Downregulation of PSI regulates photosynthesis in early successional tree species. Evidence from a field survey across European forests

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ABSTRACT

Photosynthesis rates and electron transport are key physiological traits that distinguish the successional status of plants (early successional – ES, and late successional – LS) in a community. ES plants can respond quickly to sudden changes in sunlight radiation exposure and display a greater photosynthetic plasticity, with respect to the LS ones, thanks to their ability to regulate the photosynthesis. Photosynthesis regulation is connected to the efficiency of the two photosystems (PSI and PSII). PSI plays an important role in the photosynthetic machinery functioning to respond against sudden changes of metabolism in response to heterogeneous environmental conditions. It can be downregulated under high pressure of reductants, to maintain the balance between the reductant pressure and the metabolic demand. The efficiency of PSII and of PSI can be estimated, respectively, by the maximum quantum yield of photosystem II (F_V/F_M) and the contribution of the thermal phase in the chlorophyll fluorescence emission (expressed by the parameter ΔV_{IP}, that quantify the I-P phase in the fluorescence emission transient). Both parameters are evaluated in dark-adapted samples and can be measured by prompt fluorescence technique. Here, we analyzed a large dataset from a European-wide survey on mature forests, considering four representative forest tree species growing at six sampling sites distributed along a latitudinal gradient. Two early successional (*Pinus sylvestris* and *Betula pendula*) and two late successional (*Picea abies* and *Fagus sylvatica*) species were considered. The correlations between F_V/F_M and I-P phase were mostly positive for different plant species at each sampling site for F_V/F_M values < 0.82 (assumed as optimal threshold for this parameter), but not for F_V/F_M values > 0.82. In this last case, the correlations were mostly negative for early successional species, and not significant for late successional species. Foliar nitrogen concentration plays a relevant role for the PSI regulation in early successional species: trees with high nitrogen content have higher I-P phase values and negative correlations between F_V/F_M and I-P phase for F_V/F_M > 0.82. An opposite pattern was found in tree species with low foliar nitrogen level. We conclude that the late successional tree species show a substantial regulation and stability of the photosynthetic machinery and photosystem stoichiometry, whereas early successional species have more dynamic behavior of PSI. Early successional species can modulate the photosynthetic efficiency and are able both to up-regulate and down-regulate the PSI concentration and activity in relation to quantum yield capacity and leaf nitrogen content.

1. Introduction

Early successional (ES) and late successional (LS) plant species differ for a large array of morphological and physiological features. Early successional plant species have higher SLA (Specific Leaf Area), lower leaf thickness, higher stomatal conductance, higher electron transport rate (ETR), higher net photosynthetic (*P*n) rates; moreover, ES species are less affected to photoinhibition than LS ones [6, 7, 23]. Early

successional plants have higher photosynthetic flexibility, i.e., more dynamic responses to light, with rapid adaptation capacity to sudden changes in quality and intensity of irradiation [20, 23, 47, 62].

The photosynthetic efficiency and responses of a plant is traditionally described through the structure and properties of the photosystem II (PSII) by mean of chlorophyll fluorescence analysis. Photosystem I (PSI), however, also plays a central role in the photosynthetic machinery to respond against sudden changes of plant metabolism and physiology in response to heterogeneous environmental conditions and stress factors [9, 13, 45]. Empirical evidence obtained in field studies suggests that the net photosynthesis (*Pn*) is connected to the content and efficiency of PSI more than PSII [16, 22, 45].

The two photosystems work in a coordinated way to regulate the fluxes of electrons decreasing, when necessary, the overall rate of linear electron transport so preventing an excess of reductant potential within the photosynthetic system and then avoid photoinhibition both at PSI and PSII sides [61]. The two photosystems have different sensitivity to the various stress factors [13]. PSII is quite stable and insensitive to moderate drought [11], tropospheric ozone pollution [12] and heavy metal soil pollution [8] compared to PSI [17, 36, 37, 40]. According to Yadavalli et al. [60] PSI is sensitive to iron deficiency in the soil, whereas Nikiforou and Manetas [35] and Cetner et al. [18] evidenced its sensitivity to nitrogen and phosphorus deficiency. PSI is generally more sensitive and responds quickly to environmental stress than PSII, and its recovery is slower than that of PSII [65]. The efficiency of the two photosystems is regulated on daily and season basis [63], according to the availability of environmental resources, as light intensity, ensuring a balance between available energy and photochemical capacity [53]. Several studies [21, 42] evidenced that high light induces PSII photoinhibition [1] but enhances PSI formation and activity.

The flux of electrons in the linear electron transport chain (ETC) is limited by the capacity of the end acceptors beyond PSI (i.e., NADPH) to feed the Calvin-Benson cycle [19]. The photosynthetic electron transport is regulated by the cyclic electron transport (CET) to maintain the balance between the reductant pressure and the metabolic demand [54] and to avoid photoinhibition caused by the accumulation of reactive oxygen substances (ROS) [32]. Cyclic Electron Flow around PSI, i.e., the cyclic transport of electrons involving PSI only, is known to play an important role in the photoprotection of higher plants when there is unbalance between electron flow and CO_2 availability [49]. The capacity of photoprotection is dependent also by availability of nitrogen within the crown of trees [31].

The relative efficiency of PSII and PSI can be assessed with the analysis of the chlorophyll fluorescence (ChlF). Within the constellation of the prompt ChlF parameters (i.e., JIP test, [55–57]), F_V/F_M (quantum yield efficiency in the dark-adapted samples) is a suitable proxy for the whole PSII efficiency [13]. I-P phase (ΔV_{IP}, connected to the thermal phase of the transient), on the other hands, is an indicator of the relative abundance of the PSI with respect to PSII and is related to the electron transport chain beyond PSI [50, 51]; [17]. Although the connection between I-P phase and PSI was questioned by Zivcak et al. [64], our previous findings [45, 48] showed a highly significant positive correlation between I-P phase and the PSI parameters specifically measured with M-PEA (Multi-Function Plant Efficiency Analyser, Hansatech), that combines fast fluorescence kinetic and $P700^+$ absorbance measurements.

The present paper is aimed at exploring the relationships between PSI and PSII in ES and LS trees growing in European forests, within a large-scale survey. The hypotheses to be tested in this study are: (i) the different photosynthetic plasticity of ES and LS species is related to their relative concentration of PSI, measured with the I-P phase; (ii) ES plants have greater ability than LS to modulate the relationships between the two photosystems and to regulate the overall electron flux by downregulating the I-P phase; (iii) the capacity of downregulation depends on the ecological conditions, being more effective on leaves with higher N content. We assume that very high values of quantum yield at the PSII

side (F_V/F_M) are potentially able to feed the electron flux exceeding the capacity to fix the CO_2 beyond the end acceptors at the PSI side. For this reason, the value of $F_V/F_M = 0.82$, the optimal value for the quantum yield efficiency in dark-adapted samples according to Baker and Rosenqvist [5], was considered the threshold for downregulation processes.

2. Materials and methods

2.1. Sampling sites and sampling collection

The dataset used in this study includes 209 observational plots established across Europe within the project FuDivEUROPE (*Functional Significance of the Forest Diversity in Europe*, European Union, 7th Framework Program, [4]). The project has been carried out on natural and near-natural heigh forests in Italy, Spain, Romania, Poland, Finland, Germany. The sampling for chlorophyll fluorescence (ChlF) analysis was carried out in the years 2012 and 2013, during the summer months (July and August), when the leaves of trees were fully developed and before the onset of foliar senescence processes in the fall. The leaf sampling was carried out on three to six trees per species in each plot. Trees were up to 30 m tall, and leaf sampling was carried out by means of tree climbers, extension loppers and gun shooters according to the height of the trees, the stand structure, and the operational conditions in each country. Sampling was performed in accordance with a strict safety protocol. Sun leaves from the top of the canopies were considered for this analysis of the photochemical properties of forest tree species. The following precautions were adopted for proper conservation of the samples. After sampling, branchlets 40–50 cm long, with attached leaves, were immediately placed in hermetic plastic bags and humidified to avoid leaf dehydration. The bags were then kept at constant temperature in an insulated box, where samples began the dark-adaptation period, necessary before to carried out the chlorophyll fluorescence measurements. The effectiveness of the protocol was tested prior to the sampling. More details on sampling and sample treatment can be found in Pollastrini et al. [43, 44].

2.2. Chlorophyll a fluorescence measurement

In this research we have applied the techniques related to prompt fluorescence (PF) and JIP-test [55–57], that demonstrated to be particularly appropriate for large scale ecophysiological surveys [15]. Chlorophyll fluorescence (ChlF) measurement was made using a HandyPEA fluorimeter (PEA series, Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, UK). The measurement of ChlF was done after four-five hours of sample dark-adaptation on 16 leaves for each tree. Since sampling was done at different hours in the day, a long dark-adaptation period was necessary to remove the effects of leaf photoinhibition and of the daily hours of solar radiation exposure [43].

Chlorophyll fluorescence emitted by the leaf after one second pulses of red light (650 nm, 3500 µmol m^{-2} s^{-1}) rises following a time-dependent curve (i.e., transient). Plotted on a logarithmic time scale, the fluorescence transient of the leaves shows a polyphasic shape; the key points of this curve are indicated as: O for the initial fluorescence level, K (fluorescence emitted at 300 µs), J (fluorescence at 2 – 3 ms) and I (30 ms), and P, the peak level of fluorescence, emitted at 500–800 ms. The latter indicates the highest, or maximal, fluorescence intensity (F_M), when saturating light is applied to the leaf. The fluorescence OKJIP transients were analysed using the method of the JIP-test [55–57, 59].

After a preliminary explorative analysis of the whole dataset (about 18,000 recordings) by means of Principal Component Analysis (PCA) (cf. [13]), the parameters F_V/F_M, i.e., the ratio between variable and maximal fluorescence [$F_V/F_M = (F_M - F_0) / F_M = \phi_{Po}$], that expresses the maximum quantum yield of primary photochemistry of a dark-adapted sample [38], and I-P phase, i.e. the amplitude of the relative contribution of the I to P rise of the OKJIP transient [56, 59], were chosen to represent the functionalities of PSII and PSI, respectively.

Table 1

Consistency of sampling at each site and tree species. Number of plots and trees sampled are indicated.

Species	Site	Plots n	Trees n
Pinus sylvestris	All	54	189
	Finland	16	60
	Poland	23	75
	Spain	15	54
Picea abies	All	62	222
	Finland	16	60
	Germany	9	36
	Poland	23	75
	Romania	14	51
Betula pendula	All	38	132
	Finland	16	60
	Poland	22	72
Fagus sylvatica	All	46	156
	Germany	27	93
	Romania	19	63

2.3. Foliar nitrogen data

Twenty fully expanded leaves and needles were sampled from the top part of the canopy (sunlit leaves) of each sampled tree for the analysis of nitrogen content (mass-based, N, %). After oven-drying at 60 °C for 48 h, the foliar samples of each tree were pooled together and were finely ground. To determine the leaf N content, 2.5 mg of the dry sample were used. The samples were analysed using near-infrared spectroscopy (NIRS), as described by Niederberger et al. [34]. More details on the applied methodology for foliar N content determination are given by Pollastrini et al. [41]. Leaf N contents at species level, for each stand, were calculated as average values of three to six trees per species sampled in the stand.

2.4. Data analysis

For the analyses shown in this paper, four species were selected from the whole dataset: two conifers (*Pinus sylvestris* L. in Finland, Poland, and Spain, and *Picea abies* (L.) Karst. in Finland, Poland, Germany, and Romania), and two broadleaves (*Betula pendula* L. in Finland and Poland, and *Fagus sylvatica* L. in Germany and Romania). *Pinus sylvestris* and *Betula pendula* are early successional; *Picea abies* and *Fagus sylvatica* are late successional tree species. In total, 699 trees on 200 sampling plots were examined (Table 1).

Statistical analyses were mostly correlative. Correlations between F_V/F_M and I-P phase (overall, below and above the threshold value of $F_V/F_M = 0.82$), were carried out by applying the non-parametric Spearmann test. Levels of significance were established for $p < 0.05$ (*, significant); $p < 0.01$ (**, very significant); $p < 0.001$ (***, highly significant). Significance of differences between functional groups and successional position of the species were tested with two-way Anova and post-hoc Tukey test. All analyses were carried out with software

Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

The tree species assessed in this study were analyzed separately and grouped according to functional groups (conifers *vs.* broadleaved trees) and successional stages (ES *vs.* LS species) (Table 2). The foliar parameters analyzed (N content, F_V/F_M, I-P phase) allowed to discriminate some groups of tree species and/or functional groups. N was significantly higher (p< 0.001) in broadleaved tree species (*F. sylvatica* and *B. pendula*) than in conifers (*P. sylvestris* and *P. abies*). F_V/F_M and I-P phase followed the opposite pattern, with values significantly higher (<0.001) in conifers than in broadleaves. Concerning the successional position, only I-P phase was effective to significantly separate ES from LS species. The values of I-P phase followed a species-specific pattern: they were higher in *P. sylvestris* and *B. pendula* in comparison to *P. abies* and *F. sylvatica* (*P. sylvestris* > *B. pendula* > *P. abies* > *F. sylvatica*), i.e., ES species have higher I-P phase values than LS species (Table 2). The two-way Anova, moreover, evidenced significant interactions between functional groups and successional position (N content: p = 0.021; F_V/F_M: p = 0.044; I-P phase: p < 0.001). Within the same species, the values of the parameters analyzed (N content, F_V/F_M, I-P phase) show significant differences among sites (Fig. 1). The highest values were found in Poland and in the other central European sites (Germany and Romania) and lowest in the Northernmost (Finland) and Southernmost (Spain) sites.

The correlation analyses (Table 3) indicated a general pattern with significant positive correlations between the two ChlF parameters tested. This frame, however, became more complex when the data were analyzed separately per species, site, and F_V/F_M value threshold (below and above the 0.82 value). *Pinus sylvestris* and *B. pendula* (ES species) showed a clear negative correlation between F_V/F_M and I-P phase for the threshold of $F_V/F_M > 0.82$. *Picea abies* and *F. sylvatica* (LS species) have prevalently not significant, or even positive, correlations between F_V/F_M and I-P phase for $F_V/F_M > 0.82$.

Pinus sylvestris shows a more evident change of the sign of correlation beyond the 0.82 F_V/F_M threshold (Table 3) with respect to the other tree species. This behavior is different in the three sites where this species was sampled. Although the correlation between F_V/F_M and I-P phase was positive for F_V/F_M values <0.82 in all sites, above this threshold that correlation was negative in Poland, positive in Spain and not significant in Finland. Poland was the site where I-P phase and nitrogen content in the leaves of *P. sylvestris* showed the highest values, whereas Spain was the site where I-P phase and nitrogen showed the lowest values. *Betula pendula* showed a similar behavior to *P. sylvestris*, with negative correlation (for F_V/F_M values < 0.82) between F_V/F_M and I-P phase in Poland (higher I-P phase and nitrogen foliar content) and not significant in Finland (lower I-P phase and nitrogen foliar content). The values of I-P phase were lower and less variable (coefficient of variation, CV = 14%) in late than in early (CV = 26%) successional species (Fig. 2).

Table 2

Mean values of foliar nitrogen content (N%) and the chlorophyll fluorescence parameters (F_V/F_M and I-P phase), for tree species, functional groups and successional position. Different letters indicate significant differences, according to the Tukey-test (HSD – Honest Significant Difference).

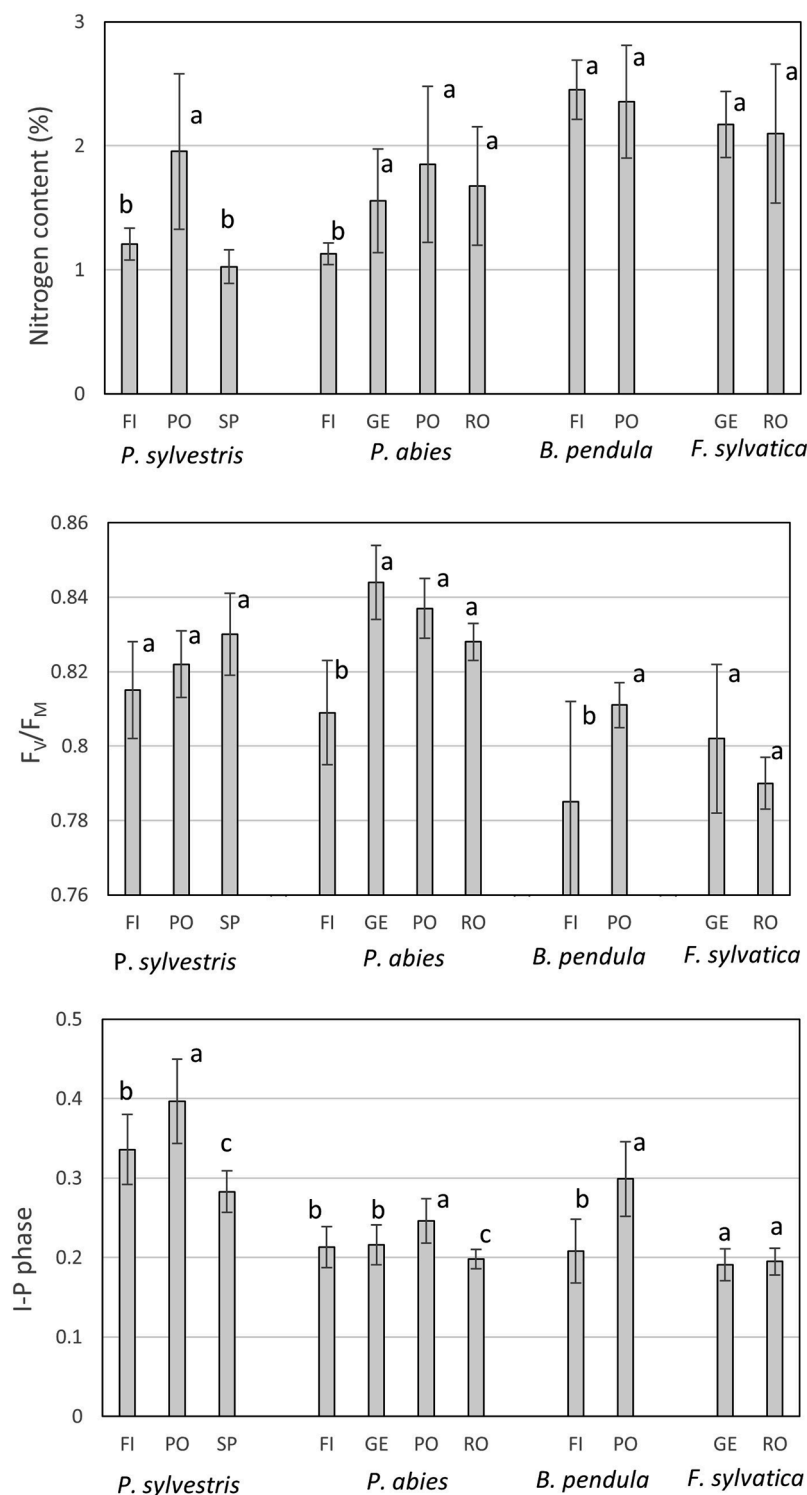| Species	N%			F_V/F_M			I-P phase		
Tree species									
Pinus sylvestris	1.510	±0.623	c	0.824	±0.015	a	0.356	±0.069	a
Picea abies	1.548	±0.621	c	0.827	±0.021	a	0.242	±0.032	c
Betula pendula	2.538	±0.468	a	0.801	±0.027	b	0.272	±0.063	b
Fagus sylvatica	2.169	±0.498	b	0.785	±0.028	c	0.214	±0.023	d
Functional groups									
Conifers	1.529	±0.619	b	0.823	±0.028	a	0.293	±0.054	a
Deciduous broadleaves	2.366	±0.513	a	0.792	±0.018	b	0.239	±0.077	b
Successional position									
Early successional	1.934	±0.758	a	0.814	±0.023	a	0.320	±0.078	a
Late successional	1.778	±0.650	a	0.809	±0.032	a	0.230	±0.032	b

Fig. 1. Average values of foliar nitrogen content (N%, mass-based) and the chlorophyll fluorescence parameters (F_V/F_M and I-P phase), for each tree species at the different sites. Bars indicate standard deviation. Different letters indicate significant differences between sites (FI = Finland, PO = Poland, SP = Spain, GE = Germany, RO = Romania) within each species, according to the Tukey-test (HSD – Honest Significant Difference) at $p < 0.05$.

4. Discussion and conclusions

The quantum yield efficiency of PSII (F_V/F_M) is the most widely chlorophyll parameter used as a sensitive indicator of plant photosynthetic performance. Differences in the values of this parameter are connected to genotype, provenance of plants and prevalent environmental factors [24, 27, 33]. F_V/F_M is very robust and stable, and it fluctuates, in healthy plants, in a narrow range of values (0.75 – 0.84, [33]) when assessed with the usual commercial devices, operating at room temperature with wavelengths longer than 700 nm. In this study, in the device utilized, Handy PEA (Hansatech Ltd.), the emitter LED array is centered at 650 nm, whereas the detector is a photosensor for wavelengths longer than 700 nm. In these conditions the contribution of PSI to the values of F_0 and F_M is estimated, respectively, to be the 30%

Table 3

Correlation (r coefficient of Spearmann) between F_V/F_M and I-P phase, considering the values of F_V/F_M <0.82 and >0.82. The number of recordings is indicated. *** = $p < 0.001$ =highly significant; ** = $p < 0.01$ =very significant; ** = $p < 0.05$ = significant; ns = not significant.

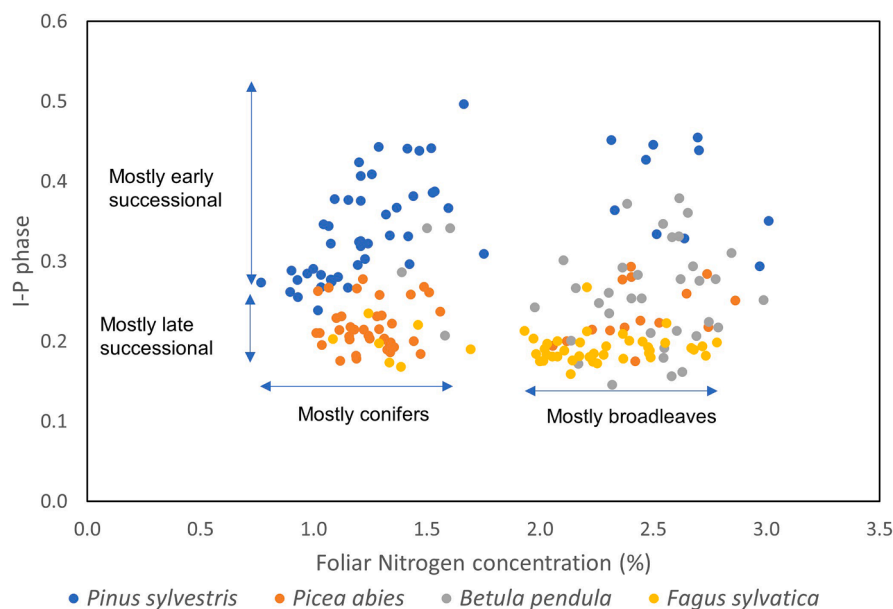|	All F_V/F_M values			F_V/F_M <0.82			F_V/F_M >0.82		
	n	r	p	N	r	p	n	r	p
All species	18186	0.116	***	13380	0.112	***	4806	−0.049	***
Pinus sylvestris									
All sites	2654	0.046	*	920	0.341	***	1734	−0.152	***
Finland	937	0.246	***	411	0.423	***	526	−0.069	ns
Spain	758	0.249	***	157	0.345	***	601	0.160	***
Poland	959	−0.137	***	352	0.084	ns	607	−0.139	***
Picea abies									
All sites	3293	0.112	***	903	0.041	ns	2390	0.1252	***
Finland	924	0.011	ns	514	0.025	ns	410	−0.017	ns
Poland	1049	0.036	ns	128	−0.028	ns	921	0.053	ns
Romania	798	0.068	ns	209	0.078	ns	589	0.046	ns
Germany	522	0.001	ns	52	0.028	*	470	−0.018	ns
Betula pendula									
All sites	4136	0.421	***	3146	0.488	***	990	−0.127	***
Finland	950	0.412	***	810	0.133	**	140	−0.257	***
Poland	799	−0.085	*	557	0.419	***	242	0.041	ns
Fagus sylvatica									
All sites	2387	−0.105	***	1779	0.096	ns	608	0.009	ns
Germany	1382	−0.072	***	806	−0.020	ns	576	0.003	ns
Romania	1005	0.085	**	973	0.076	*	32	0.229	ns

Fig. 2. Distribution of the I-P phase values in relation with the foliar nitrogen content.

and 6% in C3 plants and the 50% and 12% in C4 plants. Therefore, the values F_V/F_M are underestimated [2, 25, 27, 29, 39, 52, 58]. Methods to correct parameters like F_V/F_M for the contribution of PSI fluorescence where searched, but that has not led to a simple formula applicable routinely [28]. Several JIP-test parameters, concerning both the photochemical and thermal phases of the whole ChlF emission curve, are not affected by the contribution of PSI fluorescence [28], thus the efficiency of PSII could be expressed with parameters strictly related to F_V/F_M, like Ψ_{Eo} (probability that a photon trapped by the PSII reaction center enters the electron transport chain) [13]. In our data analysis the values of R^2 in the correlation between F_V/F_M and Ψ_{Eo} is 0.48, with $p <$ 0.001. We assume, therefore, that in comparative experiments (stressed *vs.* non stressed plants) and in field surveys, the values of F_V/F_M measured with commercial fluorimeters maintain their proportionality between F_0 and F_M, and reliability to express the differences in photosynthetic efficiency among samples. Based on the proportionality between F_0 and F_M and the relationships among ChlF parameters, models

of neuronal networks and artificial intelligence were built for the classification of genotypes and plant responses to stress factors [3, 26, 30]. Moreover, F_V/F_M is robust, less variable than other ChlF parameters, comparable between different instruments [14] and research activities. Differently, I-P phase is less popular then other ChlF parameters, but its use and application are growing among the users of the prompt fluorescence and JIP test. This parameter is not influenced by the emission of fluorescence from PSI [28].

Concerning the results reported in this contribute, the quantum yield efficiency (F_V/F_M) and leaf nitrogen content are parameters able to discriminate between plant functional groups (conifers vs. broadleaves), but not between successional positions. Unlike, the I-P phase is a good discriminator for successional position, functional group and for individual tree species. The I-P phase shows the highest values in ES species, both conifers and broadleaves, then confirming the first hypothesis formulated in introduction, i.e., the greater PSI concentration and efficiency in ES plant species. In ES species, moreover, I-P phase shows

greater plasticity and variability among sampling sites than F_V/F_M, highlighting the role of PSI in the acclimatization to environmental conditions [9].

The two parameters (F_V/F_M and I-P phase) were positively correlated when the data were aggregated for species, site, and functional groups, but their relationships are not constant and, in many cases, the correlations became negative or not significant for $F_V/F_M > 0.82$ because of the downregulation of PSI that occurred especially in ES species. High PSII photochemical efficiency (F_V/F_M) releases more electrons by PSII into the electron transport chain [10], thus the limitation of PSI may be due to a limitation of the end acceptors or the substrate to be reduced (CO_2). The downregulation capacity is connected to the content of foliar nitrogen [31], that plays a fundamental role in the construction of the photosynthetic machinery [35]. Downregulation on ES species was more effective in sites with high nitrogen supply, suggesting that in resources poor environments ES plants can be more sensitive to photoinhibition. With low nitrogen supply PSI downregulation may fail and excessive ROS may be produced, particularly under high light intensity [46]. Our findings evidence a substantial regulation and stability of the photosynthetic machinery in late successional tree species, and a more dynamic behavior of PSI in early successional species, that are also accredited to have higher photosynthetic rates and a prompt response to changes in radiation intensity. The results here presented can contribute to understand the ability of early successional species to adapt in heterogeneous sunlight environments and to colonize open spaces.

We have emphasized the role of PSI in the responses of plants to environmental changes by regulating the pressure of the photon flux with the capacity to reduce the CO_2, and we aim to promote the use of the related parameters in applied ecological research and environmental monitoring. Although biased by the contribution of a fraction of fluorescence coming from PSI, the use of F_V/F_M, as it is measured by commercial devices, remains essential to compare the findings in applied studies. It may be advisable the development of devices and concepts for a more precise determination of this parameter by the users and, at the same time, to make possible the re-interpretation of past data.

Authors' contributions

FB: wrote the first draft; MP: wrote, reviewed and editing the manuscript, made sample collection and took chlorophyll fluorescence measurements; MF: made sample leaf collection, Nitrogen content data responsible, writing and editing; WB: writing and editing. All the authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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