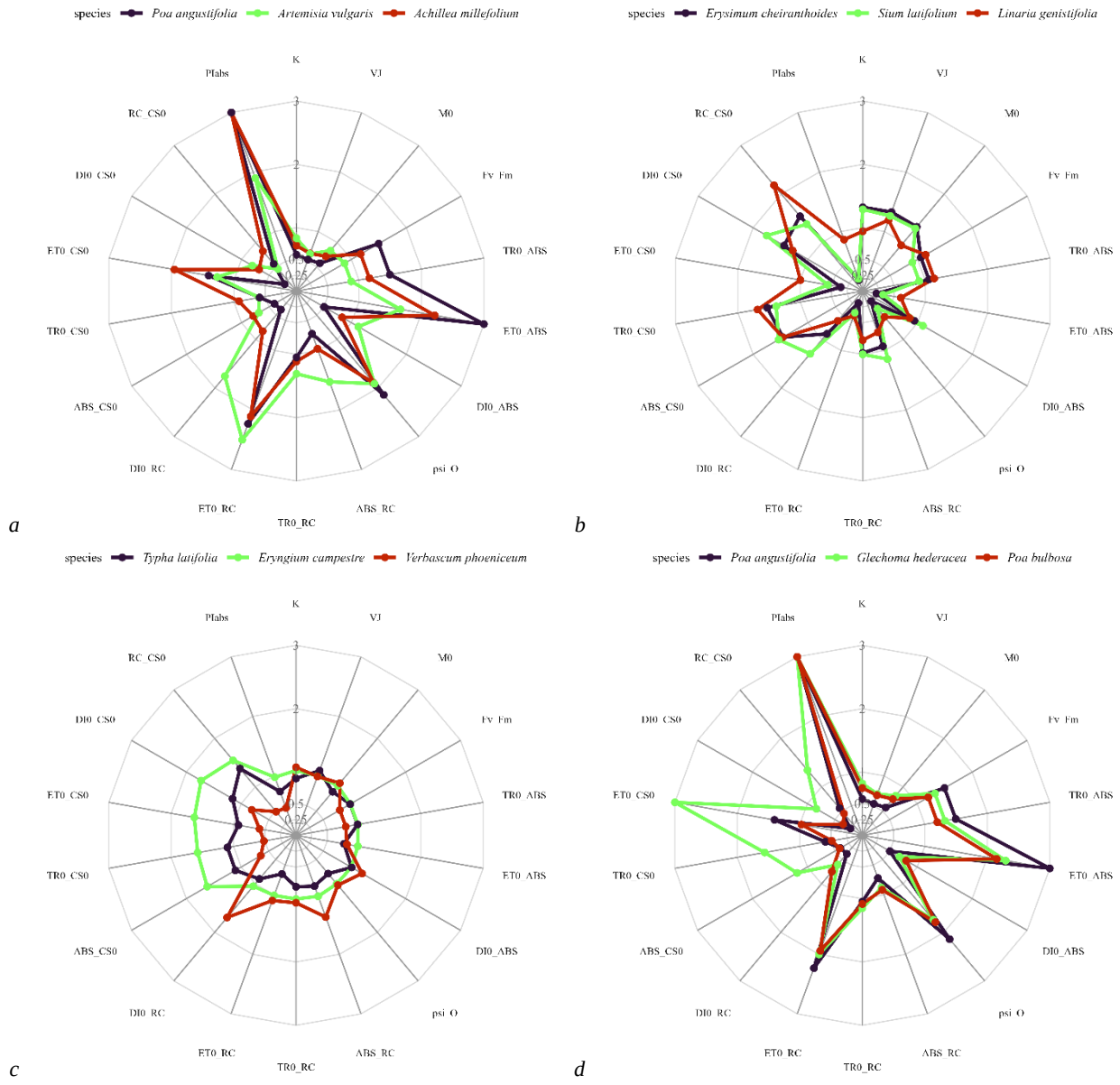

Fig. 4. Spider plots of JIP-test parameters for species exhibiting extreme scores along RDA axes: spider (radar) plots showing standardised JIP-test chlorophyll fluorescence parameters for the three species with the highest absolute RDA scores along each canonical axis of redundancy analysis (RDA); panels correspond to: (*a*) species with the most negative scores on RDA2; (*b*) species with the most positive scores on RDA2; (*c*) species with the most negative scores on RDA3; (*d*) species with the most positive scores on RDA3. Values are normalised using a global scaling approach across all species (0–3 scale), allowing direct comparison of multivariate photosynthetic trait profiles; the plotted variables include key parameters of PSII photochemistry and energy fluxes derived from the JIP-test: K – relative variable fluorescence at 300 μs (donor side limitation of PSII); VJ and VI – relative variable fluorescence at J- and I-steps (electron transport bottlenecks at QA and PQ pool level); M$_0$ – initial slope of fluorescence rise (rate of QA reduction); Fv/Fm – maximum quantum yield of PSII photochemistry; TR$_0$/ABS – trapping efficiency per absorbed energy; ET$_0$/ABS – electron transport efficiency; DI$_0$/ABS – energy dissipation per absorbed energy; ABS_RC, TR$_0$_RC, ET$_0$_RC, DI$_0$_RC – energy fluxes per active reaction centre; ABS_CS$_0$, TR$_0$_CS$_0$, ET$_0$_CS$_0$, DI$_0$_CS$_0$ – energy fluxes per cross-section; RC_CS$_0$ – density of active PSII reaction centres; and PIabs – performance index based on absorption

Poa angustifolia, *Glechoma hederacea*, and *Poa bulbosa* form the positive pole of RDA3 and are distinguished by consistently elevated PIabs together with increased TR_0/RC. This combination indicates a PSII configuration with high integrated photochemical performance and effective primary energy trapping at the level of active reaction centres. The remaining JIP-test parameters show broadly similar profiles across the three species, without a marked increase in acceptor-side limitation or energy-dissipation traits. Thus, the positive side of RDA3 reflects a high-performance photochemical configuration rather than a stress-related shift in PSII regulation. *Typha latifolia*, *Eryngium campestre*, and *Verbascum phoeniceum*, representing the negative pole of RDA3, shared a common chlorophyll fluorescence profile characterised by consistently elevated DI_0/ABS and DI_0/CS_0. Despite moderate differences in other JIP-test parameters, all three species exhibited a similar relative increase in energy dissipation both per absorbed energy and per excited cross-section. This common pattern indicates that enhanced dissipation of excitation energy represents the principal functional feature shared by species associated with the negative end of the third ordination axis. RDA3 can be interpreted as a gradient in the final partitioning of absorbed excitation energy between photochemical use and dissipation. The positive side of the axis is marked by higher PIabs and TR_0/RC, indicating greater integrated PSII performance and more effective primary energy trapping by active reaction centres. In contrast, the negative side is defined by elevated DI_0/ABS and DI_0/CS_0, reflecting a larger fraction of absorbed energy being diverted into dissipative pathways. Thus, RDA3 summarises the balance between productive photochemical energy conversion and non-photochemical energy loss within PSII.

Discussion

This study shows that plant ecological specialization is reliably indicated by chlorophyll fluorescence traits. OJIP-derived parameters serve as effective markers for both immediate physiological stress and long-term ecological differences. The relationships observed between fluorescence traits and ecological indicator values indicate that variations in PSII function align with species' positions along key environmental gradients, such as light availability, moisture, nutrient levels, habitat disturbance, and ecological strategies. In particular, species associated with open, high-light habitats tend to exhibit fluorescence profiles characterised by enhanced energy dissipation and increased excitation pressure, reflecting the need for photoprotective regulation under excess irradiance. This pattern is consistent with the established view that plants exposed to excess irradiance enhance thermal dissipation of absorbed excitation energy to protect photosystem II from photoinhibition and oxidative damage (Demmig-Adams & Adams, 1992, 2006). Conversely, species from more humid and nutrient-rich habitats, where dense vegetation often intensifies competition for light, show higher photochemical efficiency and a greater density of active PSII reaction centres, suggesting optimisation for efficient use of limited or heterogeneous irradiance. This interpretation is consistent with previous ecological and physiological studies showing that productive habitats often shift plant competition from below-ground resources toward above-ground competition for light. Nutrient enrichment and reduced disturbance can increase plant cover and canopy density, thereby decreasing light availability in the understory and intensifying light limitation for subordinate species (Hautier et al., 2009; Eskelinen et al., 2022). Under such conditions, selection should favour species capable of maintaining efficient photochemical energy conversion under low or heterogeneous irradiance. Classical studies on sun and shade plants have shown that shade-adapted plants are generally more efficient at low light intensities, even if they have lower maximum photosynthetic capacity under full light (Boardman, 1977). More recent chlorophyll fluorescence studies also indicate that moderate shading can increase Fv/Fm and Fv/Fo, reflecting improved PSII photochemical efficiency and potential activity of PSII reaction centres under reduced irradiance (Xue et al., 2023). The association between humid, nutrient-rich habitats and higher RC/CS_0 in our study is also physiologically plausible. JIP-test studies show that RC/CS_0 represents the density of active PSII reaction centres per excited cross-section and is sensitive to resource availability and stress. Under nitrogen deficiency, drought, or other limiting conditions, the number of active reaction centres and electron transport efficiency often decline, whereas better nitrogen supply and adequate water availability can support a higher number of functional reaction centres and more efficient use of absorbed energy. The higher photochemical efficiency and increased RC/CS_0 observed in species from humid and nutrient-rich habitats may reflect an ecological strategy optimised for maintaining PSII functionality and efficiently using limited light within dense vegetation. Thus, OJIP parameters capture more than transient responses to environmental stress. They reflect integrated functional syndromes shaped by species' ecological niches and evolutionary or long-term adaptive responses to habitat conditions. Differences in parameters such as DI_0/ABS, DI_0/RC, RC/CS_0, TR_0/ABS, VJ, VI, M_0, and PIabs indicate that plant species differ in how they partition absorbed excitation energy between photochemistry, electron transport, and non-photochemical dissipation. These differences align with broader ecological strategies: competitive species tend to be associated with efficient light utilisation in productive habitats; ruderal and highly hemerobic species with rapid excitation dynamics and acceptor-side regulation under disturbed open conditions; and stress-tolerant species with more conservative patterns of PSII energy processing. Consequently, chlorophyll fluorescence traits can be interpreted as species-level functional traits that link photosynthetic performance with ecological specialisation across environmental gradients.

The strongest ecological correlate of interspecific variation in OJIP fluorescence was the light regime. Species associated with high-light habitats were characterised by increased energy dissipation parameters (DI_0/ABS, DI_0/RC and φDo). In contrast, species occupying comparatively shaded or more humid environments showed higher values of photochemical efficiency indices, including Fv/Fm, Fv/Fo and TR_0/ABS. This pattern indicates that long-term adaptation to contrasting light environments is accompanied by substantial reorganisation of PSII functioning, shifting the balance between photochemical energy conversion and photoprotective energy dissipation. The observed association between high-light species and increased energy dissipation parameters is consistent with the well-established role of photoprotective energy dissipation under excess irradiance. Previous studies have demonstrated that exposure to high light increases the contribution of energy dissipation pathways and modifies OJIP fluorescence parameters within species (Tsimilli-Michael, 2020). Our results extend this concept to the interspecific level, suggesting that these physiological adjustments are reflected in long-term ecological specialization of plant species to contrasting light environments.

Increased water and nutrient availability generally enhance ecosystem productivity, resulting in greater above-ground biomass, denser canopies, and stronger competition for light. Consistent with this ecological framework, species associated with humid and nutrient-rich habitats in our dataset also exhibited a stronger competitive (C) strategy. Under these conditions, light becomes the principal limiting resource despite favourable edaphic conditions. Accordingly, species occupying such habitats were characterised by higher photochemical efficiency (TR_0/ABS and RC/CS_0), indicating an optimisation of PSII for efficient utilisation of low, heterogeneous irradiance within closed canopies. Our results are consistent with recent syntheses of chlorophyll fluorescence responses to water availability, which indicate that Fv/Fm is one of the most stable physiological indicators of plant water status. Water deficit typically causes a decline in Fv/Fm owing to impaired PSII photochemistry, whereas recovery of water supply restores higher Fv/Fm values (Peco et al., 2025). While these conclusions have largely been derived from experiments manipulating water availability within individual species, our results extend this concept to the interspecific level. Species occupying naturally more humid habitats exhibited higher photochemical efficiency, as reflected in increased Fv/Fm values, suggesting that corresponding differences in PSII function accompany long-term ecological specialisation along the moisture gradient. Thus, the physiological responses observed under experimental drought appear to scale up to persistent ecological

differentiation among plant species, indicating that chlorophyll fluorescence traits capture not only short-term acclimation but also long-term ecological adaptation. Our results are also consistent with experimental evidence demonstrating the sensitivity of Fv/Fo to plant water status (Zhang et al., 2026). Compared with Fv/Fm, the Fv/Fo ratio is considered an even more sensitive indicator of PSII functionality because it reflects the potential activity of the water-splitting complex and the efficiency of electron donation to reaction centres. Under drought conditions, Fv/Fo typically decreases owing to the progressive inactivation of PSII reaction centres and impairment of electron transport. Conversely, improved water availability restores PSII functionality and increases Fv/Fo. For example, a recent study on sunflowers showed that improving soil water availability through biochar application significantly increased Fv/Fo, indicating recovery of PSII performance and enhanced photochemical efficiency. The association between higher humidity preferences and increased TR_0/ABS is consistent with previous experimental evidence showing that water deficit reduces the efficiency of excitation energy trapping in PSII reaction centres (Umar et al., 2019). TR_0/ABS reflects the maximum quantum yield of primary photochemistry and therefore characterises the efficiency with which absorbed energy is trapped by active PSII reaction centres. In sunflowers, salt and drought stress reduced TR_0/ABS and were associated with the inactivation of PSII reaction centres, whereas better-performing cultivars maintained more efficient photochemistry under stress. Our results extend this physiological pattern from controlled stress experiments to a broader ecological context. Species associated with more humid habitats showed higher TR_0/ABS values, suggesting that improved water availability in natural environments is linked to more efficient PSII energy trapping. Moreover, because humid and nutrient-rich habitats usually support greater community biomass and denser canopies, this pattern may also reflect stronger interspecific competition for light. Under such conditions, selection may favour species that efficiently capture and use limited light energy rather than dissipate excess excitation energy.

RC/CS_0 represents the density of active PSII reaction centres per excited leaf cross-section and is therefore considered a structural indicator of photosystem II integrity rather than only a measure of photochemical efficiency. Under prolonged drought, a substantial fraction of PSII reaction centres becomes inactive or damaged owing to photoinhibition and oxidative stress (Umar et al., 2019). Consequently, RC/CS_0 decreases because fewer functional reaction centres remain available to utilise the absorbed excitation energy, while a progressively larger proportion of absorbed photons is dissipated as heat. This response has been consistently reported in OJIP analyses of drought-stressed plants and is regarded as a characteristic signature of chronic water stress. In a study of sunflower cultivars, prolonged drought and combined drought-salinity stress markedly reduced RC/CS values, along with declines in TR_0/ABS and PIABS, indicating the conversion of active into inactive PSII reaction centres and inhibition of electron transport beyond QA. The authors interpreted this decrease as evidence of structural inactivation of PSII rather than merely a reversible decline in photochemical efficiency. Our results considerably broaden this interpretation. Along the natural ecological gradient, species associated with more humid habitats exhibited higher RC/CS_0 values, suggesting that they maintain a greater density of active PSII reaction centres under their typical environmental conditions. This pattern is consistent with experimental evidence showing that improved water availability preserves the structural integrity of PSII. At the ecological scale, however, it also reflects adaptation to productive habitats where dense vegetation increases competition for light. Maintaining a larger population of active reaction centres enables more efficient utilisation of the limited photons penetrating the canopy. It may therefore represent an important component of the competitive strategy of mesophytic species.

Ruderal strategy and hemeroby are ecologically related categories, since both reflect species association with disturbed, open, and anthropogenically transformed habitats (Kunakh et al., 2026; Tutova et al., 2026). In our ordination, the ruderal component of Grime's CSR strategy and Hemeroby were oriented in a similar direction and were associated with increased VJ, VI, and M0 values. These parameters characterise the early phases of the OJIP transient and reflect the redox state of the PSII acceptor side, especially the accumulation of reduced QA and limitations in electron transport beyond QA. Therefore, ruderal and highly hemerobic species appear to be characterised by a more dynamic and potentially more overloaded acceptor side of PSII. This pattern may reflect a photosynthetic strategy typical of species adapted to frequently disturbed habitats. Such species often occur in open environments with high irradiance, rapid resource turnover, and unstable conditions. Under these circumstances, fast light capture and rapid primary photochemical activity may be advantageous, but they can also lead to a higher excitation pressure on PSII. Increased VJ, VI, and M0 may therefore indicate a strategy associated with rapid photosynthetic response and high metabolic turnover, accompanied by a greater need to regulate electron transport and prevent over-reduction of the photosynthetic electron transport chain.

An important aspect of the present study is that the relationship between chlorophyll fluorescence and ecological specialisation was revealed not through isolated pairwise associations, but through a multivariate structure of coordinated OJIP traits. Although individual parameters such as Fv/Fm, TR_0/ABS, RC/CS_0, DI_0/ABS, or VJ can be interpreted separately, their ecological meaning becomes much clearer when they are analysed as components of integrated photosynthetic syndromes. The PCA/RDA approach showed that species do not differ solely by increases or decreases in single fluorescence indices. Instead, they occupy positions along multivariate axes that combine several functionally related parameters of PSII energy absorption, trapping, electron transport, reaction centre density, and energy dissipation. This is particularly important because OJIP parameters are physiologically interdependent. For example, an increase in energy dissipation cannot be fully understood without considering absorption fluxes, reaction-centre density, and electron-transport efficiency. Similarly, changes in VJ or M_0 acquire different ecological interpretations depending on whether they are accompanied by increased ABS/CS_0, reduced ψ_0, higher DI_0/RC, or enhanced ET_0/RC. Therefore, the multivariate approach avoids oversimplifying interpretations in which each fluorescence parameter is treated as an independent stress marker. Instead, it allows the detection of coherent functional configurations that reflect the organisation of the photosynthetic apparatus as a whole.

The RDA results indicate that ecological differentiation among species is expressed through several partly independent axes of PSII functional variation. This is important because multivariate variation in OJIP traits can be structured in different ways depending on the analytical approach. In an unconstrained ordination such as PCA, the extracted axes summarise the dominant internal covariance structure of fluorescence parameters, but these axes are not necessarily linked to any predefined ecological gradient. Such an approach is useful for identifying major patterns of covariation among JIP-test parameters, as shown by Bussotti et al. (2020), but the ecological meaning of PCA axes must be inferred only after ordination. In contrast, the RDA approach used in the present study extracts axes under explicit ecological constraints. The ordination therefore identifies the component of PSII functional variation that is statistically associated with ecological indicator values, hemeroby, and components of the CSR strategy. This provides a more direct basis for ecological interpretation, because the canonical axes are not merely mathematical summaries of fluorescence covariation, but represent the part of multivariate OJIP variation that can be explained by species' ecological differentiation. Consequently, RDA enables one to distinguish ecologically structured fluorescence syndromes from the much larger pool of species-specific, developmental, genetic, or microenvironmental variation. Thus, while PCA is valuable for describing general patterns of functional organisation within the photosynthetic apparatus, RDA is particularly suitable for testing whether these patterns correspond to ecological gradients. In the present study, this constrained ordination framework showed that the organisation of PSII energy absorption, trapping, electron transport, reaction centre density, and energy dissipation is partly aligned with species differences in light preference, moisture and nutrient conditions, disturbance affinity, and CSR strategy. Therefore, the main contribution of the RDA analysis is not only

the detection of multivariate OJIP structure, but also the demonstration that this structure has a statistically supported ecological interpretation.

The first RDA axis primarily reflected the dominant ecological gradient associated with light availability and related habitat conditions. Along this axis, species from open, high-light environments were characterised by a coordinated shift toward higher energy dissipation and altered excitation pressure. In contrast, species from more humid and nutrient-rich habitats showed traits associated with higher photochemical efficiency and a greater density of active reaction centres. Thus, the main ecological signal was not represented by a single parameter, but by a coordinated reorganisation of the balance between photochemical energy use and photoprotective dissipation. The subsequent ordination axes revealed additional dimensions of variation associated with plant ecological strategies and disturbance affinity. Ruderal and highly hemerobic species tended to share fluorescence profiles marked by increased VJ, VI, M_0, and ABS/CS_0, suggesting rapid excitation build-up and stronger regulation of the PSII acceptor side. In contrast, stress-tolerant or more conservative functional strategies were associated with distinct combinations of reaction-centre-based energy fluxes and electron-transport traits. These patterns suggest that ecological strategies are not reflected solely in the absolute level of photosynthetic efficiency, but also in how absorbed energy is distributed among competing pathways within PSII. Thus, the main value of the PCA/RDA approach lies in showing that chlorophyll fluorescence traits form ecologically meaningful complexes. These complexes represent integrated modes of PSII functioning rather than isolated physiological responses. Such an interpretation is especially relevant for comparative studies across many species, where variation in fluorescence traits may reflect not only current environmental conditions, but also long-term ecological specialisation. The ordination framework, therefore, supports the view that OJIP parameters can be used as species-level functional traits, provided they are interpreted in their multivariate context. Consequently, the results of this study should not be reduced to individual relationships such as the association between Fv/Fm and moisture or between DI_0/ABS and light availability. These relationships are important, but they represent only parts of a broader functional structure. The central finding is that plant species differ in coordinated photosynthetic strategies, and these strategies correspond to ecological gradients of light, moisture, nutrient supply, disturbance, and CSR differentiation. In this sense, multivariate analysis provides a more realistic interpretation of chlorophyll fluorescence variation, as it captures the integrated organisation of photosystem II as a functional system shaped by ecological specialisation.

The ability of OJIP chlorophyll fluorescence parameters to reflect ecological specialisation stems from the integrative nature of the OJIP transient itself. The OJIP curve is not a single measure of photosynthetic performance, but a dynamic signal that summarises several interconnected components of photosystem II functioning. It contains information about the structural organisation of PSII, the density and activity of reaction centres, the efficiency of excitation energy trapping, electron transport beyond QA, the redox state of the acceptor side, and the balance between photochemical use and non-photochemical dissipation of absorbed energy. Therefore, OJIP parameters are sensitive not only to immediate stress effects, but also to persistent differences in how species organise their photosynthetic apparatus under their typical ecological conditions. This integrative character explains why ecological strategies are expressed through whole fluorescence profiles rather than through single diagnostic variables. Species adapted to open, high-light habitats are expected to require stronger photoprotective regulation, because excess irradiance increases excitation pressure on PSII and the risk of photoinhibition. This ecological context is reflected in higher dissipation-related parameters and changes in acceptor-side dynamics. In contrast, species from humid, nutrient-rich, and densely vegetated habitats often experience stronger competition for light. Their fluorescence profiles therefore tend to show higher photochemical efficiency and greater functional capacity of PSII reaction centres, reflecting an optimisation for the use of limited or heterogeneous light. Similarly, ruderal and highly hemerobic species may combine rapid light capture with more dynamic regulation of electron transport, whereas stress-tolerant species may display more conservative patterns of energy processing. Thus, OJIP works as an ecological indicator because it links several physiological processes that are directly involved in plant responses to habitat conditions. Light availability, water supply, nutrient status, disturbance regime, and plant ecological strategy all influence the balance between energy absorption, trapping, electron transport, reaction centre maintenance, and energy dissipation. Since these processes are functionally coupled within PSII, ecological specialisation becomes visible as a coordinated photosynthetic syndrome. This is why the most informative interpretation of OJIP data is multivariate: ecological differentiation is reflected in the configuration of parameters, not merely in the value of Fv/Fm, PIabs, or any other single index.

The ecological interpretation of OJIP traits should be approached cautiously. Although ecological predictors significantly explained part of the variation in fluorescence parameters, their contribution was relatively small. This is expected since OJIP fluorescence is affected by numerous factors beyond ecological preferences, such as phenological stage, plant age, leaf development, leaf position within the canopy, recent microclimate conditions, measurement time, local site history, genetic differences, and phylogenetic relationships among species. Additionally, even among the same species, fluorescence traits can fluctuate due to short-term acclimation, previous stress events, and the physiological state of individual leaves. Consequently, the relatively low percentage of explained variance does not diminish the ecological significance of the findings. Instead, it suggests that ecological specialization is just one of many factors within a complex hierarchy influencing OJIP fluorescence. The main point is that, despite the effects of phenology, ontogeny, microenvironment, genotype, and phylogeny, ecological traits still explain a meaningful and interpretable portion of interspecific variation. This validates the use of OJIP parameters as indicators of ecological differentiation, as long as they are viewed as part of an integrated photosynthetic profile rather than as standalone, universal habitat markers.

The present findings also highlight a broader conceptual implication of chlorophyll fluorescence measurements for plant functional ecology. OJIP parameters have traditionally been used as indicators of photosynthetic performance and short-term physiological responses to environmental stress. However, our results demonstrate that a substantial part of their variation is consistently associated with stable ecological characteristics of species. This suggests that OJIP-derived traits may be considered functional traits that describe species-level strategies for photosynthetic energy utilisation, complementing classical trait categories such as leaf morphology, specific leaf area, plant height, and leaf economics spectrum characteristics. Such an approach opens several promising directions for future research. First, the ecological significance of OJIP traits should be evaluated across broader taxonomic, climatic, and biogeographical gradients to determine the generality of the observed relationships. Second, future studies should explicitly partition the relative contributions of ecological specialisation, phylogenetic history, phenological stage, plant ontogeny, canopy position, and environmental acclimation to the overall variation in fluorescence parameters. Integrating phylogenetic comparative methods with chlorophyll fluorescence measurements may help distinguish evolutionary conservatism from ecological adaptation in photosynthetic functioning. Finally, repeated observations across seasons and environmental conditions will allow assessment of the temporal stability and plasticity of OJIP-derived functional profiles.

From a practical standpoint, viewing OJIP parameters as functional traits broadens their usefulness in ecological studies. Chlorophyll fluorescence offers a quick, non-harmful, and standardized way to analyze plant strategies, making it valuable for large-scale biodiversity surveys, vegetation ecology, ecosystem restoration, and environmental change assessments. Because OJIP profiles combine various aspects of PSII activity into a single physiological picture, they can supplement traditional trait data and assist in developing high-throughput phenotyping methods that connect plant physiology with ecological specialization. This integration can enhance trait-based

ecology by including functional traits that reflect the physiological processes driving species adaptation to environmental gradients. Instead of just indicating short-term stress, OJIP fluorescence traits can be seen as functional traits that link the structure of photosystem II with long-term ecological adaptations.

Conclusion

This study shows that chlorophyll fluorescence traits give valuable insights into stable ecological differences among plant species. Relationships between OJIP-derived parameters and ecological indicator values indicate that variations in photosystem II function reflect long-term species adaptations to different habitats. Species with preferences for varying light, moisture, nutrients, disturbance levels, and CSR strategies displayed corresponding differences in PSII energy absorption, excitation trapping, electron transport, reaction centre density, and non-photochemical energy dissipation. A key finding is that ecological specialization is represented by coordinated fluorescence profiles composed of multiple related OJIP parameters. Multivariate analysis identified distinct photosynthetic syndromes aligned with ecological gradients, revealing that species differ in their balance of photochemical energy conversion, electron transport, and photoprotective dissipation. Species in open, disturbed habitats generally showed fluorescence patterns indicating higher excitation pressure and photoprotection, while those in humid, productive, or densely vegetated areas exhibited traits suggesting greater efficiency and light utilization. These patterns suggest that the functional organization of photosystem II is an important element of plant ecological strategy. Ecological specialization is one of several factors influencing chlorophyll fluorescence variation. Other factors such as phenological stage, plant age, leaf position, microenvironment, past stress, genetics, geography, and phylogeny also affect OJIP traits. Therefore, ecological traits account for a moderate yet statistically significant and biologically relevant portion of interspecific differences in PSII function, demonstrating that ecological adaptation leaves a detectable functional signature amid other sources of variation. The findings support viewing OJIP chlorophyll fluorescence parameters as species-level functional traits. Within a multivariate framework, these traits describe integrated photosynthetic syndromes connecting the physiological organization of photosystem II with ecological specialization. This approach broadens OJIP analysis from purely physiological assessment to a tool for functional and comparative plant ecology, offering a quantitative method to explore how photosynthetic organization facilitates species adaptation across environmental gradients. By linking physiological performance and ecological specialisation, OJIP fluorescence traits act as a functional bridge between photosynthetic and trait-based plant ecology, enabling the study of adaptive differentiation across environmental gradients.

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