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Functional variation in PSII traits along post-disturbance successional gradient in montane springs

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Abstract

The effects of post-windstorm succession on mountain spring vegetation were studied in the High Tatra Mountains (Slovakia, Central Europe) through field sampling conducted over the 2020–2025 period, assessing chlorophyll *a* fluorescence to compare windthrow-affected and unaffected plots. Variance partitioning revealed that structural PSII parameters (absorption flux per RC, relative variable fluorescence at the J step) are primarily species-dependent, whereas maximum quantum yield is environment-driven. Within-site analyses validated that species-specific PSII architecture persists under uniform conditions. The invasive *Impatiens parviflora* displayed the largest antenna size per reaction center (RC) but the lowest performance index and the highest dissipation flux per active RC. Community-weighted mean analysis revealed a successional trade-off: shaded, closed-canopy sites exhibited high photosynthetic performance but low species richness, whereas open sites prioritized biodiversity over photosynthetic optimization. These results suggest a shift in the balance between community-level physiological efficiency and species diversity in mountain spring habitats, with significant implications for the conservation of these sensitive ecosystems.

Keywords: chlorophyll fluorescence; community-weighted mean; functional diversity; mountain spring; post-disturbance succession; species diversity.

Introduction

Global climate change is rapidly transforming the physical environment of terrestrial ecosystems and presenting plants

with unprecedented changes in temperature, precipitation patterns, and CO₂ concentrations. Climate-induced changes are more pronounced in mountainous areas than in low-altitude regions (Pepin *et al.* 2015). They are often

Highlights

- Species identity is the dominant driver of PSII structural parameter variation
- Post-disturbance succession creates a trade-off between PSII optimization and diversity
- Invasive *I. parviflora* shows high light absorption but also high heat dissipation

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Abbreviations: ABS/RC – absorption flux per RC; Chl – chlorophyll; CWM – community-weighted mean; DI₀/RC – dissipation flux per active RC, dissipated in the form of heat, fluorescence, or transfer to other systems, at time *t* = 0; ET₀/RC – electron transport flux (further than Q_A⁻) per RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; RC – reaction center; RE₀/RC – electron flux reducing end electron acceptors at the PSI acceptor side, per RC; RWC – relative water content; TR₀/RC – trapped energy flux per RC; V_J – relative variable fluorescence at the J step (2 ms); φDo – quantum yield of energy dissipation in PSII antennae and reaction centers; φEo – quantum yield for electron transport (ET); φPo – maximum quantum yield for primary photochemistry at any time *t*; φRo – quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE); ψEo – efficiency/probability that an electron moves further than Q_A⁻.

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manifested as catastrophic natural disturbances, which may trigger successional processes (Fleischer *et al.* 2017), facilitating the coexistence of plant species with different ecological niches and resource-acquisitive strategies. The successional mosaic of plant communities represents an ideal natural system for studying changes in plant species assemblages and their associated functional traits along a light availability gradient, *i.e.*, from open forest clearings characterized by high irradiance to structurally complex and canopy-closed forest habitats (Fleischer *et al.* 2017, Schulz 2023).

Spring forests are usually small-patch communities developed on waterlogged soils saturated by groundwater discharges. These habitats occur in montane catchments at stream sources, at the base of slopes within floodplains, or in gently sloping headwater areas on hillslopes. They are considered ecological and biodiversity hotspots, whose functioning may be largely independent of stream water and channel-related processes (Pielech *et al.* 2015), due to the predominant role of groundwater supply (Kuglerová *et al.* 2014). Active groundwater discharge maintains relatively stable soil moisture and thermal regimes, thereby enhancing the resilience of spring forests to climate-driven hydrological extremes, such as intra-seasonal variability in streamflow or shifts in snowmelt dynamics. The overstorey of spring forests is characterized by a mosaic structure with a fine-scale partitioning of light resources (Pielech *et al.* 2015). This pattern is attributed to the well-documented susceptibility of unstable waterlogged soil surfaces to recurrent tree-fall events and the associated formation of canopy gaps (Slezák *et al.* 2024). However, extensive tree canopy removal induced by natural disturbance events may lead to pervasive microclimate changes that impose physiological stress primarily on shade-tolerant species through elevated temperatures and increased light availability. Conversely, the release of understory vegetation from light competition with overstorey trees may facilitate rapid colonization and growth of light-demanding species, including invasive plant species. Interspecific competitive hierarchies and the redistribution of resources (mainly light availability) undergo marked reorganization along post-disturbance successional gradients. Plant functional traits, especially those related to the photosynthetic apparatus (Picorel *et al.* 2017, Miller *et al.* 2021), may therefore differ between high-irradiance microhabitats in open clearings and shaded environments beneath mature or regenerating forest canopies.

These contrasting light environments impose fundamentally different photochemical challenges on co-occurring species, from shade acclimation in hygrophytic forbs to high-light stress tolerance in species colonizing open post-disturbance patches. Chlorophyll *a* (Chl *a*) fluorescence transient analysis (the JIP-test) offers a high-throughput, nondestructive approach capable of capturing the full PSII energy flux cascade under field conditions (Strasser *et al.* 2004, Stirbet and Govindjee 2011, Živčák *et al.* 2014, Konôpková *et al.* 2020, Hammami *et al.* 2024). To date, this approach has not been applied to natural mountain spring plant communities, despite its

potential to disentangle structural photosynthetic traits linked to species identity from functional indicators that respond directly to environmental pressures (Bussotti *et al.* 2010, Strasser *et al.* 2010).

Four species that are abundant across our study sites represent markedly different functional types: *Alnus incana* (L.) Moench is a deciduous tree and actinorhizal nitrogen-fixer; *Caltha palustris* L. is the dominant hygrophytic forb present in 74% of all vegetation plots; *Crepis paludosa* (L.) Moench is a subdominant herb of intermediate ecological amplitude. Moreover, the colonization of these springs by invasive therophyte species (*e.g.*, *Impatiens parviflora*) after canopy disturbance adds a new functional dimension to this process (Hejda 2012).

The co-occurrence of functionally distinct species within the Tatra mountain spring also provides an opportunity to test whether community composition generates predictable community-level photosynthetic properties. Although existing studies have applied the community-weighted mean (CWM) approach (Lavorel and Garnier 2002, Garnier *et al.* 2004) to relate functional traits to productivity in grassland and forest ecosystems (Yan *et al.* 2023), the photochemical profile of spring species remains uniquely underexplored. Integrating these physiological parameters with diversity metrics allows us to assess how community-level photosynthetic performance is traded off against species diversity along successional trajectories (Bongers *et al.* 2021).

The objectives of this study were: (1) to assess the physiological status of four co-occurring spring plant species representing different functional types; (2) to compare the relative contribution of species identity and environmental factors in determining PSII energy allocation, and (3) to investigate how community-level photosynthetic optimization is affected by climate change associated with mountain forest disturbances.

Materials and methods

Study area and sample collection: The study was conducted across five spring sites categorized by canopy cover and/or disturbance history in the High Tatra Mountains (northern Slovakia) during the vegetation periods of 2020–2025 (Fig. 1). Sampling sites comprised three forested springs with a well-developed tree canopy (Štôla, Nová Lesná, Tatranská Lomnica; 870–950 m a.s.l.; hereinafter termed “shaded”) and two open spring areas with disturbed tree canopy in the last two decades (Javorová dolina, Bielovodská dolina; 1,050–1,200 m a.s.l.; hereinafter termed “open”). Habitat selection was based on local experts’ knowledge of spring distribution and data retrieved from the literature. The pre-selected sites were checked using available orthophotomaps (Geodetic and Cartographic Institute Bratislava, National Forest Centre Zvolen, Slovakia) and verified *in situ* by visual assessment. These spring stands were clearly delineated from adjacent (usually mesic) vegetation patches by characteristic plant species assemblages associated with waterlogged soils with a developed gleyic horizon and groundwater

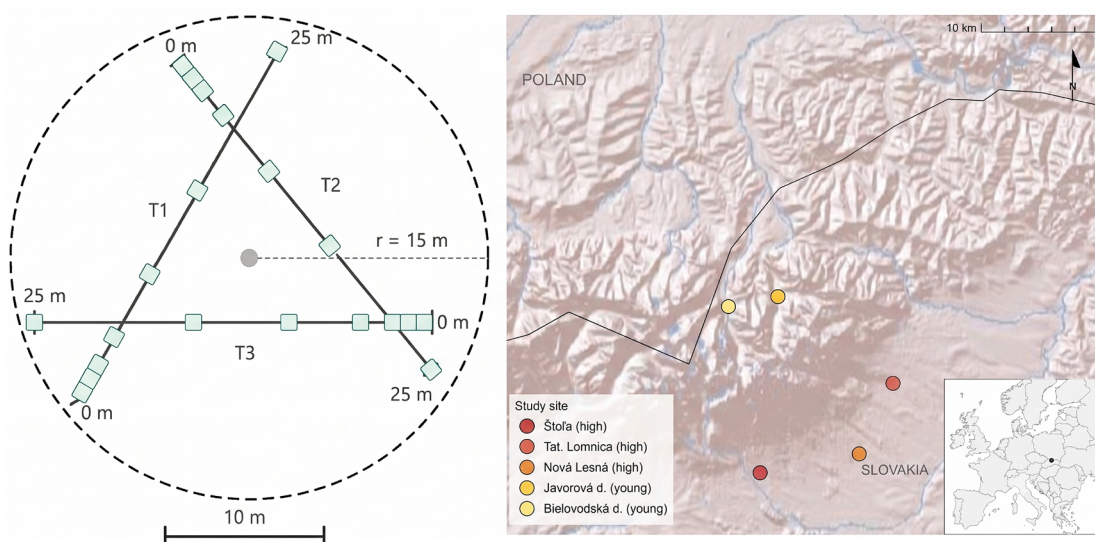


Fig. 1. Distribution of the research plots with a triangular sampling design with three 25-m transects (T1–T3) and 21 square micro-plots (1 m × 1 m; seven per transect), nested within a circular plot with a 15-m radius, was established at each spring site.

discharge. At each sampling site, the vegetation was recorded using a triangular sampling design (Brownstein *et al.* 2012) consisting of three 25-m crossed transects, with seven micro-plots (1 m × 1 m) established at fixed center distances of 0.5, 1.5, 2.5, 4.5, 9.0, 15.0, and 25.0 m along each transect. The transects were embedded in a circular plot of 15-m radius, centered on the intersection point of the triangular arrangement (Fig. 1). The final dataset contained 105 micro-plots (5 sites × 21 micro-plots). All vascular plant species and their percentage cover were recorded within each micro-plot. While circular plots were used to measure functional traits of four target species, species records from the micro-plots were used to calculate species diversity metrics (*i.e.*, species richness, Shannon diversity index), and CWM.

Chlorophyll *a* fluorescence measurement: Chlorophyll *a* fluorescence transients were measured in July using a HandyPEA portable fluorimeter (Hansatech Instruments, King's Lynn, UK). This mid-growing-season period reduces potential bias arising from intra-seasonal variability in photochemical performance. It coincides with the peak in above-ground biomass of herb-layer vegetation, a period critical for community–diversity assessment (Vymazalová *et al.* 2012). Fully expanded leaves were dark-adapted for 30 min using leaf clips before application of a saturating red-light pulse [3,000 μmol(photon) m⁻² s⁻¹, peak 650 nm, 1-s duration]. A total of 373 measurements were obtained across four species: *Alnus incana* (*n* = 96), *Caltha palustris* (*n* = 93), *Crepis paludosa* (*n* = 94), and *Impatiens parviflora* (*n* = 90), with 15–20 individuals per species per locality measured on different individuals.

Total chlorophyll content: Total chlorophyll content expressed as a chlorophyll index was measured using the portable CL-01 Chlorophyll Content Meter (Hansatech, UK), providing a rapid, nondestructive estimate of leaf pigment contents by measuring relative optical

absorbance at two wavelengths: a chlorophyll-absorbing red waveband (660 nm) and a nonabsorbing near-infrared reference waveband (940 nm) to account for leaf thickness and structural variation. Fifty measurements were done for each species per locality. The highest Chl index values were recorded in *A. incana* (42.0 ± 4.3), followed by *C. palustris* (37.8 ± 4.2), *C. paludosa* (33.4 ± 4.3), and *I. parviflora* (23.7 ± 2.3).

Stomatal morphology and relative water content:

Stomatal density (SD, number per 1 mm²) and guard cell length (LA, μm) were determined from nail varnish impressions of the abaxial leaf surface examined at 200× and 400× magnification using a microscope with a digital camera Levenhuk MED D35T (Levenhuk Inc., USA) and Image J software (*n* = 85 measurements, 15–25 per species). Leaf relative water content (RWC) was measured gravimetrically: $RWC = (FM - DM)/(TM - DM) \times 100$ (*n* = 100 measurements, 5 per species per locality), where FM = fresh mass, DM = dry mass, TM = turgid mass representing fully hydrated leaf.

Community-weighted means:

Community-weighted means (CWM) of JIP-test parameters were calculated for each vegetation plot following Garnier *et al.* (2004): $CWM_i = \Sigma(p_i \times t_i) / \Sigma p_i$, where *p_i* is the percentage cover of species, *t_i* is the mean trait value of species *i* at the given locality. Trait values were calculated as species × locality means from the HandyPEA data (*n* = 18 species–locality combinations). CWM was computed for *F_v/F_m*, *PI_{ABS}*, *φE_o*, *φDo*, *DI_o/RC*, and *ABS/RC*. Only the four measured species contributed to CWM calculations; their combined cover averaged 28.0% per plot, and at least one was present in 91 of 96 plots (95%). The CWM was therefore calculated using only those species actually present at each plot. When *p_i* = 0 (species absent), it contributes nothing to the numerator or denominator, and we are weighting by actual abundance. We acknowledge that CWM values

based on four species represent a partial characterization of community-level photosynthetic properties; however, these species span the full functional range present in the community (tree, dominant forb, subdominant forb, invasive annual), and their cover represents a substantial fraction of the herb layer.

Functional diversity of PSII traits: Functional diversity of PSII traits was quantified as the variance among trait values of co-occurring measured species (weighted by cover) within each plot. This metric captures the spread of photosynthetic strategies presented in the community and is analogous to functional dispersion (Laliberté and Legendre 2010) but restricted to the four measured species.

Statistical analysis: All statistical analyses were performed using *Python 3.11* using *SciPy 1.11*, *scikit-learn 1.3*, and *pandas 2.0*. Interspecific differences were tested using the *Kruskal–Wallis* H-test with *Dunn’s* post-hoc comparisons. Effect sizes were quantified as $\eta^2 = (H - k + 1) / (N - k)$, where k is the number of groups and N is the total sample size (Tomczak and Tomczak 2014). Species effects were additionally tested within each site type separately to control for the elevation-light confound between shaded (870–950 m a.s.l.) and open (1,050–1,200 m a.s.l.) localities. Correlations between CWM traits and diversity metrics (species richness, *Shannon’s* H') were assessed using *Spearman’s* rank correlation (ρ). Significance was set at $\alpha = 0.05$ throughout.

Results

Relative contributions of species identity and locality to variation in PSII fluorescence parameters: Variance partitioning using η^2 effect sizes from *Kruskal–Wallis* tests revealed a clear contrast in the relative contributions of species identity and locality to variation in JIP-test parameters (Table 1). Parameters describing PSII structural organization were predominantly determined by species identity: ABS/RC showed 49-fold greater η^2 for species than for locality ($\eta^2 = 0.438$ vs. 0.009), and V_J showed a 9.5-fold difference ($\eta^2 = 0.142$ vs. 0.015).

In contrast, the maximum quantum yield F_v/F_m was primarily environment-driven ($\eta^2_{\text{locality}} = 0.406$ vs. $\eta^2_{\text{species}} = 0.128$), confirming that this widely used stress indicator reflects site conditions more than constitutive species properties in field settings. PI_{ABS} and DI_0/RC showed intermediate patterns (η^2 ratios of 2.5 and 3.3, respectively), consistent with their integrative character, as they incorporate both structural and functional components of PSII performance. This distinction between constitutive PSII architecture (species-driven) and acclimation-responsive efficiency (environment-driven) has important implications for interpreting field fluorescence data and underpins the CWM patterns described below.

Species-specific PSII energy allocation profiles: The *Kruskal–Wallis* tests revealed clear species-specific differences in the performance of PSII across the four studied spring species. Several JIP-test parameters showed highly significant variation among species, indicating contrasting physiological strategies. For example, F_v/F_m , ϕEo , and PI_{ABS} exhibited strong species effects ($p < 0.001$), with *Alnus incana* and *Caltha palustris* generally maintaining higher values associated with more efficient photochemical energy conversion. In contrast, *Impatiens parviflora* showed comparatively lower performance in these parameters, but the highest thermal dissipation ($DI_0/RC = 0.36 \pm 0.05$).

Species differences were accompanied by moderate to strong effect sizes (η^2), suggesting biologically meaningful divergence in PSII functioning. Locality effects were also significant for several fluorescence traits, although generally weaker than species effects. Sites that had undergone successional overgrowth (Štôla, Nová Lesná, Tatranská Lomnica) tended to show different fluorescence responses compared to the two open, high-elevation post-disturbance sites. These patterns indicate that shading and microhabitat conditions modulate PSII performance across species.

Species $\times$ locality variation in PSII parameters: Species $\times$ locality boxplots (Fig. 2) revealed consistent

Table 1. Mean $\pm$ SD of JIP-test parameters for four spring fen species ($n = 373$). *Kruskal–Wallis* H-statistics and η^2 effect sizes [in brackets] for species and locality effects (*** $p < 0.001$, ** $p < 0.01$, ns = not significant). ABS/RC – absorption flux per RC; DI_0/RC – dissipation flux per active RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; V_J – relative variable fluorescence at the J step (2 ms); ϕDo – quantum yield of energy dissipation in PSII antennae and reaction centers; ϕEo – quantum yield for electron transport (ET).

Parameter	<i>Alnus incana</i> (96)	<i>Caltha palustris</i> (93)	<i>Crepis paludosa</i> (94)	<i>Impatiens parviflora</i> (90)	H (species) [η^2]	H (locality) [η^2]
F_v/F_m	0.831 $\pm$ 0.034	0.830 $\pm$ 0.022	0.812 $\pm$ 0.038	0.816 $\pm$ 0.012	48.1*** [0.128]	146.0*** [0.406]
ϕEo	0.256 $\pm$ 0.023	0.269 $\pm$ 0.029	0.266 $\pm$ 0.025	0.249 $\pm$ 0.013	39.9*** [0.105]	35.5*** [0.090]
ϕDo	0.169 $\pm$ 0.034	0.170 $\pm$ 0.022	0.188 $\pm$ 0.038	0.184 $\pm$ 0.012	47.9*** [0.128]	146.0*** [0.406]
ABS/RC	1.351 $\pm$ 0.184	1.365 $\pm$ 0.239	1.556 $\pm$ 0.274	1.973 $\pm$ 0.169	156.8*** [0.438]	7.0 ns [0.009]
DI_0/RC	0.231 $\pm$ 0.072	0.236 $\pm$ 0.071	0.298 $\pm$ 0.112	0.364 $\pm$ 0.054	127.6*** [0.355]	41.3*** [0.107]
V_J	0.692 $\pm$ 0.023	0.677 $\pm$ 0.030	0.673 $\pm$ 0.029	0.695 $\pm$ 0.012	52.9*** [0.142]	9.2 ns [0.015]
PI_{ABS}	1.75 $\pm$ 0.55	1.89 $\pm$ 0.71	1.49 $\pm$ 0.52	1.02 $\pm$ 0.20	92.8*** [0.256]	39.7*** [0.102]
Chl index	42.0 $\pm$ 4.3	37.8 $\pm$ 4.2	33.4 $\pm$ 4.3	23.7 $\pm$ 2.3	229.8*** [0.644]	6.7 ns [0.008]

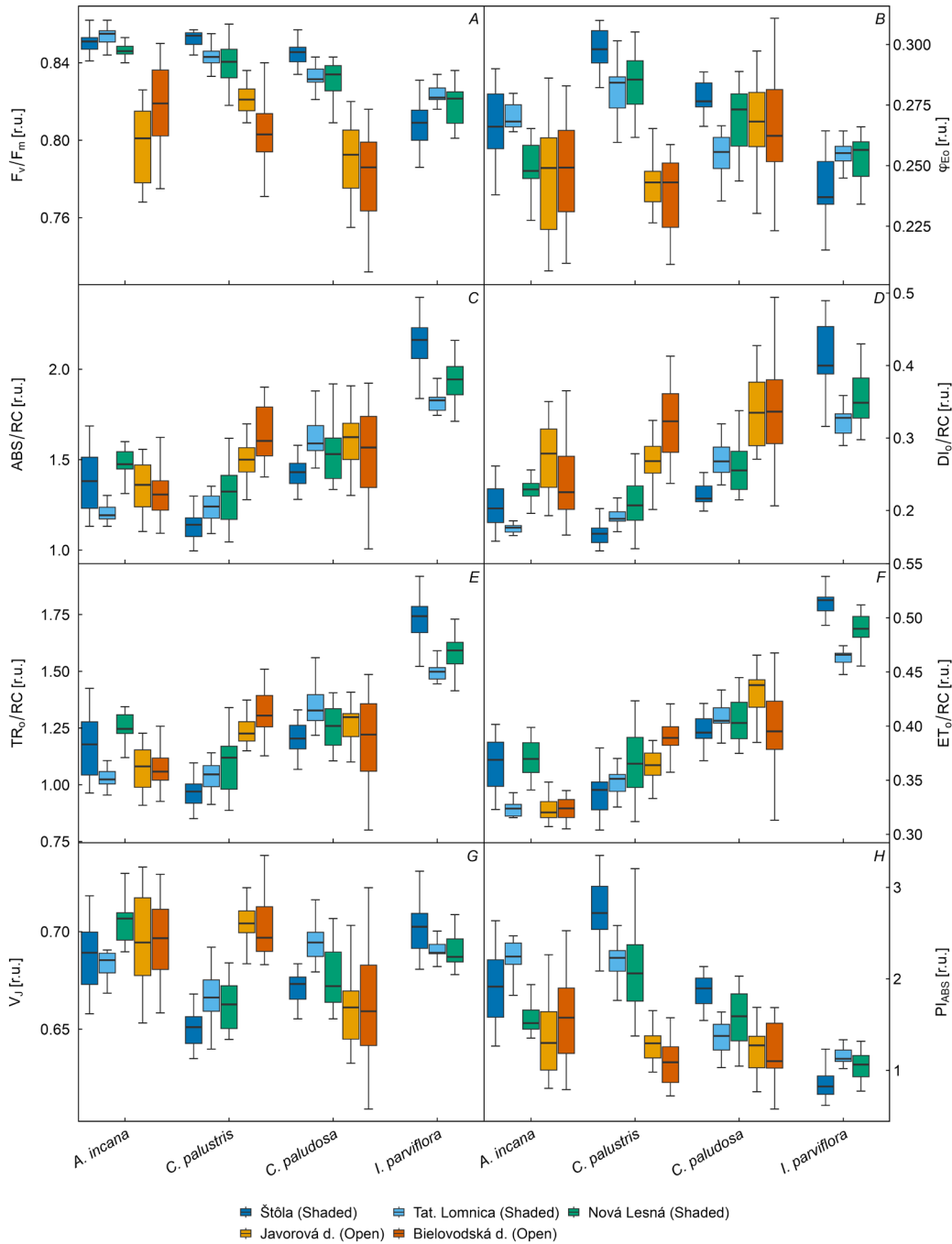


Fig. 2. Species $\times$ locality boxplots for eight JIP-test parameters ($n = 373$). Boxplots show the median (horizontal line), the interquartile range (box; 25th–75th percentiles), and whiskers extending to the most extreme values within $1.5 \times \text{IQR}$. Outliers (beyond $1.5 \times \text{IQR}$ within each locality $\times$ species combination) were removed prior to plotting. Dark blue, blue, and green box plots indicate shaded localities (Štôla, Nová Lesná, Tatranská Lomnica), orange and red box plots indicate open localities (Javorová dolina, Bielovodská dolina). Species are ordered left to right: *A. incana*, *C. palustris*, *C. paludosa*, *I. parviflora*. ABS/RC – absorption flux per RC; DI_o/RC – dissipation flux per active RC; ET_o/RC – electron transport flux (further than Q_A⁻) per RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; TR_o/RC – trapped energy flux per RC; V_j – relative variable fluorescence at the J step (2 ms); φE_o – quantum yield for electron transport (ET).

interspecific differences across all ten JIP-test parameters. *Impatiens parviflora* exhibited consistently higher ABS/RC and DI_o/RC values across all three shaded localities where it occurred, confirming the robustness of its high

energy-absorption and also dissipation strategy across environments.

The shaded-to-open gradient was most apparent in F_v/F_m, PI_{ABS}, and φE_o, where all species showed lower

values in open localities (Javorová dolina, Bielowodská dolina) compared to shaded localities (Štôla, Nová Lesná, Tatranská Lomnica). In contrast, ABS/RC and V_j showed minimal variation across localities, consistent with their species-constitutive nature ($\eta^2_{\text{locality}} = 0.009$ and 0.015 , respectively).

Community-weighted PSII performance based on focal species: Community-weighted means (based on

focal species) of PSII parameters showed significant correlations with species diversity across 91 vegetation plots (Fig. 3). CWM PI_{ABS} was negatively correlated with species richness ($\rho = -0.546$, $p < 0.001$; Fig. 3A), as was CWM F_v/F_m ($\rho = -0.557$, $p < 0.001$; Fig. 3B) and CWM ϕ_{Eo} ($\rho = -0.463$, $p < 0.001$). Conversely, CWM DI_o/RC was positively correlated with richness ($\rho = +0.531$, $p < 0.001$; Fig. 3C), indicating that species-rich communities show higher community-level energy dissipation. All six

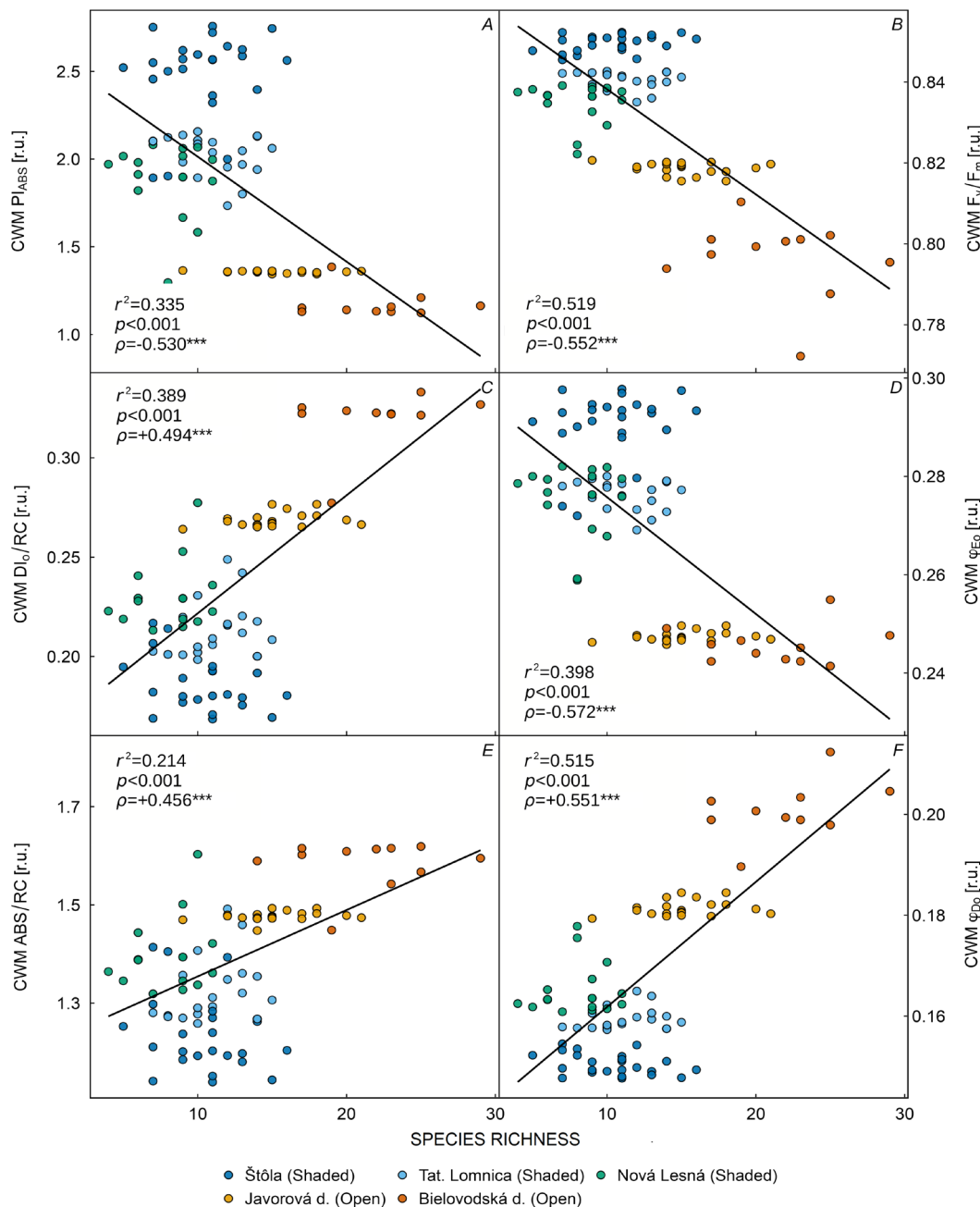


Fig. 3. Community-weighted mean (CWM) photosynthetic performance vs. species diversity ($n = 91$ plots). (A) CWM PI_{ABS} vs. species richness. (B) CWM F_v/F_m vs. species richness. (C) CWM DI_o/RC vs. species richness. (D) CWM ϕ_{Eo} vs. species richness. (E) CWM ABS/RC vs. species richness. (F) CWM ϕ_{Do} vs. species richness. All *Spearman's* correlations $p < 0.001$. ABS/RC – absorption flux per RC; DI_o/RC – dissipation flux per active RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; ϕ_{Do} – quantum yield of energy dissipation in PSII antennae and reaction centers; ϕ_{Eo} – quantum yield for electron transport (ET).

Table 2. *Spearman's* rank correlations between community-weighted mean (CWM) JIP-test parameters and species diversity metrics across 91 vegetation plots. All correlations are significant at $p < 0.001$. ABS/RC – absorption flux per RC; DI₀/RC – dissipation flux per active RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; V_J – relative variable fluorescence at the J step (2 ms); ϕDo – quantum yield of energy dissipation in PSII antennae and reaction centers; ϕEo – quantum yield for electron transport (ET).

CWM parameter	vs. Richness (ρ)	p	vs. <i>Shannon's</i> H' (ρ)	p
CWM PI _{ABS}	−0.546	<0.001***	−0.444	<0.001***
CWM F_v/F_m	−0.557	<0.001***	−0.445	<0.001***
CWM ϕEo	−0.463	<0.001***	−0.351	<0.001***
CWM ϕDo	+0.557	<0.001***	+0.443	<0.001***
CWM DI ₀ /RC	+0.531	<0.001***	+0.450	<0.001***
CWM ABS/RC	+0.476	<0.001***	+0.418	<0.001***

CWM-diversity correlations were significant at $p < 0.001$ (Table 2).

Shaded localities with closed canopy had high CWM performance but low species richness: Štôla showed the highest CWM PI_{ABS} (2.54 ± 0.16) and richness of 10.1 ± 3.1 (Fig. 4). Open localities showed the opposite pattern: Bielovodská dolina had CWM PI_{ABS} of 1.23 ± 0.07 with richness of 21.4 ± 4.6 . The *Kruskal–Wallis* test confirmed significant differences in CWM PI_{ABS} between localities ($H = 80.6, p < 0.001$). This gradient was consistent across all CWM parameters, with Tatranská Lomnica occupying an intermediate position (CWM PI_{ABS} = 2.06, richness = 9.0).

Shaded vs. open site comparison: Direct comparison between shaded and open sites revealed significant differences for most fluorescence parameters across species (Fig. 5). F_v/F_m was significantly higher in shaded than open sites for all three native species (*A. incana*: 0.849 vs. 0.800, $p < 0.001$; *C. palustris*: 0.843 vs. 0.812, $p < 0.001$; *C. paludosa*: 0.834 vs. 0.782, $p < 0.001$). PI_{ABS} showed the same pattern, with substantially higher values in shaded sites for *A. incana* (1.95 vs. 1.42, $p < 0.001$), *C. palustris* (2.31 vs. 1.26, $p < 0.001$), and *C. paludosa* (1.60 vs. 1.33, $p < 0.001$). DI₀/RC was consistently lower in shaded sites, reflecting reduced thermal dissipation under the canopy. *Impatiens parviflora* occurred exclusively in shaded localities, precluding direct comparison, but its PSII performance remained significantly inferior to native species even within shaded sites (F_v/F_m : $H = 130.4, p < 0.001$; Fig. 4, top row).

Functional diversity of PSII traits: Functional diversity of PSII traits was significantly higher in open than in shaded sites. The variance of F_v/F_m among co-occurring species was 1.6 times higher in open sites (0.000224 vs. 0.000140, $p = 0.009$). The functional diversity of F_v/F_m correlated positively with species richness ($\rho = +0.271, p = 0.024$), indicating that species-rich open communities reflect a wider range of photosynthetic strategies, while shaded communities respond with a more balanced PSII performance.

Chlorophyll content, stomatal traits, and water relations: Chlorophyll content, expressed as the Chl index

(Table 1), differed markedly between species ($H = 229.8, p < 0.001, \eta^2 = 0.644$), while no significant effect of locality was observed ($H = 6.7, ns, \eta^2 = 0.008$). The highest Chl index values were recorded in *A. incana* (42.0 ± 4.3), followed by *C. palustris* (37.8 ± 4.2) and *C. paludosa* (33.4 ± 4.3), whereas *I. parviflora* exhibited substantially lower values (23.7 ± 2.3). These results indicate strong interspecific variation in chlorophyll content, with minimal influence of site conditions.

Stomatal traits showed strong species-level differentiation ($\eta^2 = 0.457$ for stomatal density, 0.284 for guard cell length; both $p < 0.001$), yet were not significantly affected by site type (stomatal density: $p = 0.055$; guard cell length: $p = 0.836$), confirming their constitutive nature (Table 1). *Alnus incana* had the highest stomatal density (124.8 ± 34.2 per 1 mm²) with the smallest guard cells (10.6 ± 3.1 μ m), consistent with its deciduous tree habit requiring efficient gas-exchange regulation. *Caltha palustris* showed the opposite strategy: few, large stomata (48.4 ± 26.0 per 1 mm², 19.9 ± 7.8 μ m), reflecting its hygrophytic ecology. *Crepis paludosa* (51.96 ± 21.5 per 1 mm², 12.04 ± 3.70 μ m) and *Impatiens parviflora* (47.74 ± 27.56 per 1 mm², 13.74 ± 3.18 μ m) had similar low densities with intermediate guard cell sizes. A significant negative trade-off between stomatal density and guard cell length was confirmed at the individual level ($\rho = -0.220, p = 0.044, n = 85$). In contrast, leaf relative water content (RWC) differed significantly between site types (shaded: $83.9 \pm 8.3\%$ vs. open: $76.5 \pm 10.0\%$, $p = 0.001$) but only marginally among species ($p = 0.053$), and correlated positively with F_v/F_m across species-locality means ($\rho = 0.531, p = 0.023, n = 18$), suggesting that water availability contributes to the higher CWM quantum yield in shaded sites.

Discussion

Our investigation of the post-disturbance successional gradient in the High Tatra Mountains (Fleischer *et al.* 2017) enabled us to examine how the photochemical characteristics of springs change among different forest canopies. The variance partitioning analysis revealed that several structural parameters of PSII organization (e.g., ABS/RC, V_J) were predominantly determined by species identity rather than by locality. Such patterns are

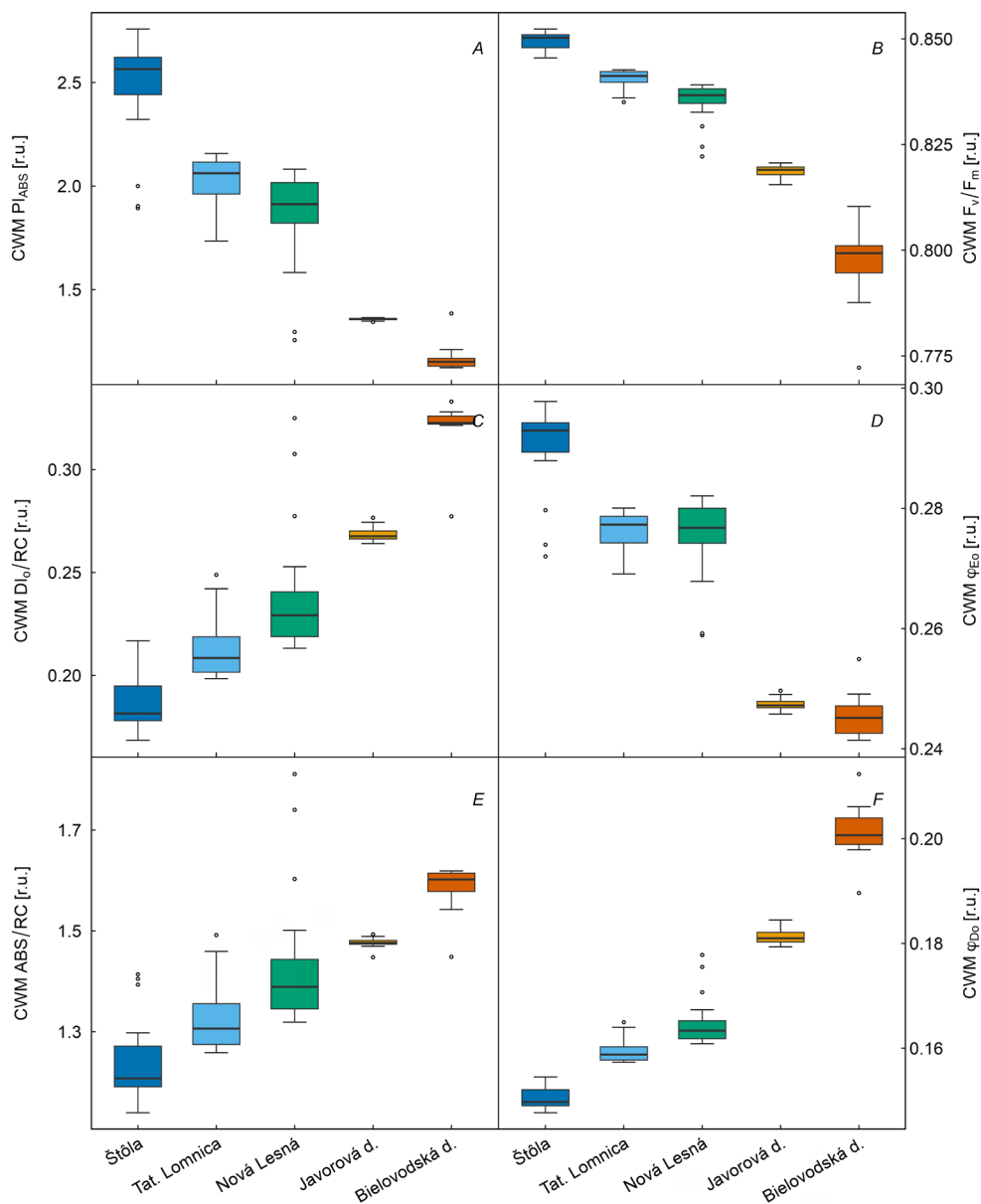


Fig. 4. Community-weighted mean (CWM) JIP-test parameters by locality showing the successional gradient from shaded (*dark blue, blue, and green box plots*) to open (*orange and red box plots*) sites. (A) CWM PI_{ABS} . (B) CWM F_v/F_m . (C) CWM DI_0/RC . (D) CWM ϕ_{Eo} . (E) CWM ABS/RC . (F) CWM ϕ_{Do} . Boxplots show the median (horizontal line), the interquartile range (box; 25th–75th percentiles), and whiskers extending to the most extreme values within $1.5 \times IQR$. ABS/RC – absorption flux per RC; DI_0/RC – dissipation flux per active RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; ϕ_{Do} – quantum yield of energy dissipation in PSII antennae and reaction centers; ϕ_{Eo} – quantum yield for electron transport (ET).

consistent with the interpretation that parameters describing antenna size, reaction center density, or the redox state of Q_A reflect inherent properties of the photosynthetic apparatus that differ among species due to evolutionary and functional trait divergence (Wang *et al.* 2022). Structural JIP-test parameters such as ABS/RC represent the apparent antenna size per active PSII reaction center and are therefore linked to species-specific organization of the light-harvesting complex and PSII stoichiometry. Similar species-driven patterns in fluorescence parameters have been reported in comparative studies across cultivars

or genotypes (Kaltrina *et al.* 2020, Konôpková *et al.* 2020, Húdoková *et al.* 2023) where JIP-test metrics successfully differentiated inherent photosynthetic characteristics even when plants were exposed to similar environmental conditions.

In contrast to the structural parameters, F_v/F_m showed a strong locality effect, indicating that this widely used fluorescence metric primarily reflects environmental conditions *in situ*. Because F_v/F_m represents the potential efficiency of primary photochemistry rather than the structural organization of the PSII, it is expected to

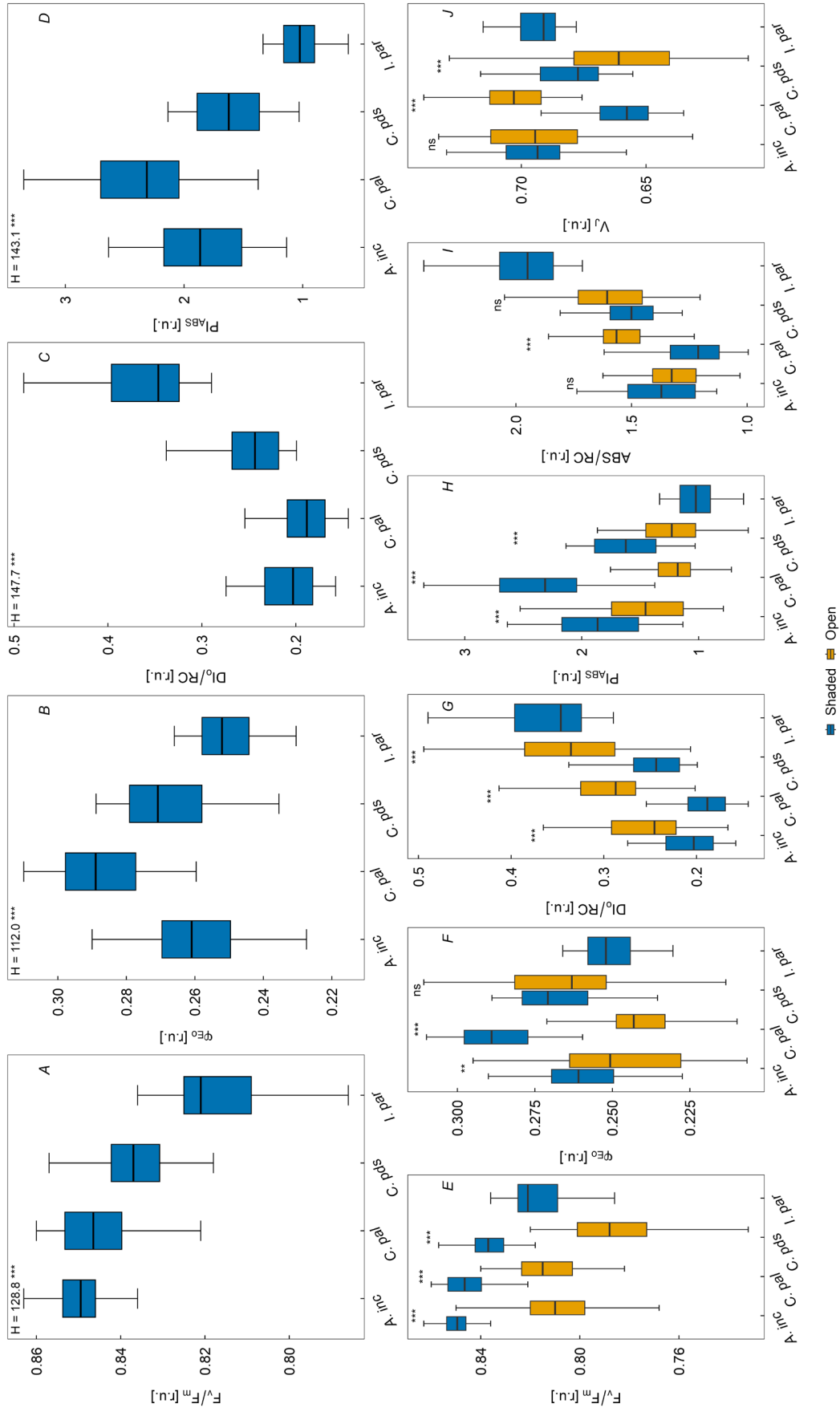


Fig. 5. Comparison of PSII performance among species. *Top row (A–C)*: Within shaded sites only, controlling for elevation and light confounding. *Bottom row (D–F)*: Shaded (*blue*) versus open (*orange*) comparison for each species. Boxplots show the median (horizontal line), the interquartile range (box; 25th–75th percentiles), and whiskers extending to the most extreme values within 1.5 $\times$ IQR. Outliers (beyond 1.5 $\times$ IQR within each locality $\times$ species combination) were removed prior to plotting. ***, $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant (*Mann-Whitney U*-test). ABS/RC – absorption flux per active RC; F_v/F_m – maximum quantum yield; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; V_j – relative variable fluorescence at the J step (2 ms); ϕEo – quantum yield for electron transport (ET).

respond rapidly to environmental variation. Consequently, differences in shading, temperature regime, or microhabitat moisture across the studied localities likely explain the strong site-related variance observed in this parameter. Similar patterns have been documented in field studies where F_v/F_m varied primarily with stress intensity or light environment, whereas structural JIP-test parameters remained relatively stable (Kalaji *et al.* 2025). The 30-min dark adaptation is sufficient for reliable F_v/F_m determination (Demmig-Adams and Adams 2006), as the rapidly reversible q_E component fully relaxes within 5–10 min. However, slowly reversible components (q_H , q_I) relax only over hours to days, hence the lower F_v/F_m at open sites may partly reflect residual photoprotection rather than intrinsic PSII efficiency alone (Malnoë 2018, Krause and Winter 2020). Since the same protocol was applied across all species and localities, these effects represent biologically meaningful variation rather than methodological bias. Moreover, F_v/F_m , as a measure of the maximal quantum efficiency of PSII, reflects the capacity of vegetation to convert absorbed light into chemical energy, making it directly relevant to carbon assimilation metrics such as gross primary production (GPP) (Perez-Priego *et al.* 2015). Canopy-level studies indicate that F_v/F_m is strongly linked to ecosystem-scale GPP across heterogeneous landscapes (Nichol *et al.* 2019), and its temporal variations may serve as an early-warning indicator of photosynthetic downregulation. Although it is not a direct measure of carbon assimilation, unlike net photosynthetic rate, changes in F_v/F_m often precede detectable shifts in ecosystem carbon fluxes across diverse climatic regions (Hernández-Clemente *et al.* 2019).

PI_{ABS} , as a composite parameter integrating both structural and functional components of the photosynthetic apparatus, occupied an intermediate position (η^2 ratio = 2.5). This pattern suggests that the index reflects not only environmental influences but also species-specific characteristics (Bussotti *et al.* 2020), which explains why it can be ecologically informative yet difficult to interpret without species-resolved data. Such a dual sensitivity is consistent with the conceptual framework proposed by Kalaji *et al.* (2017), who distinguished between parameters associated with rapid acclimation to environmental conditions (e.g., quantum yields) and those reflecting the constitutive architecture of the photosynthetic machinery (e.g., specific energy fluxes). Similarly, DI_0/RC reflects the amount of absorbed excitation energy dissipated as heat per reaction center and therefore represents a photoprotective mechanism that can increase under stress or excess light. Variation in this parameter among species and localities likely reflects differences in photoprotective capacity, e.g., *Impatiens parviflora* vs. native species, as well as acclimatory responses to microhabitat conditions (opened vs. shaded mountain springs).

The clear interspecific differences detected in several fluorescence parameters indicate contrasting PSII energy-allocation strategies among the studied spring species. Higher values of F_v/F_m , ϕE_0 , and PI_{ABS} in *Alnus incana* and *Caltha palustris* suggest a greater capacity for efficient photochemical energy conversion, which may

reflect adaptation to relatively stable or moist riparian habitats where light limitation rather than photoinhibition constrains photosynthesis. Moreover, *Alnus incana* demonstrates a remarkable ability to maintain metabolic activity despite low groundwater temperatures. Through actinorhizal nitrogen fixation, it compensates for nutrient-limited conditions, accumulating leaf nitrogen to fuel high photosynthetic capacities and a high photochemical capacity, which also supports a higher transpiration rate and stomatal conductance. Simultaneously, the species employs a robust xanthophyll cycle to mitigate photoinhibition, effectively dissipating excess light energy as heat. This dual strategy of thermal regulation and photoprotection ensures sustained, high rates of carbon fixation even under the extreme irradiance levels characteristic of high-altitude habitats (Körner 2021).

On the other hand, elevated ABS/RC and DI_0/RC values observed in invasive species *Impatiens parviflora* provided an interesting perspective on these dynamics. Despite its ability to colonize these sites (Hejda 2012), we found that it exhibited a distinct JIP-test signature characterized by relatively high ABS/RC , increased thermal dissipation per RC , and lower photochemical performance indices compared to co-occurring native species. The occurrence of *Impatiens parviflora* was associated with higher plot-level CWM PI_{ABS} values; however, this relationship reflects the preferential occurrence of the species in shaded sites at advanced successional stages, where native species attain their optimal photosynthetic performance. Thus, this pattern represents an environmental (confounding) effect rather than a positive functional contribution of the invasive species to community-level performance. Although species from the genus *Impatiens* would benefit from the increased light conditions, the full irradiation causes severe damage to the photosynthetic apparatus (from degradation of chlorophyll molecules through increased heat dissipation, ROS accumulation, to visible damages such as bleaching of thin leaves) (Leuschner and Ellenberg 2017).

The observed patterns may instead reflect a shade-associated functional strategy, balancing light capture and energy dissipation under low irradiance conditions. Because the species was absent from open-canopy sites, its physiological responses to high irradiance could not be evaluated in this study, which limits broader inference regarding its performance across the full successional light gradient.

A lower Chl index in *I. parviflora* may be associated with a different ecological strategy, potentially linked to shade tolerance or resource allocation pattern. The negligible effect of locality further supports the stability of this parameter across varying site conditions. Overall, these results suggest that the comparatively lower photosynthetic performance of *I. parviflora* is more likely related to a specific shade-associated strategy under the observed conditions rather than a universal or strictly intrinsic species characteristic across all light environments. Similar patterns, in which invasive species benefit from favorable habitat conditions without exhibiting superior physiological performance relative to native species, have

been reported in the broader context of invasion functional ecology (e.g., Daehler 2003, Funk 2013).

Light environment is a key driver of PSII acclimation, affecting both photochemical performance and photoprotective energy dissipation (Bielczynski *et al.* 2016, Zhang *et al.* 2020). Under high-light intensity conditions, excess excitation can partially inactivate PSII reaction centers, altering energy fluxes within the photosynthetic apparatus. As a result, OJIP-derived parameters often show increased energy dissipation (DI_0/RC), reduced electron transport beyond Q_A (ET_0/RC), and changes in apparent antenna size (ABS/RC), reflecting the accumulation of reduced Q_A^- and the limitation of electron transport (Kalaji *et al.* 2017, Mlinarić *et al.* 2017, Bayat *et al.* 2018, Janeeshma *et al.* 2024).

Locality effects along the shaded-to-open gradient of mountain springs were pronounced. Open, high-irradiance sites such as Bielovodská dolina exhibited lower community-weighted means for F_v/F_m , PI_{ABS} , and ϕE_o values, suggesting that higher light intensity and associated environmental stress reduce PSII photochemical efficiency compared to shaded late-successional sites such as Štôla. The observed increase in ABS/RC in open spring habitats can be interpreted as a consequence of partial inactivation of PSII reaction centers rather than an actual increase in antenna size. When reaction centers become inactivated under high light and stress, the remaining active centers receive a larger share of absorbed energy, leading to elevated ABS/RC (Strasser *et al.* 2004, Mihaljević *et al.* 2024). This pattern has also been demonstrated under various abiotic stresses, where decreases in active center density correspond with increases in ABS/RC and related flux parameters (e.g., DI_0/RC). We suppose this shift is driven by the fact that canopy closure protects plants from intense light stress and photoinhibition (Strasser *et al.* 2010, Kalaji *et al.* 2014), which are common in open areas after a disturbance. Moreover, the positive correlation between RWC and F_v/F_m , combined with the significant difference in RWC between site types (shaded: 83.9% vs. open: 76.5%), suggests that water availability is a proximate mechanism linking canopy closure to higher CWM quantum yield.

Stomatal traits in our study exhibited pronounced species-level differentiation, as reflected by high effect sizes for stomatal density ($\eta^2 = 0.457$) and guard cell length ($\eta^2 = 0.284$), despite showing no significant responses to site type. Stomatal anatomy, particularly stomatal density, can be highly plastic and responsive to dynamic environmental fluctuations such as light availability, atmospheric (CO_2), and localized stressors (Franks and Beerling 2009, Marengo *et al.* 2017, Madhavan *et al.* 2025). For instance, in highly dynamic systems such as intertidal environments, plants like mangroves exhibit significant anatomical adaptations and plastic stomatal shifts to cope with shifting environmental stresses (Madhavan *et al.* 2025). High plasticity in stomatal density was observed among tropical species depending on light conditions, water availability, and air humidity (Marengo *et al.* 2017, Azevedo *et al.* 2025). However, within the specific environmental gradient examined in this study,

our findings suggest that interspecific genetic variation outweighs local microhabitat plasticity. This indicates that for these particular species, stomatal characteristics function primarily as constitutive traits shaped by evolutionary strategies to balance water use and carbon gain (Sperry *et al.* 2017), rather than undergoing rapid plastic adjustments across the studied site types. The high stomatal density and short guard cells observed in *Alnus incana* align with its deciduous tree growth form and the associated demand for rapid gas exchange to support high photosynthetic rates during favorable periods (Brodribb and Feild 2000). In contrast, *Caltha palustris* displayed a strategy characterized by fewer but larger stomata, consistent with its hygrophytic ecology, where abundant water availability reduces the necessity for high stomatal densities and may favor large pore sizes to facilitate efficient diffusion under saturated conditions. Similar trait syndromes have been reported in wetland vs. upland species, where stomatal morphology co-varies with habitat water status rather than ambient light or temperature (Sack *et al.* 2003, Muir 2015).

One of the most notable patterns in our study was the apparent trade-off between photosynthetic performance and species richness. As communities progress toward a closed canopy, they tend to be dominated by a few highly efficient species, which enhances overall light-use efficiency but reduces diversity (Jiang *et al.* 2020, Yang *et al.* 2023).

In contrast, open sites supported higher species richness and greater variability in photosynthetic strategies. Functional diversity of PSII traits, particularly the variance of F_v/F_m among co-occurring species, was 1.6 times higher in open habitats, suggesting that high-irradiance environments allow species to exploit contrasting approaches to light capture and photoprotection (Tilman 1999, Niinemets 2010). The positive relationship between functional diversity of F_v/F_m and species richness further indicates that species-rich open communities encompass a wider spectrum of PSII efficiencies and acclimation strategies. Shaded communities exhibit more uniform and balanced PSII performance, reflecting the constraints imposed by low-light environments on divergent photosynthetic traits (Valladares and Niinemets 2008). Higher functional variability of F_v/F_m observed in open-canopy sites may reflect increased heterogeneity of photosynthetic acclimation strategies among co-occurring species. However, given the observational and temporally limited nature of this study, this pattern may equally represent a transient successional stage characterized by incomplete competitive filtering. Distinguishing between these alternative mechanisms would require long-term monitoring or experimental approaches.

This study shows that variation in PSII functioning among spring forest species reflects the combined effects of intrinsic species-specific traits and environmental conditions. Parameters related to PSII structural organization (e.g., ABS/RC) were predominantly species-driven, reflecting constitutive differences in photosynthetic architecture, whereas functional efficiency indicators (most notably F_v/F_m) were strongly influenced by locality,

confirming their sensitivity to environmental conditions *in situ*. Integrative parameters (such as PI_{ABS} and DI_0/RC) captured both components, reinforcing their value as comprehensive indicators of PSII performance.

Consistent interspecific differences across all JIP-test parameters highlight distinct physiological strategies among species. Native species, particularly *Alnus incana* and *Caltha palustris*, exhibited higher photochemical efficiency, while *Impatiens parviflora* displayed a divergent physiological response. *Impatiens parviflora* appears to follow an acquisitive resource-use strategy, characterized by rapid resource capture even under shaded conditions. This is reflected in its relatively high light absorption capacity, coupled with lower photochemical efficiency. Such a pattern suggests a high-throughput system, where resources are captured and utilized quickly, with less emphasis on minimizing losses. This strategy may confer an advantage in dynamic or resource-limited understory environments, where rapid exploitation of transient light availability is beneficial.

The observed patterns may instead reflect a shade-associated functional strategy, balancing light capture and energy dissipation under low irradiance conditions. Because the species was absent from open-canopy sites, its physiological responses to high irradiance could not be evaluated in this study, which limits broader inference regarding its performance across the full successional light gradient.

Importantly, the results indicate that post-disturbance open localities represent a comparatively higher-risk environment for species dependent on a stable water supply. Lower F_v/F_m , PI_{ABS} , and ϕE_o values at open sites, together with elevated DI_0/RC , indicate increased photoinhibition and a greater reliance on protective energy dissipation under high irradiance. This pattern is consistent with the reduced leaf relative water content observed in open habitats, suggesting that increased evaporative demand and limited moisture retention exacerbate physiological stress. For species such as *Alnus incana* and *Caltha palustris*, which are adapted to moist, shaded microhabitats, these conditions likely constrain photosynthetic efficiency and may reduce competitive performance. In contrast, shaded localities buffer against radiation and desiccation stress, providing more favorable microclimatic conditions that support higher PSII efficiency and water status. At the community level, this gradient is further reflected in a clear trade-off: open, post-disturbance sites tend to support higher species richness, but this is accompanied by reduced community-level photosynthetic performance, suggesting a shift towards stress-tolerant and opportunistic species rather than highly efficient, moisture-dependent taxa. Conversely, shaded, less diverse communities maintain higher photosynthetic efficiency, supported by more stable and buffered environmental conditions.

From a conservation perspective, these results suggest that no single canopy state is universally optimal. Rather than promoting either complete canopy closure or persistent openness, management strategies should aim to maintain a mosaic of successional stages and canopy conditions within mountain spring ecosystems. Such structural heterogeneity is likely to preserve both the high

physiological performance associated with shaded habitats and the elevated taxonomic and functional diversity characteristic of open-canopy sites. This approach may be particularly important in mountain spring ecosystems facing ongoing environmental change and increasing disturbance pressures.

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