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Research

Taxonomic and ecological relevance of the chlorophyll *a* fluorescence signature of tree species in mixed European forests

Martina Pollastrini[1], Vera Holland[2,3], Wolfgang Brüggemann[2,3], Helge Bruelheide[4,5], Iulian Dănilă[6], Bogdan Jaroszewicz[7], Fernando Valladares[8] and Filippo Bussotti[1]

[1]Department of Agri-Food Production and Environmental Science, University of Florence, Piazzale delle Cascine 28, Florence 50144, Italy; [2]Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Max-von-Laue-Str. 13, Frankfurt/M. D-60438, Germany; [3]Biodiversity and Climate Research Centre, Frankfurt, Senckenberganlage 25, Frankfurt/M. D-60325, Germany; [4]Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, Halle D-06108, Germany; [5]German Centre of Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, Leipzig D-04103, Germany; [6]Laboratory of Applied Ecology, Faculty of Forestry, Stefan cel Mare University of Suceava, Universităţii 13, Suceava 720229, Romania; [7]Białowieża Geobotanical Station, Faculty of Biology, University of Warsaw, ul. Sportowa 19, Białowieża 17-230, Poland; [8]Museo Nacional de Ciencias Naturales, MNCN-CSIC, Serrano 115 dpdo, Madrid E-28006, Spain

Author for correspondence:
Martina Pollastrini
Tel: +39 0552755850
Email: martina.pollastrini@unifi.it

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Summary

- The variability of chlorophyll *a* fluorescence (ChlF) parameters of forest tree species was investigated in 209 stands belonging to six European forests, from Mediterranean to boreal regions.
- The modifying role of environmental factors, forest structure and tree diversity (species richness and composition) on ChlF signature was analysed.
- At the European level, conifers showed higher potential performance than broadleaf species. Forests in central Europe performed better than those in Mediterranean and boreal regions. At the site level, homogeneous clusters of tree species were identified by means of a principal component analysis (PCA) of ChlF parameters. The discrimination of the clusters of species was influenced by their taxonomic position and ecological characteristics. The species richness influenced the tree ChlF properties in different ways depending on tree species and site. Tree species and site also affected the relationships between ChlF parameters and other plant functional traits (specific leaf area, leaf nitrogen content, light-saturated photosynthesis, wood density, leaf carbon isotope composition).
- The assessment of the photosynthetic properties of tree species, by means of ChlF parameters, in relation to their functional traits, is a relevant issue for studies in forest ecology. The connections of data from field surveys with remotely assessed parameters must be carefully explored.

Introduction

A tree species can be described by means of its genetic, morphological and physiological features, according to taxonomic, ecological and/or functional points of view (Bussotti & Pollastrini, 2015; Bussotti *et al.*, 2015). It is assumed that taxonomic signals can be obtained from functional traits (Kirova *et al.*, 2014; Sardans *et al.*, 2015). Wilhelm & Wirth (2015) proposed the term 'physiodiversity' to indicate that differences in physiological behaviours among species have evolutionary significance. Aiken *et al.* (2008) proposed the use of bio-optical traits to discriminate between taxonomic groups of phytoplankton, and introduced the concept of 'bioenergetic taxonomy' (see also Drinovec *et al.*, 2011). Among the physiological features that can be derived by simple foliar analysis, Sardans *et al.* (2015) demonstrated that the elemental composition (macronutrient concentration) is a useful tool to discriminate between individual species and functional groups. The taxonomic relevance of the photosynthetic properties is still an object of discussion. Climate determines the evolution of adaptive photosynthetic strategies (Walters, 2005) in different plant species. However, it is not obvious whether chlorophyll *a* fluorescence (ChlF) parameters are able to reflect taxonomic differences among plant species. The photosynthetic apparatus is a conservative element of the plant cell (Ke, 2001), and its reaction centres (RCs) have low specific characterization, but the features (size, pigment composition) of the antenna complexes are highly variable and specific to different groups of plant species (Kirova *et al.*, 2014). Most of the ChlF at the minimum basal value (F_0) is emitted by the antenna Chl*a* molecules when all RCs are open, whereas the variable fluorescence (F_V) arises from the back transfer of excitation energy from the closed RCs to the antenna Chl (Krause & Weis, 1991). Differences among taxa, moreover, derive from specific strategies for the use and conservation of energy, and from interactions between genotypes and their environment (Rousseau *et al.*, 2013). Starting from these considerations, ChlF has been used as a tool for taxonomic

classification (Kirova *et al.*, 2014). Salvatori *et al.* (2014) demonstrated that a multivariate statistical analysis of ChlF parameters allows the discrimination of plant species according to their respective functional groups.

A large body of literature claims that ChlF parameters are useful for the characterization of the responses of plants to a variety of environmental stress factors, and for phenotyping (for a review, see Maxwell & Johnson, 2000; Baker & Rosenqvist, 2004; Papageorgiou & Govindjee, 2004; Tsimilli-Michael & Strasser, 2008a; Murchie & Lawson, 2013; Rousseau *et al.*, 2013). Intrinsic differences between tree species have been evidenced in plants coexisting in the same environment (Li *et al.*, 2004; Mänd *et al.*, 2012; Pollastrini *et al.*, 2014), but the consistency of these differences across wide geographical and ecological gradients and varying environmental conditions has not yet been thoroughly tested. Photosynthetic properties and ChlF parameters are connected to the ecological behaviour of a given species and, with other leaf traits, represent an important tool in plant ecology studies (Meng *et al.*, 2015). According to de Miguel *et al.* (2014), leaf traits related to photosynthesis and water use efficiency are genetically determined.

The most well-known ChlF parameter for the comparison of different species and physiological responses is the maximum quantum yield of primary photochemistry of a dark-adapted sample. The equations by which the quantum yields are linked with fluorescence signals are simple applications of the general equation of Paillotin (1976) ($\varphi_{Po} = TR_0/ABS = [F_M - F_0]/F_M = F_V/F_M$). This parameter is defined as the fraction of the total energy flux trapped by the photosystem II (PSII) RCs. The quantum yield of primary photochemistry is a function of the photochemical (k_P) and nonphotochemical (k_N) de-excitation constants [$F_V/F_M = k_P/(k_P + k_N)$] and represents the 'steady' structure of PSII (Strasser *et al.*, 2000). To describe the overall efficiency of photosystems, however, it is also necessary to take into account the processes of acceptor feeding and electron transport (Strasser *et al.*, 2004). Among ChlF techniques, the analysis of the fluorescence transient by the so-called JIP-test (Strasser *et al.*, 2000, 2004; Tsimilli-Michael & Strasser, 2008b) is the most efficient for screening purposes, as it combines speed of execution with manoeuvrability of the instrument in field conditions. The JIP-test is applied to dark-adapted samples, and measures the maximum potential performance of the photosystems, that is, the capacity to do work.

Large-scale remote sensing surveys consider the solar-induced fluorescence, and the parameter F_S' (steady-state fluorescence in light-adapted conditions) is probed to correlate with the net photosynthesis (P_N), measured at ground level, and with the productivity of the vegetation (Zarco-Tejada *et al.*, 2013; Yang *et al.*, 2015). Another remote sensing parameter, the photochemical reflectance index (PRI), is a proxy for F_V/F_M (Weng *et al.*, 2006). The latter does not correlate necessarily with the actual photosynthetic rates, but, in optimal environmental conditions, high values of F_V/F_M are related to high photosynthetic capacity (Zhao *et al.*, 2015). The assessment of the dark fluorescence on trees, therefore, may be considered as a tool to estimate the potential photosynthetic performance and growth capacity of individual species and forest ecosystems.

The ChlF characteristics of a given plant species can be modified by several factors, including developmental stage (ontogeny), leaf age (phenology) and environmental conditions (Čaňová *et al.*, 2008; Kalaji *et al.*, 2014). In plants sharing the same environment, the respective physiological features can be modified by reciprocal interactions. In forest populations, tree species mixture and combination determine different individual physiological responses (Gayler *et al.*, 2006; Kaitaniemi & Lintunen, 2010; Drössler *et al.*, 2015). In species-rich stands, the niche partitioning among the species might relieve trees from stress, which should be reflected in ChlF parameters. Competition at belowground level may provoke drought stress (Grossiord *et al.*, 2014a) and nutrient deficiency (Kreuzwieser & Gessler, 2010). Conversely, the presence of nitrogen (N)-fixing species is a cause of N enrichment, fostering photosynthesis and growth (Forrester *et al.*, 2012). Aboveground competition affects space and light availability with consequent deficiency or excess of light (Kozovits *et al.*, 2005; Ishii & Asano, 2010). These responses are probably reflected in changes in photosynthetic properties and ChlF characteristics (Pollastrini *et al.*, 2014).

The study presented here was carried out within the FunDivEUROPE project, whose goal was to evaluate the functional significance of tree diversity in European forests. Our main aim in this project was to assess the role of tree diversity as a factor modifying the photosynthetic efficiency of the co-occurring tree species in forest ecosystems. Moreover, this survey allowed us to test specific points: the differences in the ChlF properties among tree species and among functional groups (i.e. conifers, temperate deciduous broadleaved species, Mediterranean oaks), according to geographical and ecological gradients in Europe; and the differences in ChlF properties among species growing at the same site, in relation to their taxonomic position (genus and/or family) and ecological similarity and/or difference. Finally, a preliminary evaluation of the relationships between ChlF parameters and functional traits (directly measured in the field and/or collected from the literature) was conducted.

Materials and Methods

Sampling sites

This survey was carried out in six of the major European forest types, from boreal to Mediterranean regions (Supporting Information Fig. S1): North Karelia, Finland (FI); Białowieża, Poland (PL); Hainich, Germany (GE); Râşca-Suceava, Romania (RO); Colline Metallifere, Italy (IT); and Alto Tajo, Spain (SP). The main features of these forests were reported by Baeten *et al.* (2013) and are summarized in Table 1. Leaf area index (LAI) of the stands was measured during the same time period as foliar sampling. Five measurements of LAI in each stand were carried out using a Plant Canopy Analyzer LAI-2000 (Li-Cor Inc., Lincoln, NE, USA). Measurements were made early in the morning (shortly after sunrise) or late in the evening (shortly before

Table 1 Main features of the six study sites belonging to the explorative platform of the FunDivEUROPE project (Baeten *et al.*, 2013, modified)

Forest name	North Karelia	Białowieża	Hainich	Râşca-Suceava	Colline Metallifere	Alto Tajo
Country	Finland	Poland	Germany	Romania	Italy	Spain
Latitude/longitude	62.60°/29.83°	52.72°/23.95°	51.10°/10.51°	47.32°/26.03°	43.27°/11.26°	40.77°/1.95°
Altitude (m asl)	100–150	135–185	500–600	600–1000	250–550	960–1400
Main forest type	Boreal	Hemiboreal	Beech	Mountainous beech	Therm. deciduous	Cont. Medit.
No. of forest stands	28	43	38	28	36	36
Tree species	*Betula pendula* *Picea abies* *Pinus sylvestris*	*Betula pendula* *Carpinus betulus* *Picea abies* *Pinus sylvestris* *Quercus robur*	*Acer pseudoplatanus* *Fagus sylvatica* *Fraxinus excelsior* *Picea abies* *Quercus* sp.	*Abies alba* *Acer pseudoplatanus* *Fagus sylvatica* *Picea abies*	*Castanea sativa* *Ostrya carpinifolia* *Quercus cerris* *Quercus ilex* *Quercus petraea*	*Pinus nigra* *Pinus sylvestris* *Quercus faginea* *Quercus ilex*
Mean leaf area index	2.77	5.59	6.32	5.73	3.94	1.72
Mean basal area (m^2 ha^{-1})	22.70	37.57	35.59	51.32	27.348	22.19
MAT (°C)	2.1	6.9	6.8	6.8	13.5	10.2
MAP (mm)	629	627	775	800	850	499
GSR (kJ m^{-2} d^{-1}) April–September	14907	16634	16018	17917	21022	23428
Martonne aridity index (mm °C^{-1})	62.59	52.73	51.06	47.288	43.208	40.73
Topography	Flat	Flat	Flat	Medium to steep slopes	Medium to steep slopes	Flat to medium slopes
Soil depth	Deep	Deep	Medium	Deep	Deep	Shallow

MAT, mean annual temperature; MAP, mean annual precipitation; GSR, global solar radiation, daily averaged for the period 2009–2013 (April–September). GSR data from the CGMS database of interpolated meteorological data (AGRI4CAST, http://mars.jrc.ec.europa.eu/mars). Martonne Aridity Index: (annual precipitation/(mean annual temperature + 10)).

sunset) in order to work in the presence of diffuse solar radiation and thus reduce the effect of scattered blue light in the canopy.

Tree species diversity of the forest stands was quantified using the Shannon diversity index (Shannon, 1948), based on the basal area of each target species within a plot, using Eqn 1:

$$\text{Shannon diversity index} = -\sum_{i=1}^{n} \frac{BA_i}{BA_T} \ln\left(\frac{BA_i}{BA_T}\right) \qquad \text{Eqn 1}$$

where n is the number of target tree species in the plot, BA_i is the basal area of the target species in the plot and BA_T is the total basal area of the plot.

The tree species studied were: *Abies alba* Mill., *Acer pseudoplatanus* L., *Betula pendula* Roth., *Castanea sativa* Mill., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Ostrya carpinifolia* Scop., *Picea abies* (L.) Karst., *Pinus nigra* L., *Pinus sylvestris* L., *Quercus cerris* L., *Quercus faginea* Lam., *Quercus ilex* L., *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. In each forest stand, between six and 15 dominant trees for each local species were selected. Six trees were selected in monospecific stands, and three trees were selected per species in all other species mixtures. The trees were randomly selected among those with the largest diameter at breast height. Branches, with attached leaves, were sampled in the highest southern exposed part of the crown. The sampling was performed in the summer period, between June and August, when leaves were fully developed. In 2012, we sampled the forest stands in Italy, Germany and Finland; in 2013, leaves were collected in Spain, Romania and Poland. The 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 region. Sampling was performed in accordance with a strict safety protocol.

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, humidified to avoid leaf dehydration. The bags where then kept at constant temperature in an insulated box, where samples began the dark adaptation period. The effectiveness of the protocol was tested before sampling.

Chl *a* fluorescence measurements

ChlF measurements were made using a HandyPEA fluorimeter (in all sites except Germany) and a PocketPEA fluorimeter (in Germany). Both instruments belong to the PEA series, Plant Efficiency Analyser (Hansatech Instruments Ltd, Petney, Norfolk, UK). To avoid bias caused by differences between instruments, a comparison of the fluorimeters was performed using the criteria described by Bussotti *et al.* (2011) to correct systematic differences. The measurements of ChlF were performed after 4–5 h of sample dark adaptation on 16 leaves for each tree. As sampling was performed at different hours in the day, a long dark adaptation period was necessary to reduce the effects of leaf photoinhibition and of the daily hours of solar radiation exposure (Desotgiu *et al.*, 2012, 2013).

Fluorescence rise OJIP curves were induced by 1-s pulses of red light (650 nm, 3500 μmol m^{-2} s^{-1}). Plotted on a logarithmic

time scale, the fluorescence transients show a polyphasic shape. '*O*' refers to the initial fluorescence level, *K* (300 μs), *J* (*c.* 2–3 ms) and *I* (*c.* 30 ms) are intermediate levels of the fluorescence emission, and *P* (500–800 ms to 1 s) is the peak level of fluorescence. The latter indicates the highest, or maximal, fluorescence intensity (F_M) when saturating light is applied to the leaf.

The fluorescence OJIP transients were analysed using the JIP-test (Strasser *et al.*, 2000, 2004). The JIP-test defines the maximal (subscript '0') energy fluxes in the energy cascade for the events absorption (ABS), trapping (TR_0), electron transport (ET_0), dissipation (DI_0) and reduction of end acceptors of photosystem I (PSI) (RE_0). The parameters used in this survey and the relative equations are reported in detail in Table S1.

The maximum quantum yield for primary photochemistry of a dark-adapted sample (F_V/F_M) is a widely used parameter that allows comparison of our data with the published literature. Other parameters applied for fluorescence analysis were as follows: the number of active RCs per total Chl content in the antennae of PSII (RC/ABS); the probability of an electron to reduce the primary quinone acceptor and to move into the electron transport chain (Ψ_{Eo}) beyond PSII; the amplitude of the relative contribution of the I to P rise to the OJIP transient (ΔV_{IP}). ΔV_{IP} is an indicator of the abundance of PSI with respect to PSII, and is related to the electron transport chain beyond PSI (Ceppi *et al.*, 2012). The Performance Indices (PIs) measure the potential energy conservation of photons in the intersystem between PSII and PSI (PI_{ABS}) and the potential energy conservation from photons absorbed by PSII to the reduction flux of end-electron acceptors of PSI (PI_{TOT}).

Supporting data

Chlorophyll fluorescence data were combined with other foliar traits related to photosynthetic function (Sperlich *et al.*, 2015): the leaf N content mass-based (N, %); the specific leaf area (SLA, i.e. the one-sided area of a fresh leaf divided by its oven-dry mass, $cm^2\,g^{-1}$); and the light-saturated rate of photosynthesis (A_{max}, $\mu mol\,m^{-2}\,s^{-1}$). We also considered the following plant traits related to environmental stress: the foliar stable carbon isotope composition ($\delta^{13}C$, ‰) and the wood density (WD, $g\,cm^{-3}$). Foliar $\delta^{13}C$ is a key parameter for the investigation of carbon sequestration and strategies for an efficient water use of trees under water stress conditions (Farquhar *et al.*, 1982; Gessler *et al.*, 2001). WD is an important evolutionary trait concerning the adaptation of tree species to light. Its relationships with drought stress have been discussed (Swenson & Enquist, 2007).

Foliar N content and carbon isotope composition were determined on the same leaf sample as used for ChlF analysis. After oven drying, the samples were analysed for N by near infra-red spectroscopy (NIRS), as described by Niederberger *et al.* (2015) (data provided by M. Fotelli). For the analysis of $\delta^{13}C$, we used *c.* 1.0 mg of dried powdered leaf material from each sample (samples from all trees of the same species per plot were pooled). The analyses were performed by the Technical Platform of Functional Ecology at the INRA Forest Ecology and Ecophysiology Unit (Champenaux, France), with an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). The isotopic composition of the sample was reported in delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite standard (data provided by C. Grossiord, Earth and Environmental Sciences Division, MS-J495, Los Alamos National Lab, Los Alamos, NM 87545, USA; D. Bonal, INRA, UMR EEF, 54280 Champenoux, France; A. Gessler, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Long-term Forest Ecosystem Research (LWF), Birmensdorf, Switzerland).

Specific leaf area was measured on the same trees as sampled for ChlF measurement at the Finnish, Romanian and Spanish study sites (data provided by R. Benavides, Albert-Ludwings-Universitat Freiburg, Schanzlestrasse 1, 79104 Freiburg, Germany). For the other sites and tree species, SLA values were obtained from the literature, taking into account the geographical provenance of the trees (Gratani & Foti, 1998; Bussotti *et al.*, 2000; Bréda, 2003; Bruschi *et al.*, 2003; Legner *et al.*, 2014).

Wood density data were derived from the global WD database (Chave *et al.*, 2009; Zanne *et al.*, 2009). The light-saturated rate of photosynthesis data were obtained from the literature (Luoma, 1997; Morecroft & Roberts, 1999; Urban *et al.*, 2007; Aphalo *et al.*, 2009; Legner *et al.*, 2014).

Statistical analyses

ChlF data were calculated per tree and per stand by averaging the values of the trees belonging to the same species in each forest stand. Nonparametric tests were applied (the assumptions of parametric tests were checked in a preliminary data analysis). The differences between more than two independent samples (species and species mixtures at each site; tree species functional groups and sites in the analysis at the European scale) were tested using the Kruskal–Wallis test of medians. The significance of the differences ($P < 0.05$) was analysed using a two-sample Kolmogorov–Smirnov test. Principal component analysis (PCA, Sokal & Rohlf, 1995) was used to analyse the correlations between the response variables (ChlF parameters) in relation to the predictors (tree species, mixture level, site). The first two components of PCA were considered. The PCA analysis was also used to analyse the relationships between ChlF parameters and other plant functional traits. Cluster analysis was used to select and characterize homogeneous groups of tree species based on their fluorescence responses. The *k*-means algorithm was used to identify the composition of the different clusters (MacQueen, 1967). At each site, we selected a number of clusters equal to the number of tree species assessed.

The univariate correlation of ChlF parameters (Fv/F_M, ΔV_{IP}) with stand characteristics (LAI, Shannon diversity index), and with environmental parameters (solar radiation, Martonne aridity index), was tested, for coniferous species and broadleaf species separately, with the Spearman rank correlation test. Linear mixed models were used to assess the influence of the explanatory variables (tree species, LAI, Shannon diversity index, Martonne aridity index, solar radiation) and their interactions on the variability of the ChlF parameters. A number of alternative fixed models, with different combinations of the explanatory variables, were fitted and compared using Akaike's Information Criterion. Models for

coniferous species and broadleaf species were fitted separately. Before running the models, the correlations between the explanatory variables were checked to avoid multicollinearity. The linear models were implemented in R v3.1.2 (R Core Team, 2014), with the package LME4 (Bates *et al.*, 2015). The other statistical analyses were performed with STATISTICA 7.1 (Statsoft Inc., Tulsa, OK, USA).

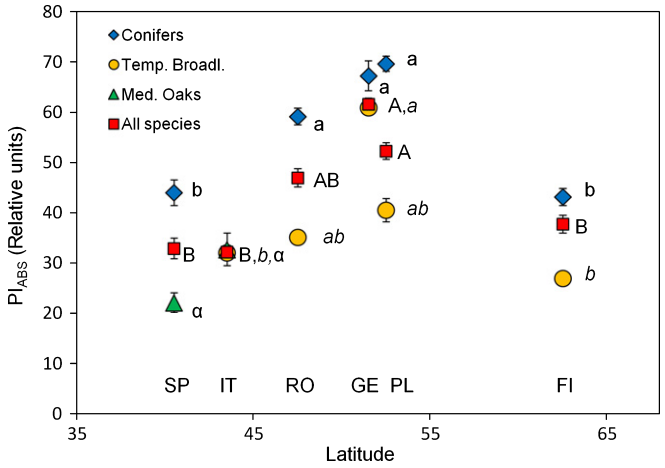

Fig. 1 Latitudinal trend of the Performance Index on absorption basis (PI_{ABS}) in European forest stands (subdivided per tree species functional group, and all species in all mixtures in each site). SP, Spain; IT, Italy; RO, Romania; GE, Germany; PL, Poland; FI, Finland. The bars indicate ± SE. Different letters indicate significant differences ($P < 0.05$) in PI_{ABS} between the sites. Uppercase letters, all species and all mixtures together in each site; lowercase letters, conifers; italic lowercase letters, temperate broadleaves; Greek letters, Mediterranean oaks.

Results

Chl *a* fluorescence patterns at the European scale

To analyse the behaviour of ChlF parameters of trees at the European scale, the tree species were assigned to three functional groups: conifers, temperate broadleaves and Mediterranean oaks (Table S2). PI_{ABS} is the parameter that most effectively describes the continental pattern of the photochemical properties of tree species (Table S2; Fig. 1). PI_{ABS} and related parameters (RC/ABS, F_V/F_M, Ψ_{Eo}) were higher in Central European forest stands (Poland, Germany, Romania) than in populations at the edges of the studied region (northern edge in Finland, and southern edges in Italy and Spain).

The PCA applied to ChlF parameters (Fig. 2) showed two distinct clusters: PI_{ABS} and RC/ABS, F_V/F_M, Ψ_{Eo} were related on PC1, whereas PI_{TOT} and ΔV_{IP} were related on PC2. F_V/F_M and ΔV_{IP} were thus selected as key parameters to investigate the relationships between ChlF parameters and ecological and structural stand factors. The linear models applied (Table S3a–d) showed significant relationships between ChlF parameters and the multiple interacting environmental factors (explanatory variables), with $R^2 = 0.43$ and 0.38 for F_V/F_M in broadleaf and coniferous species, respectively, and $R^2 = 0.82$ and 0.64 for ΔV_{IP} in broadleaf and coniferous species, respectively. The ChlF

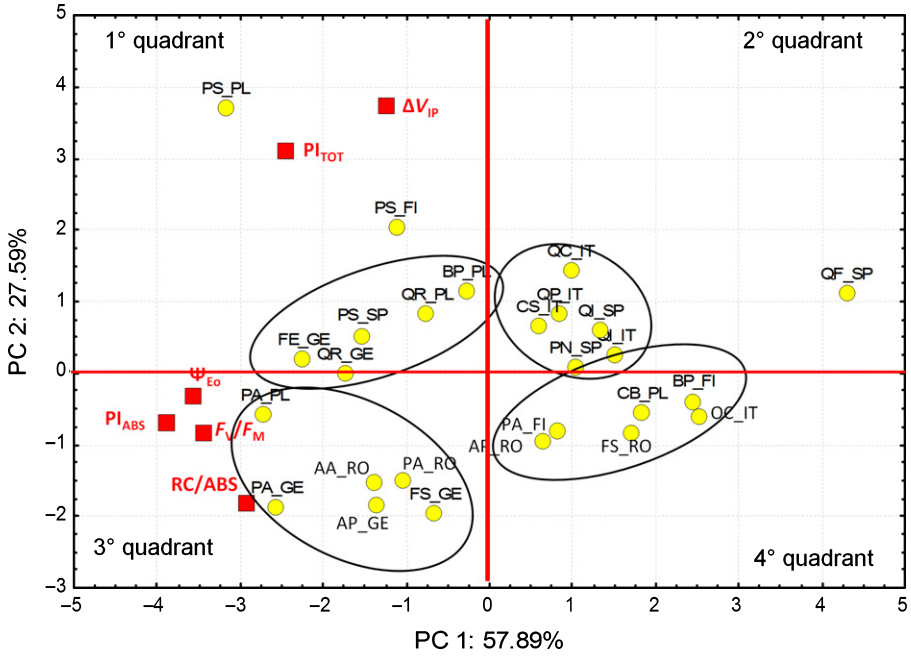

Fig. 2 Representation of the principal component analysis (PCA) space (cases) of the chlorophyll *a* fluorescence parameters examined, defined by the first two components (PC1 and PC2), for all tree species and tree species mixture levels across all sites (yellow circles). The PC1 and PC2 loadings with values > 0.6 are reported in Supporting Information Table S6. The species are indicated as follows: AA, *Abies alba*; AP, *Acer pseudoplatanus*; BP, *Betula pendula*; CB, *Carpinus betulus*; CS, *Castanea sativa*; FS, *Fagus sylvatica*; FE, *Fraxinus excelsior*; OC, *Ostrya carpinifolia*; PA, *Picea abies*; PN, *Pinus nigra*; PS, *Pinus sylvestris*; QC, *Quercus cerris*; QF, *Quercus faginea*; QI, *Quercus ilex*; QP, *Quercus petraea*; QR, *Quercus robur*; QRP, *Quercus* spp. (*Q. robur–Q. petraea*). The sites are indicated as follows: FI, Finland; GE, Germany; IT, Italy; PL, Poland; RO, Romania; SP, Spain. The main homogeneous clusters are identified graphically (black open circles). In the same figure is shown the distribution of the variables (chlorophyll *a* fluorescence (ChlF) parameters, red squares) according to the first two PC axes.

properties of trees were influenced by both environmental factors (solar radiation and precipitation : temperature ratio) and forest diversity (tree species and, only in broadleaf species for F_V/F_M, by Shannon diversity index). The univariate correlations between F_V/F_M and ΔV_{IP} with environmental and stand factors (Table S3e) showed a negative effect of solar radiation on F_V/F_M, and a positive effect on ΔV_{IP} in broadleaf species. Martonne aridity index and LAI affected positively F_V/F_M and negatively ΔV_{IP} in both coniferous and broadleaf species.

Chl *a* fluorescence responses of tree species

Different tree species growing at the same site showed differences in ChlF parameters (Table 2). Taking into account the parameters related to the events of electron trapping and delivery to the electron transport chain (ETC) (F_V/F_M, RC/ABS, Ψ_{Eo} and PI_{ABS}), coniferous species had higher values than broadleaved species at all sites. Among coniferous species, *P. sylvestris* showed the highest values of ΔV_{IP} and PI_{TOT}, whereas some deciduous broadleaved species, such as *B. pendula* (FI), *C. betulus* (PL), *F. sylvatica* (GE and RO) and *O. carpinifolia* (IT), had the lowest values of these parameters.

The Performance Indices (PI_{ABS} and PI_{TOT}) were used in this study as response variables to compare different tree species. The application of the nonparametric Kruskal–Wallis test (Tables S4, S5) showed that differences between species were highly significant at each site ($P < 0.001$), whereas the differences between samples of the same species growing in different mixture levels were generally not significant. The two-sample Kolmogorov–Smirnov test, applied to each species at each site, to test the significance of differences between tree species mixture levels, showed significant responses related to species and sites (Tables 3, 4). *Picea abies* in FI had the highest values of PI_{ABS} and PI_{TOT} in monospecific stands (Fig. S2a), whereas, in RO, values were highest in species-rich stands (Fig. S2b). PI_{ABS} increased with increasing species mixture in *Q. cerris* in IT. In relation to PI_{TOT}, *P. nigra* in SP showed the highest values at a high level of species mixtures, whereas *P. abies* (FI), *P. sylvestris* (SP), *B. pendula* (PL) and *Q. robur* (PL) showed the opposite response.

A ranking of the tree species by ChlF parameters, in general, did not vary at a given site according to the level of species mixture (e.g. the rank of PI_{ABS} and PI_{TOT} in Poland was *P. sylvestris* > *P. abies* > *Q. robur* > *B. pendula* > *C. betulus* at all mixture levels, Tables 3, 4).

Table 2 Chlorophyll *a* fluorescence (ChlF) parameters (mean ± SD) for species (in all mixture levels, pooled as a whole) at each site

Site	Species	RC/ABS Mean ± SD	F_V/F_M Mean ± SD	Ψ_{Eo} Mean ± SD	ΔV_{IP} Mean ± SD	PI_{ABS} Mean ± SD	PI_{TOT} Mean ± SD
Finland	*Betula pendula* (16)	5.19 ± 0.69b	0.78 ± 0.03b	0.53 ± 0.08c	0.2 ± 0.05c	26.08 ± 11.94b	16.59 ± 8.66c
	Picea abies (16)	6.14 ± 0.60a	0.81 ± 0.02a	0.56 ± 0.04b	0.21 ± 0.03b	39.24 ± 11.35a	24.77 ± 8.88b
	Pinus sylvestris (16)	5.81 ± 0.67a	0.82 ± 0.02a	0.63 ± 0.05a	0.33 ± 0.05a	49.61 ± 13.22a	60.44 ± 22.94a
Poland	*Betula pendula* (22)	5.08 ± 0.51b	0.81 ± 0.01b	0.66 ± 0.04b	0.3 ± 0.06b	43.55 ± 8.06b	38.39 ± 13.40b
	Carpinus betulus (23)	4.95 ± 0.35b	0.79 ± 0.01b	0.61 ± 0.03cb	0.2 ± 0.03c	30.74 ± 6. 80b	15.72 ± 4.98c
	Picea abies (23)	6.9 ± 0.57a	0.84 ± 0.01a	0.66 ± 0.03b	0.25 ± 0.04b	72.62 ± 14.39a	45.19 ± 14.41b
	Pinus sylvestris (23)	5.43 ± 0.62b	0.82 ± 0.01ab	0.72 ± 0.03a	0.4 ± 0.06a	67.22 ± 13.57a	86.51 ± 27.85a
	Quercus robur (23)	6.43 ± 0.69a	0.8 ± 0.01b	0.62 ± 0.04b	0.3 ± 0.04bc	46.87 ± 14.12b	44.9 ± 17.47b
Germany	*Acer pseudoplatanus* (19)	6.97 ± 0.51a	0.81 ± 0.01b	0.69 ± 0.04a	0.2 ± 0.03b	63.87 ± 9.21ab	19.45 ± 8.45b
	Fagus sylvatica (22)	6.97 ± 0.60a	0.8 ± 0.02b	0.66 ± 0.04b	0.19 ± 0.02c	56.43 ± 8.74b	16.23 ± 5.49b
	Fraxinus excelsior (28)	6.97 ± 0.39a	0.81 ± 0.02b	0.69 ± 0.05a	0.29 ± 0.05a	65.34 ± 12.62a	43.99 ± 12.98a
	Picea abies (10)	6.98 ± 0.46a	0.84 ± 0.01a	0.7 ± 0.04b	0.22 ± 0.02b	67.28 ± 9.39a	23.14 ± 6.80b
	Quercus sp. (14)	6.97 ± 0.52a	0.81 ± 0.02b	0.67 ± 0.06b	0.28 ± 0.06b	59.32 1 ± 3.40b	40.51 ± 16.27b
Romania	*Abies alba* (15)	6.96 ± 0.66a	0.83 ± 0.02a	0.63 ± 0.04a	0.2 ± 0.02a	62.21 ± 15.68a	30.71 ± 9.41a
	Acer pseudoplatanus (12)	5.92 ± 0.38ab	0.79 ± 0.01b	0.63 ± 0.03a	0.21 ± 0.03a	41.06 ± 7.07b	20.82 ± 4.94ab
	Fagus sylvatica (19)	5.39 ± 0.38b	0.79 ± 0.01b	0.6 ± 0.02a	0.2 ± 0.02a	31.78 ± 5.54b	16.3 ± 4.71b
	Picea abies (15)	6.69 ± 0.57a	0.83 ± 0.01a	0.61 ± 0.03a	0.2 ± 0.02a	55.91 ± 11.95b	28.02 ± 6.81ab
Italy	*Castanea sativa* (15)	5.88 ± 0.43a	0.79 ± 0.01a	0.6 ± 0.04a	0.28 ± 0.04a	36.19 ± 7.96a	33.75 ± 10.77a
	Ostrya carpinifolia (14)	4.76 ± 0.33a	0.79 ± 0.01a	0.58 ± 0.05a	0.19 ± 0.04b	25.74 ± 6.96a	13.45 ± 5.94b
	Quercus cerris (16)	5.96 ± 0.62a	0.78 ± 0.02a	0.57 ± 0.06a	0.31 ± 0.05a	31.33 ± 10.48a	39.64 ± 15.93a
	Quercus ilex (18)	5.69 ± 1.28a	0.78 ± 0.02a	0.58 ± 0.06a	0.26 ± 0.05a	34.34 ± 17.27a	28.23 ± 14.44a
	Quercus petraea (13)	5.83 ± 0.56a	0.79 ± 0.02a	0.59 ± 0.05a	0.29 ± 0.04a	34.29 ± 10.99a	35.44 ± 14.91a
Spain	*Pinus nigra* (20)	5.39 ± 0.45b	0.81 ± 0.02b	0.57 ± 0.05b	0.25 ± 0.02b	34.03 ± 10.89b	25.74 ± 8.52b
	Pinus sylvestris (15)	6.11 ± 0.49a	0.83 ± 0.01a	0.63 ± 0.05a	0.28 ± 0.03a	56.4 ± 15.55a	46.6 ± 16.21a
	Quercus faginea (21)	4.88 ± 0.59c	0.75 ± 0.04c	0.46 ± 0.07c	0.27 ± 0.04a	15.54 ± 7.64c	21.68 ± 10.23b
	Quercus ilex (15)	5.81 ± 0.90b	0.79 ± 0.03b	0.55 ± 0.06b	0.28 ± 0.04a	32.13 ± 15.31b	32.77 ± 17.65a

The significance of differences was analysed by means of the Kolmogorov–Smirnov test. Different letters indicate significant ($P < 0.05$) differences between species at the same site. The number of forest stands for each species is indicated in parentheses (both monocultures and mixtures). RC/ABS, number of active reaction centres per total chlorophyll content in the antennae of photosystem II; F_V/F_M, maximum quantum yield for primary photochemistry of dark-adapted sample; Ψ_{Eo}, the probability of an electron to reduce the primary quinone acceptor and to move into the electron transport chain beyond PSII; ΔV_{IP}, the amplitude of the relative contribution of the I to P rise to the OJIP transient; PI_{ABS}, index of potential energy conservation of photons in the intersystem between PSII and PSI; PI_{TOT}, index of potential energy conservation from photons absorbed by PSII to the reduction flux of end-electron acceptors of PSI.

Identification of tree species clusters

A multivariate analysis (PCA) allowed the identification of the main clusters in the whole sample (all species, all sites together) and at each site. In the whole sample (Table S6), principal component 1 (PC1) explained 57.89% of the variance and represented the parameters RC/ABS, F_V/F_M, Ψ_{Eo} and PI_{ABS}. PC2, which explained 27.59% of the variance, is represented by ΔV_{IP}. PI_{TOT} is present in both PC1 and PC2. The distribution of groups is shown in Fig. 2. The first quadrant comprises the 'best performing' species (i.e. those presenting the highest values of all the studied parameters). It includes *P. sylvestris* and a cluster of broadleaved trees from PL and GE. The remaining conifers (in particular, *P. abies* and *A. alba*) are clustered in the third quadrant, with higher values of the parameters in PC1 and lower values of the parameters in PC2. In the second quadrant, characterized by lower values of the parameters in PC1 and higher values in PC2, there is a cluster including evergreen and deciduous broadleaves from the Mediterranean sites (IT and SP), together with *P. nigra* from SP. At the extreme lower values of PC1 in the second quadrant, we find *Q. faginea*. Finally, the fourth quadrant hosts a cluster of 'low-efficiency' broadleaves, including also *P. abies* from FI.

At the site level, the composition of the factors in the PCA (loadings > 0.6 in PC1 and PC2) followed basically the same pattern, with some exceptions site by site (Table S6). In Figs 3 and 4, it was possible to identify homogeneous clusters per species (i.e. including different mixture levels of the same species) and a trend from low- to high-performing groups of species. Some overlaps between clusters were observed in several cases. In FI, there was a partial overlap between *P. abies* and *B. pendula* and, in PL, between *Q. robur* and *B. pendula*; in RO, the functional groups conifers and broadleaves were clearly separated, but *P. abies* and *A. alba* on the one hand, and *A. pseudoplatanus* and *F. sylvatica* on the other, were partially overlapping. In GE, *P. abies* was clearly distinct from the broadleaved species, whereas *F. sylvatica* and *A. pseudoplatanus* (low performing) formed a group separated from *F. excelsior* and oak species (*Q. robur* and *Q. petraea*, high performing). In IT, it was possible to discriminate *O. carpinifolia* and, to a lesser extent, *C. sativa*, whereas the oak species (*Q. cerris*, *Q. petraea*, *Q. ilex*) formed a group that was relatively indistinct. In SP, the two pine species were separated from each other, and separated from oaks, especially with reference to *Q. faginea*.

The clusters of the tree species shown in Figs 3 and 4 were compared with the clusters identified using *k*-means analysis (Table 5). In many cases, a good accordance was found. The

Table 3 Values of Performance Index on absorption basis (PI_{ABS}) (mean ± SD) per site, species and tree species mixture level (from one species, 1-sp, to five species, 5-sp)

		Mixture level				
	Species	1-sp Mean ± SD	2-sp Mean ± SD	3-sp Mean ± SD	4-sp Mean ± SD	5-sp Mean ± SD
Finland	*Betula pendula*	22.3 ± 12.7c	30.4 ± 10.0c	25.0 ± 12.0b		
	Picea abies	43.4 ± 11.4b,A	39.1 ± 10.7b,AB	30.5 ± 7.6b,B		
	Pinus sylvestris	52.6 ± 12.2a	48.0 ± 14.6a	46.7 ± 12.2a		
Poland	*Betula pendula*	43.2 ± 10.0b	42.6 ± 8.1b	44.3 ± 5.8b	43.4 ± 9.2b	44.6 ± 7.6b
	Carpinus betulus	28.8 ± 4.9c	33.9 ± 8.7c	33.0 ± 6.0c	28.4 ± 6.8c	30.7 ± 4.2c
	Picea abies	71.1 ± 10.1a	77.1 ± 17.4a	76.2 ± 15.5a	68.1 ± 13.6a	72.2 ± 13.3a
	Pinus sylvestris	73.3 ± 15.3a	56.8 ± 11.6a	71.4 ± 14.3a	64.4 ± 11.2a	65.1 ± 8.3a
	Quercus robur	44.9 ± 7.4b	61.2 ± 17.0a	48.9 ± 13.6b	41.3 ± 13.4b	43.3 ± 11.5b
Germany	*Acer pseudoplatanus*		68.3 ± 27.7a	64.8 ± 21.5a	59.3 ± 16.8b	
	Fagus sylvatica	56.4 ± 13.3b	47.8 ± 19.4a	59.8 ± 23.5a	56.6 ± 11.8b	
	Fraxinus excelsior	75.5 ± 25.8a	57.8 ± 30.7a	68.0 ± 22.4a	69.8 ± 22.7a	
	Picea abies	66.1 ± 15.5a	60.1 ± 26.6a	70.1 ± 15.4a	75.0 ± 14.2a	
	Quercus	41.3 ± 18.1b,B	49.8 ± 7.6a,B	65.8 ± 23.5a,A	53.3 ± 28.5b,B	
Romania	*Abies alba*	64.8 ± 13.0a	54.9 ± 14.7a	63.0 ± 17.6a	69.6 ± 14.2a	
	Acer pseudoplatanus	41.4 ± 4.3b	46.6 ± 8.0a	37.2 ± 7.2b	40.3 ± 6.1b	
	Fagus sylvatica	34.1 ± 7.6c	31.2 ± 3.3b	32.5 ± 6.1b	28.5 ± 4.3c	
	Picea abies	49.9 ± 9.0b,B	52.3 ± 8.7a	58.5 ± 13.8a,A	65.6 ± 11.0a,A	
Italy	*Ostrya carpinifolia*	25.2 ± 7.8a	25.2 ± 9.4a	25.7 ± 7.2a	27.2 ± 4.7a	
	Quercus ilex	42.1 ± 27.7a	36.2 ± 20.6a	29.7 ± 6.5a	31.2 ± 10.4a	
	Quercus cerris	23.5 ± 6.7a,B	37.1 ± 7.4a,A	29.6 ± 7.6a,AB	35.1 ± 12.3a,A	
	Quercus petraea	35.7 ± 14.7a,A	33.2 ± 4.8a,AB	29.0 ± 10.6a,B	38.9 ± 5.8a,A	
	Castanea sativa	32.1 ± 5.3a	37.7 ± 8.1a	36.5 ± 7.6a	38.4 ± 9.7a	
Spain	*Pinus nigra*	29.6 ± 9.0b	37.2 ± 10.7b	30.2 ± 9.1b	40.2 ± 13.3a	
	Pinus sylvestris	55.3 ± 14.1a	61.8 ± 17.1a	59.0 ± 13.0a	44.8 ± 11.7a	
	Quercus faginea	14.2 ± 6.6c	14.9 ± 7.7c	18.5 ± 8.8c	14.6 ± 6.6b	
	Quercus ilex	29.5 ± 13.3b	30.4 ± 13.3b	26.9 ± 11.9b	44.2 ± 20.5a	

Significance of differences was analysed by means of the Kolmogorov–Smirnov test. Significances are reported for $P < 0.05$. Lowercase letters indicate differences between species at the same site and tree species mixture level (on columns). Uppercase letters indicate differences between mixture levels for the same species (on rows). When not expressed, differences were not significant.

k-means analysis confirmed, alongside the distinction between conifers and broadleaves, the previously evidenced overlaps, especially between species belonging to the same genus and/or family. In these cases, the *k*-means distances between the groups were low.

A supplemental PCA was carried out on the same species (*P. sylvestris* and *P. abies*) or genus (*Quercus*) growing at different sites. The results (Fig. 5; Table S7) show that, although there are some partial overlaps, the different sites can be clearly discriminated. In coniferous species, PC1 indicates the pattern of the ChlF parameters related to PI_{ABS} (RC/ABS, F_V/F_M, Ψ_{Eo}), which decrease at edge sites (SP and FI for *P. sylvestris*; FI for *P. abies*). In oak species, the pattern of distribution of the species is less evident, but the oaks at the Mediterranean sites (*Q. faginea* in SP and *Q. ilex* in SP and IT), with lower photosynthetic efficiency, can be roughly separated from the more efficient deciduous species (*Q. cerris* in IT, *Q. robur* in PL and GE, and *Q. petraea* in IT and GE).

Relationships between ChlF parameters and functional traits

Specific leaf area, leaf N content, leaf carbon isotope composition, light-saturated photosynthesis rate, WD and selected ChlF parameters (F_V/F_M and ΔV_{IP}, chosen as proxy of the photosynthetic efficiency) were analysed using PCA (Fig. S3). The results are presented separately for the two main functional groups, that is, temperate broadleaf tree species and coniferous species.

In broadleaf species (Fig. S3a), there is a main cluster on PC1 that includes N, A_{max} and F_V/F_M. This cluster describes the behaviour of the best-performing species at PL and GE sites and, at the opposite position on PC1, some less well-performing species. On PC2, ΔV_{IP} is related to WD, $\delta^{13}C$ and, in an opposite manner, to SLA.

In coniferous species (Fig. S3b), A_{max} is related to ΔV_{IP} and WD on PC1 (with *P. sylvestris*), whereas F_V/F_M, SLA and N are grouped in a cluster on PC2 (with *P. abies*).

Discussion

Structural, chemical and physiological leaf traits (with special reference to those related to photosynthetic function) are highly variable in functional groups (Niinemets *et al.*, 2015), and amongst ecological (edaphic and climatic) site conditions (Moles *et al.*, 2014; Maire *et al.*, 2015). Concerning the analysis of the factors affecting the ChlF parameters (namely F_V/F_M) in natural field conditions, only a few papers have reported findings on mature forest trees (Hallik *et al.*, 2012), and most derive from

Table 4 Values of Performance Index total (PI_{TOT}) (mean ± SD) per site, species and tree species mixture level (from 1 species, 1-sp, to five species, 5-sp)

		Mixture level				
	Species	1-sp Mean ± SD	2-sp Mean ± SD	3-sp Mean ± SD	4-sp Mean ± SD	5-sp Mean ± SD
Finland	*Betula pendula*	13.2 ± 8.9c,B	20.1 ± 7.7b,A	16.5 ± 7.9b,AB		
	Picea abies	27.4 ± 9.0b,A	24.8 ± 8.3b,A	19.1 ± 7.6b,B		
	Pinus sylvestris	58.7 ± 20.4a,A	59.1 ± 24.2a,A	66.6 ± 26.1a,A		
Poland	*Betula pendula*	40.1 ± 12.8b,A	30.5 ± 9.7b,AB	44.0 ± 9.8b,A	38.3 ± 17.0b,AB	32.1 ± 5.0b,
	Carpinus betulus	12.6 ± 2.9c,A	17.0 ± 4.7c,A	17.6 ± 5.4c,A	15.5 ± 5.3c,A	14.1 ± 2.9c,A
	Picea abies	43.4 ± 5.7b,A	48.2 ± 15.5b,A	46.4 ± 17.5b,A	42.1 ± 14.7b,A	50.7 ± 13.2b,A
	Pinus sylvestris	91.1 ± 35.8a,A	81.6 ± 26.2a,A	91.2 ± 27.2a,A	85.4 ± 25.1a,A	70.5 ± 27.4a,A
	Quercus robur	45.4 ± 9.5b,A	59.7 ± 23.2b,A	45.7 1 ± 4.5b,A	39.9 ± 17.4b,AB	38.3 ± 23.1b,B
Germany	*Acer pseudoplatanus*		27.1 ± 14.7a,A	19.0 ± 9.1b,A	15.8 ± 7.3b,A	
	Fagus sylvatica	15.2 ± 5.7b,A	14.6 ± 7.1b,A	17.4 ± 10.6b,A	15.2 ± 4.2b,A	
	Fraxinus excelsior	47.8 ± 20.9a,A	18.6 ± 12.4a,A	26.9 ± 9.5a,A	21.9 ± 7.7a,A	
	Picea abies	25.5 ± 7.1b,A	40.2 ± 18.4a,A	44.5 ± 15.8a,A	49.7 ± 17.1a,A	
	Quercus	28.1 ± 13.6b,B	35.7 ± 4.6a,B	46.0 ± 22.0a,A	33.7 ± 23.1a,B	
Romania	*Abies alba*	30.8 ± 8.6a,A	26.9 ± 8.5a,A	31.6 ± 10.2a,A	35.3 ± 9.2a,A	
	Acer pseudoplatanus	22.5 ± 2.3ab,A	23.3 ± 6.3a,A	17.5 ± 4.5b,B	20.6 ± 4.8b,A	
	Fagus sylvatica	18.5 ± 6.1b,A	15.4 ± 2.8b,A	17.1 ± 5.0b,A	13.7 ± 4.6c,A	
	Picea abies	25.7 ± 5.1ab,A	27.2 ± 6.6a,A	28.7 ± 8.1a,A	31.4 ± 6.1a,A	
Italy	*Castanea sativa*	32.1 ± 7.1a,A	40.6 ± 14.4a,A	29.9 ± 8.7b,A	34.8 ± 11.4a,A	
	Ostrya carpinifolia	13.5 ± 5.9b,A	11.8 ± 5.6c,A	16.6 ± 8.2c,A	12.6 ± 4.3c,A	
	Quercus cerris	31.1 ± 11.2a,A	48.9 ± 17.4a,A	42.3 ± 15.9a,A	39.2 ± 15.7a,A	
	Quercus ilex	34.1 ± 16.2a,A	30.3 ± 18.7b,A	25.5 ± 9.5b,A	23.8 ± 12.1b,A	
	Quercus petraea	41.5 ± 21.1a,A	48.1 ± 17.6a,A	28.4 ± 10.0b,A	35.1 ± 8.6a,A	
Spain	*Pinus nigra*	21.3 ± 5.9b,C	26.9 ± 7.6b,B	24.6 ± 7.9b,B	33.0 ± 11.7a,A	
	Pinus sylvestris	43.1 ± 10.6a,A	54.2 ± 21.4a,A	46.5 ± 11.8a,A	37.6 ± 9.7a,B	
	Quercus faginea	22.3 ± 10.4b,A	21.6 ± 10.7c,A	23.1 ± 10.9b,A	18.3 ± 7.5c,A	
	Quercus ilex	35.2 ± 16.8a,A	30.3 ± 17.0b,A	24.6 ± 11.6b,A	42.6 ± 21.9a,A	

Significance of differences was analysed by means of the Kolmogorov–Smirnov test. Significances are reported for $P < 0.05$. Lowercase letters indicate differences between species at the same site and mixture level. Uppercase letters indicate differences between mixture levels for the same species. When not expressed, differences were not significant.

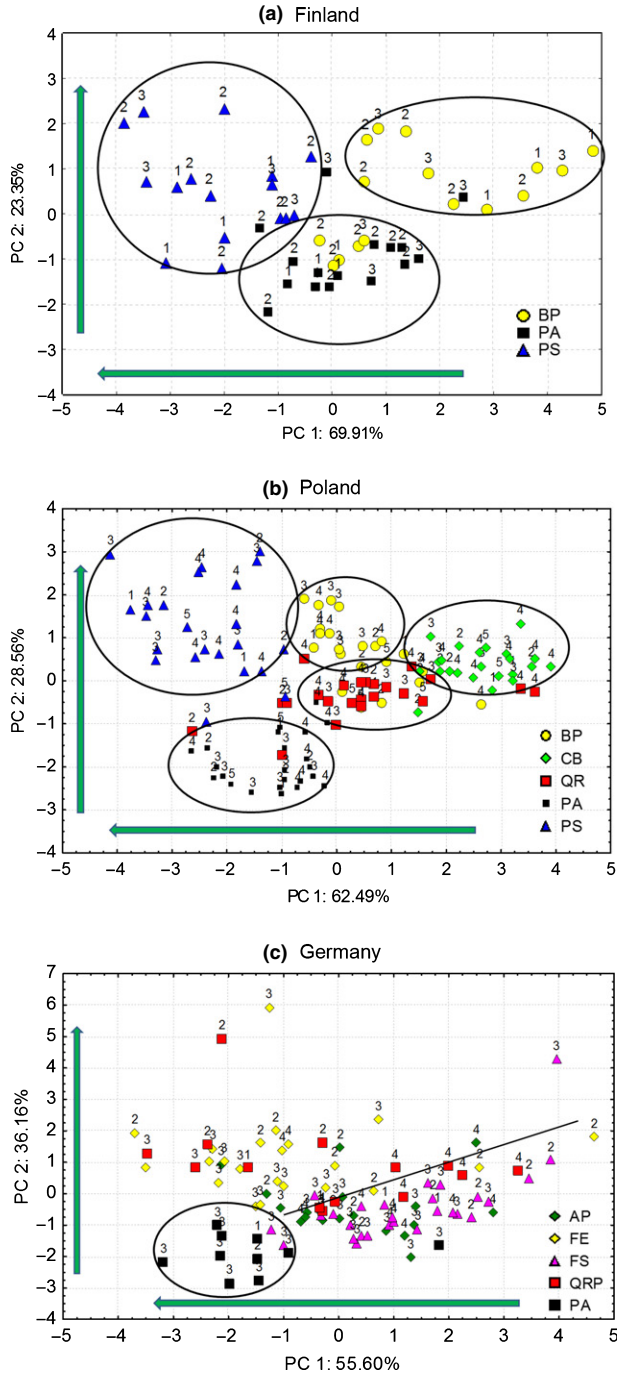

Fig. 3 Representation of the principal component analysis (PCA) space (cases) of the chlorophyll *a* fluorescence (ChlF) parameters, defined by the first two components (PC1 and PC2), per site (a, Finland; b, Poland; c, Germany). The species are indicated as follows: AP, *Acer pseudoplatanus*; BP, *Betula pendula*; CB, *Carpinus betulus*; FS, *Fagus sylvatica*; FE, *Fraxinus excelsior*; PA, *Picea abies*; PS, *Pinus sylvestris*; QR, *Quercus robur*; QRP, *Quercus* spp. (*Q. robur*–*Q. petraea*). Mixture levels (one to five species) are indicated for each point. The loadings of the principal components with values > 0.6 are reported in Supporting Information Table S6 for each site. The main homogeneous clusters are indicated graphically (black open circles). Arrows indicate the direction of increasing value of the parameters with loadings > 0.6 in each component. In (c), the straight black line indicates the separation between the two groups of broadleaf species (*F. sylvatica* and *A. pseudoplatanus* on the one hand, and *Q. robur-petraea* and *F. excelsior* on the other).

research carried out in experimental areas equipped for long-term surveys (Gielen *et al.*, 2007; Mänd *et al.*, 2012). Differences in ChlF parameters of tree species within multi-layered and multi-species forest canopies are related to light availability, N content, leaf angle, leaf mass per area, Chl content and species-specific differences in light acclimation strategies (Hallik *et al.*, 2012; Mänd *et al.*, 2012). Specific differences were documented by Hallik *et al.* (2012), and a first broad distinction indicated that conifers generally had higher F_V/F_M values than broadleaved species (Weng *et al.*, 2006; Pollastrini *et al.*, 2014; Sperlich *et al.*, 2015).

Global solar radiation, water availability and soil fertility are supposed to influence the general distribution of ChlF characteristics of trees. The photosynthetic efficiency of tree species, as well as the LAI of the stands, increases with a latitudinal gradient (coupled with a solar radiation and temperature gradient) from southern to central Europe, excluding the Finnish site. The decrease in PI_{ABS} and related parameters (F_v/F_M, Ψ_{Eo}, RC/ABS) in southernmost sites, with an increase in solar radiation, indicates a strategy for acclimation to light (Adams & Demmig-Adams, 2004), including the dissipation of excess radiation and photoinhibition processes (Gilmore, 2004). Such strategies also include a decrease in the quantum efficiency of primary photochemistry (F_V/F_M), caused by the activation of the xanthophyll cycle and damage to the D1 protein in the PSII RC. These protective processes (nonphotochemical de-excitation pathways) are combined with an increase in electron transport beyond PSI (increasing values of the IP phase, ΔV_{IP} (Cascio *et al.*, 2010; Desotgiu *et al.*, 2012, 2013). At the southernmost Mediterranean sites, the depression of PI_{ABS} may be attributed, alongside high solar radiation, to poor soils and dry climate (Peguero-Pina *et al.*, 2009). An analogous response is found in tree species at the northernmost sites, where the drop in Fv/F_M and PI_{ABS} may be explained by limiting climate and soil fertility. The downregulation between trapping and electron transport at low temperature has been described by Tsonev *et al.* (2003), whereas Rozema *et al.* (2005) reported a negative effect of UVB on photosynthetic functions at high latitudes. In general, sites hosting low-performing trees (FI, SP, IT) also have lower LAI.

If, at the European level, the ChlF features of tree species are determined by environmental conditions, at a given site, with homogeneous ecological conditions, differences can be expected in relation to specific physiological behaviours. Research carried out in tropical (Krause *et al.*, 2001; Sobrado, 2008) and Mediterranean (Sánchez-Gómez *et al.*, 2006; Valladares & Sánchez-Gómez, 2006) forests evidenced different behaviours of ChlF characteristics between early successional (light-demanding) and late successional (shade-tolerant) tree species. Early successional species have a higher SLA, and lower leaf thickness, N and Chl content, when compared with late successional species (Ribeiro *et al.*, 2005). Furthermore, they are more efficient in exploiting high light radiation when fully exposed to the sun (Krause *et al.*, 2001). Such differences are reflected in their ChlF properties, and Ribeiro *et al.* (2005) argued that ChlF might be a suitable tool for the phenotyping of the successional status of a tree. In the present survey, the species with the lowest PI_{ABS} and PI_{TOT} were the shade-

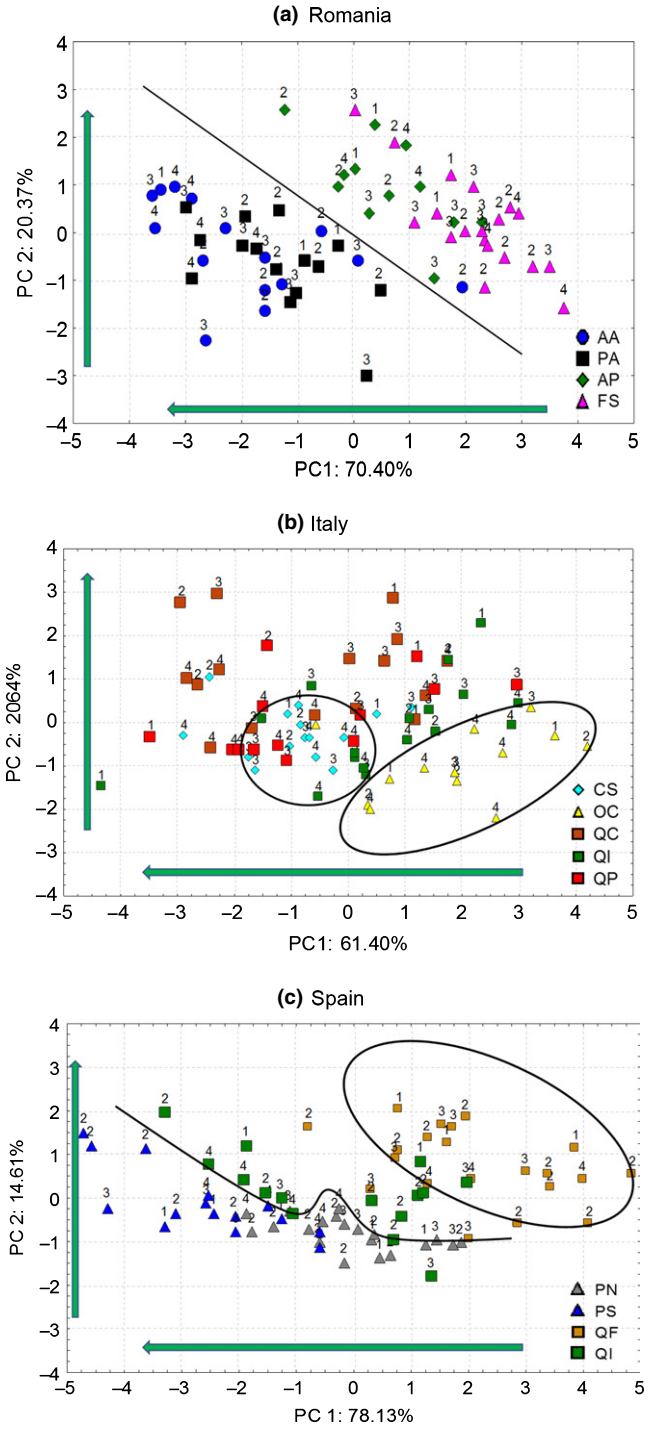

Fig. 4 Representation of the principal component analysis (PCA) space (cases) of the chlorophyll *a* fluorescence (ChlF) parameters, defined by the first two components (PC1 and PC2), per site (a, Romania; b, Italy; c, Spain). The species are indicated as follows: AA, *Abies alba*; AP, *Acer pseudoplatanus*; CS, *Castanea sativa*; FS, *Fagus sylvatica*; OC, *Ostrya carpinifolia*; PA, *Picea abies*; PN, *Pinus nigra*; PS, *Pinus sylvestris*; QC, *Quercus cerris*; QF, *Quercus faginea*; QI, *Quercus ilex*; QP, *Quercus petraea*. Mixture levels (one to four species) are indicated for each point. The loadings of the components with values > 0.6 are reported in Supporting Information Table S6 for each site. The main homogeneous clusters are identified graphically (black open circles). In (a) and (c), the straight black line indicates the separation between broadleaf and conifer species.

tolerant ones (*C. betulus* in PL, *F. sylvatica* in RO and GE; *O. carpinifolia* in IT). At the edge sites (SP and FI), the lowest PI_{ABS} and PI_{TOT} values were found in early successional, light-demanding species (*Q. faginea* and *B. pendula*, respectively), but this behaviour may be influenced by the poor soil conditions. In general, in central European sites, the forests, with higher photosynthetic efficiency than those at the edge sites, are well developed and in an advanced successional status, with high LAI and standing on deep soils.

Niinemets & Valladares (2006) considered the phylogenetic distance as a possible explanatory factor for differences in the responses of trees to environmental conditions. In IT, *O. carpinifolia* and, partially, *C. sativa* form distinct clusters, whereas the species belonging to the same genus *Quercus* are clustered together (all species are Fagaceae). In PL, *B. pendula* (Betulaceae) shows a behaviour more similar (and smaller distance) to *Q. robur* (Fagaceae) than to *C. betulus*, which belongs to the same family (Betulaceae). Although conifers and broadleaved trees are effectively discriminated by means of ChlF parameters, the distinction of singular taxa is more problematic. The overlaps of different taxa in the same groups and the distances between different groups (see Table 5) reflect taxonomic and ecological features. It is known that phylogenetically close species may have very different ecological requirements (Baraloto *et al.*, 2012). Moreover, in mature multi-layered forests, the photosynthetic behaviour of a given species depends on interactions with the site features, the role the species plays in the succession and its position with respect to the canopies.

Concerning the level of the tree species mixture, considered in this article as a possible modifying factor of the ChlF characteristics, we found differences between monocultures and mixed stands. The most interesting trends were observed in *P. abies*, in which the PIs increased with the Shannon diversity index in Romania and decreased with the Shannon diversity index in Finland. The role of structural and ecological factors leading to these two opposite trends still remains to be explored. According to the stress gradient hypothesis (He & Bertness, 2014), limiting ecological conditions enhance the positive effects of tree diversity (see also Grossiord *et al.*, 2014b), but not all the observed trends can be explained in this way.

The results presented here demonstrate that ChlF features were reasonably constant within trees belonging to the same species, and the combination of the fast kinetic fluorescence parameters may have taxonomic relevance. ChlF parameters are able to discriminate between different species, although their taxonomic (same or different genus and/or family) and ecological characteristics must be considered.

Another outcome of this study was an overall evaluation of the variability of ChlF data in forest trees and the effectiveness of a field survey, which are crucial issues for the repeatability of a survey. The variability of ChlF data observed at the tree species and site level, expressed as a coefficient of variation (CV%, Table S8), reflects the findings already described by Pollastrini *et al.* (2014), with F_V/F_M being the most robust parameter and PIs the most variable. Such variability has also

been confirmed in experiments carried out in growth chambers under controlled conditions (E. Salvatori, pers. comm.), and should be considered intrinsic and constant for plants in good vegetative conditions.

The relationships between ChlF parameters and functional traits differ in the two main tree species functional groups. The relationship of F_V/F_M with leaf N content and light-saturated photosynthesis rate (A_{max}) in deciduous broadleaved species is consistent with observations in the literature (Sperlich *et al.*, 2015), thus supporting the use of F_V/F_M in remote sensing surveys (Peñuelas *et al.*, 1995; Weng *et al.*, 2006; Serbin *et al.*, 2012). In coniferous species, A_{max} was positively related to the capacity of electron transport beyond PSI (ΔV_{IP}). The relation of ΔV_{IP} with WD, in both coniferous and broadleaved species, and with $\delta^{13}C$ in broadleaved species, can be explained by the geographical distribution of the species: trees with high values of WD and with high values of leaf $\delta^{13}C$ are more diffuse in the Mediterranean region, where drought conditions are more frequent and stronger than in central and northern Europe.

The analysis of ChlF parameters on forest trees can be a very informative tool in ecological surveys. It can contribute to explain the physiological behaviour of trees, especially when their assessment is combined with a set of other functional traits. The acquisition of reliable terrestrial data is relevant to support the interpretation of remotely detected signals. ChlF, however, is assessed with remote sensing on light-adapted canopies (Meroni *et al.*, 2009) and cannot be compared directly with the JIP-test parameters measured from the ground on dark-adapted samples. To enhance the comparability between terrestrial and remote sensing surveys, it is therefore necessary to promote further studies to identify the proxies aimed at relating JIP-test parameters and remotely assessed parameters.

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Table 5 Results of cluster analysis (*k*-means). For each site, a number of groups (Gr.) equal to the number of tree species was selected

								Distances between groups				
Site and groups	Members of groups							Gr.1	Gr.2	Gr.3	Gr.4	Gr.5
Finland												
Gr.1	PA1	PA2						0.0				
Gr.2	BP1	BP2	BP3	PA3				6.8	0.0			
Gr.3	PS1	PS2	PS3					14.6	19.9	0.0		
Poland												
Gr.1	PA1	PA2	PA3	PA4	PA5	QR2		0.0				
Gr.2	BP1	BP3	BP4	QR1	QR3	QR4	QR5	11.3	0.0			
Gr.3	BP2	BP4						13.3	4.4	0.0		
Gr.4	PS1	PS2	PS3	PS4	PS5			14.9	19.7	23.8	0.0	
Gr.5	CB1	CB2	CB3	CB4	CB5			21.7	12.4	8.6	32.2	0.0
Germany												
Gr.1	FE1	FE3	FE4	QRP3				0.0				
Gr.2	FE2	QRP2	QRP4					7.9	0.0			
Gr.3	AP4	FS1	FS2	FS3	FS4	PA2		13.7	8.4	0.0		
Gr.4	QRP1							14.0	6.1	7.9	0.0	
Gr.5	AP2	AP3	PA1	PA3	PA4			9.4	8.0	5.9	11.4	0.0
Romania												
Gr.1	AP1	AP2	AP3	AP4				0.0				
Gr.2	AA2	PA1	PA2	PA3				5.7	0.0			
Gr.3	FS1	FS2	FS3	FS4				4.4	10.1	0.0		
Gr.4	AA1	AA3	AA4	PA4				11.0	5.2	15.3	0.0	
Italy												
Gr.1	OC1	OC2	OC3	OC4				0.0				
Gr.2	CS1	CS3	QI2	QI3	QI4	QP3		6.6	0.0			
Gr.3	QC1							7.3	3.8	0.0		
Gr.4	CS2	CS4	QC4	QI1	QP4			10.9	4.3	6.5	0.0	
Gr.5	QC2	QC3	QP1	QP2				13.4	7.0	7.1	3.9	0.0
Spain												
Gr.1	PN1	PN2	PN3	QI1	QI2	QI3		0.0				
Gr.2	PN4	PS4	QI4					6.6	0.0			
Gr.3	PS1	PS2	PS3					14.4	7.8	0.0		
Gr.4	QF1	QF2	QF3	QF4				6.3	12.6	20.4	0.0	

The abbreviation of the name of the tree species is shown (see Fig. 2). The number after the species abbreviation indicates the level of the tree species mixture (from one species to five species).

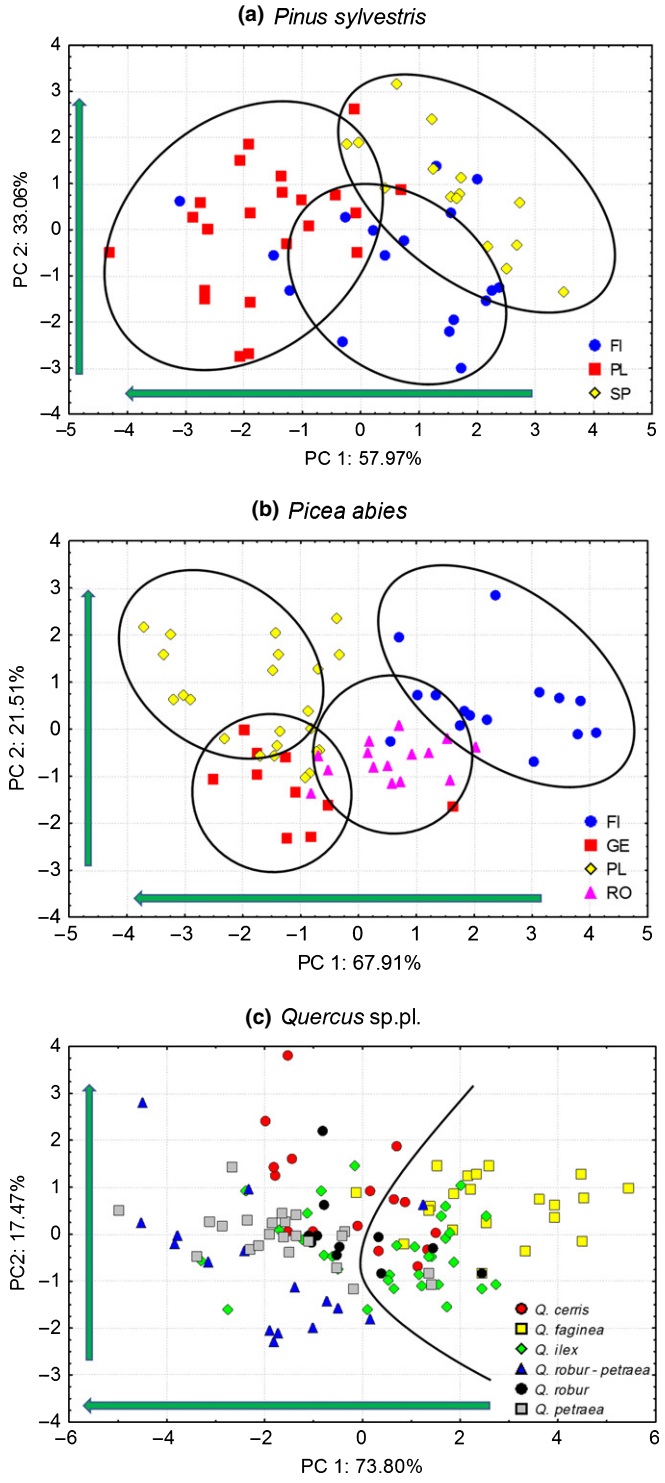

Fig. 5 Representation of the principal component analysis (PCA) space (cases) of the chlorophyll *a* fluorescence (ChlF) parameters, defined by the first two components (PC1 and PC2), in (a) *Pinus sylvestris*, (b) *Picea abies* and (c) *Quercus* sp. pl. species. FI, Finland; PL, Poland; SP, Spain; GE, Germany; RO, Romania. The loadings of the components with values > 0.6 are reported in Supporting Information Table S7 for each species. The main homogeneous clusters are identified graphically (black open circles). The direction of the green arrows indicates the increase in the value of the ChlF parameters. In (c), the black line indicates the separation between Mediterranean oak species from the central European species.

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Author contributions

M.P., V.H., W.B. and F.B. designed the research; M.P., V.H., W.B. and F.B performed the research; M.P., V.H. and F.B. analysed the data; M.P., V.H., W.B. and F.B. wrote the first version of the manuscript; M.P., V.H., W.B., F.B., H.B., B.J., F.V. and I.D. contributed substantially to revisions.

References

Adams WW III, Demmig-Adams B. 2004. Chlorophyll fluorescence as a tool to monitor plant response to the environment. In: Papageorgiou GC, Govindjee, eds. *Advances in photosynthesis and respiration series. Chlorophyll fluorescence: a signature of photosynthesis.* Dordrecht, the Netherlands: Springer, 583–604.

Aiken J, Hardman-Mountford NJ, Barlow R, Fishwick J, Hirata T, Smyth T. 2008. Functional links between bioenergetics and bio-optical traits of phytoplankton taxonomic groups: an overarching hypothesis with applications for ocean colour remote sensing. *Journal of Plankton Research* **30**: 165–181.

Aphalo PJ, Vapaavuori EM, de la Rosa TM, Lehto T. 2009. Does supplemental UV-B radiation affect gas exchange and RuBisCO activity of *Betula pendula* Roth. seedlings grown in forest soil under greenhouse conditions? *Plant Ecology & Diversity* **2**: 37–43.

Baeten L, Verheyen K, Wirth C, Bruelheide H, Bussotti F, Finér L, Jaroszewicz B, Selvi F, Valladares F, Allan E *et al.* 2013. A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. *Perspectives in Plant Ecology, Evolution and Systematics* **15**: 281–291.

Baker NR, Rosenqvist E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: examination of future possibilities. *Journal of Experimental Botany* **55**: 1607–1621.

Baraloto C, Hardy OJ, Paine CET, Dexter KG, Cruaud C, Dunning LT, Gonzalez M-A, Molino J-F, Sabatier D, Savolainen V *et al.* 2012. Using functional traits and phylogenetic trees to examine the assembly of tropical tree communities. *Journal of Ecology* **100**: 690–701.

Bates M, Maechler MM, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bréda NJJ. 2003. Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. *Journal of Experimental Botany* **54**: 2403–2417.

Bruschi P, Vendramin GG, Bussotti F, Grossoni P. 2003. Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae). *Annals of Botany* **91**: 707–716.

Bussotti F, Borghini F, Celesti C, Leonzio C, Bruschi P. 2000. Leaf morphology and macronutrients in broadleaved trees in Central Italy. *Trees* **14**: 361–368.

Bussotti F, Pollastrini M. 2015. Evaluation of leaf features in forest trees: methods, techniques, obtainable information and limits. *Ecological Indicators* **52**: 219–230.

Bussotti F, Pollastrini M, Cascio C, Desotgiu R, Gerosa G, Marzuoli R, Nali C, Lorenzini G, Pellegrini E, Carucci MG *et al.* 2011. Conclusive remarks. Reliability and comparability of chlorophyll fluorescence data from several field teams. *Environmental and Experimental Botany* **73**: 116–119.

Bussotti F, Pollastrini M, Holland V, Brüggemann W. 2015. Functional traits and adaptive capacity of European forests to climate change. *Environmental and Experimental Botany* **111**: 91–113.

Čaňová I, Ďurkovič J, Hladká D. 2008. Stomatal and chlorophyll fluorescence characteristics in European beech cultivars during leaf development. *Biologia Plantarum* **52**: 577–581.

Cascio C, Schaub M, Novak K, Desotgiu R, Bussotti F, Strasser RJ. 2010. Foliar responses to ozone of *Fagus sylvatica* L. seedlings grown in shaded and in full sunlight conditions. *Environmental and Experimental Botany* **68**: 188–197.

Ceppi MG, Oukarroum A, Çiçek N, Strasser RJ, Schansker G. 2012. The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves: a study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. *Physiologia Plantarum* **144**: 277–288.

Chave J, Coomes DA, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

Desotgiu R, Cascio C, Pollastrini M, Gerosa G, Marzuoli R, Bussotti F. 2012. Short and long term photosynthetic adjustments in sun and shade leaves of *Fagus sylvatica* L., investigated with the fluorescence transient (FT) analysis. *Plant Biosystems* **146**: 206–216.

Desotgiu R, Cascio C, Pollastrini M, Gerosa G, Marzuoli R, Bussotti F. 2013. Responses to ozone on *Populus* "Oxford" clone in an open top chamber experiment assessed before sunrise and in full sunlight. *Photosynthetica* **51**: 267–280.

Drinovec L, Flander-Putrle V, Knez M, Beran A, Berden-Zrimec M. 2011. Discrimination of marine algal taxonomic groups using delayed fluorescence spectroscopy. *Environmental and Experimental Botany* **73**: 42–48.

Drössler L, Övergaard R, Ekö PM, Gemmel P, Böhlenius H. 2015. Early development of pure and mixed tree species plantations in Snogeholm, southern Sweden. *Scandinavian Journal of Forest Research* **30**: 304–316.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121–137.

Forrester DI, Lancaster K, Collopy JJ, Warren CR, Tausz M. 2012. Photosynthetic capacity of *Eucalyptus globulus* is higher when grown in mixture with *Acacia mearnsii*. *Trees – Structure and Functions* **26**: 1203–1213.

Gayler S, Grams TEE, Kozovits AR, Winkler JB, Luedemann G, Priesack E. 2006. Analysis of competition effects in mono- and mixed cultures of juvenile beech and spruce by means of the plant growth simulation model PLATHO. *Plant Biology* **8**: 503–514.

Gessler A, Schrempp S, Matzarakis A, Mayer H, Rennenberg H, Adams M. 2001. Radiation modifies the effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). *New Phytologist* **150**: 653–664.

Gielen B, Löw M, Deckmyn G, Metzger U, Franck F, Heerdt C, Matyssek R, Valcke R, Ceulemans R. 2007. Chronic ozone exposure affects leaf senescence of adult beech trees: a chlorophyll fluorescence approach. *Journal of Experimental Botany* **58**: 785–795.

Gilmore AM. 2004. Excess light stress: probing excitation dissipation mechanisms through global analysis of time- and wavelength-resolved chlorophyll a fluorescence. In: Papageorgiou GC, Govindjee, eds. *Advances in photosynthesis and respiration series. Chlorophyll fluorescence: a signature of photosynthesis*. Dordrecht, the Netherlands: Springer, 555–581.

Gratani L, Foti I. 1998. Estimating forest structure and shade tolerance of the species in a mixed deciduous broad-leaved forest in Abruzzo, Italy. *Annales Botanici Fennici* **35**: 75–83.

Grossiord C, Gessler A, Granier A, Pollastrini M, Bussotti F, Bonal D. 2014a. Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. *Forest Ecology and Management* **318**: 54–61.

Grossiord C, Granier A, Ratcliffe S, Bouriaud O, Bruelheide H, Chećko E, Forrester DI, Dawud SM, Finér L, Pollastrini M *et al.* 2014b. Tree diversity does not always improve resistance of forest ecosystems to drought. *Proceedings of the National Academy of Sciences, USA* **111**: 14812–14815.

Hallik L, Niinemets Ü, Kull O. 2012. Photosynthetic acclimation to light in woody and herbaceous species: a comparison of leaf structure, pigment content and chlorophyll fluorescence characteristics measured in the field. *Plant Biology* **14**: 88–99.

He Q, Bertness MD. 2014. Extreme stresses, niches, and positive species interactions along stress gradients. *Ecology* **95**: 1437–1443.

Ishii H, Asano S. 2010. The role of crown architecture, leaf phenology and photosynthetic activity in promoting complementary use of light among coexisting species in temperate forests. *Ecological Research* **25**: 715–722.

Kaitaniemi P, Lintunen A. 2010. Neighbor identity and competition influence tree growth in Scots pine, Siberian larch and silver birch. *Annals of Forest Science* **67**: 604–611.

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dąbrowski P *et al.* 2014. Frequently asked questions about chlorophyll fluorescence: practical issues. *Photosynthesis Research* **122**: 121–158.

Ke B. 2001. *Photosynthesis. Photobiochemistry and photobiophysics*. Dordrecht, the Netherlands: Kluwer Academic Publisher.

Kirova M, Ceppi G, Chernev P, Goltsev V, Strasser R. 2014. Using artificial neural networks for plant taxonomic determination based on chlorophyll fluorescence induction curves. *Biotechnology & Biotechnological Equipment* **23**: 941–945.

Kozovits AR, Matyssek R, Winkler JB, Göttlein A, Blaschke H, Grams TEE. 2005. Aboveground space sequestration determines competitive success in juvenile beech and spruce trees. *New Phytologist* **167**: 181–196.

Krause GH, Koroleva OY, Dalling JW, Winter K. 2001. Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. *Plant, Cell & Environment* **24**: 1345–1352.

Krause GH, Weis E. 1991. Chlorophyll fluorescence and photosynthesis. The basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 313–349.

Kreuzwieser J, Gessler A. 2010. Global climate change and tree nutrition: influence of water availability. *Tree Physiology* **30**: 1221–1234.

Legner N, Fleck S, Leuschner C. 2014. Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance. *Trees* **28**: 263–280.

Li Y-G, Li LH, Jiang G-M, Niu SL, Liu MZ, Gao LM, Peng Y, Jiang CD. 2004. Traits of chlorophyll fluorescence in 99 plant species from the sparse-elm grassland in Hunshandak Sandland. *Photosynthetica* **42**: 243–249.

Luoma S. 1997. Geographical pattern in photosynthetic light response of *Pinus sylvestris* in Europe. *Functional Ecology* **11**: 273–281.

MacQueen JB. 1967. Some methods for classification and analysis of multivariate observations. In: *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability. Volume 1: Statistics*. Berkeley, CA, USA: University of California Press, 281–297.

Maire V, Wright I, Prentice IC, Batjes NH, Bhaskar R, van Bodegom PM, Cornwell WK, Ellsworth D, Niinemets Ü, Ordonez A *et al.* 2015. Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecology and Biogeography* **24**: 706–717.

Mänd P, Hallik L, Peñuelas J, Kull O. 2012. Electron transport efficiency at opposite leaf sides: effect of vertical distribution of leaf angle, structure, chlorophyll content and species in a forest canopy. *Tree Physiology* **33**: 202–210.

Maxwell C, Johnson GN. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* **51**: 659–668.

Meng T-T, Wang H, Harrison SP, Prentice IC, Ni J, Wang G. 2015. Responses of leaf traits to climatic gradients: adaptive variation versus compositional shifts. *Biogeosciences* **12**: 5339–5352.

Meroni M, Rossini M, Guanter L, Alonso L, Rascher U, Colombo R, Moreno J. 2009. Remote sensing of solar-induced chlorophyll fluorescence: review of methods and applications. *Remote Sensing of Environment* **113**: 2037–2051.

de Miguel M, Cabezas J-A, de María N, Sánchez-Gómez D, Guevara M-Á, Vélez M-D, Sáez-Laguna E, Díaz L-M, Mancha J-A, Barbero M-C *et al.* 2014. Genetic control of functional traits related to photosynthesis and water use efficiency in *Pinus pinaster* Ait. drought response: integration of genome annotation, allele association and QTL detection for candidate gene identification. *BMC Genomics* **15**: 464.

Moles AT, Perkins SE, Laffan SW, Flores-Moreno H, Awasthy M, Tindall ML, Sack L, Pitman A, Kattge J, Aarssen LW. 2014. Which is a better predictor of

plant traits: temperature or precipitation? *Journal of Vegetation Science* **25**: 1167–1180.

Morecroft FD, Roberts JM. 1999. Photosynthesis and stomatal conductance of mature canopy oak (*Quercus robur*) and sycamore (*Acer pseudoplatanus*) trees throughout the growing season. *Functional Ecology* **13**: 332–342.

Murchie EH, Lawson T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* **64**: 3983–3998.

Niederberger J, Todt B, Boča A, Nitschke R, Kohler M, Kühn P, Bauhus J. 2015. Use of near-infrared spectroscopy to assess phosphorus fractions of different plant availability in forest soils. *Biogeosciences* **12**: 3415–3428.

Niinemets Ü, Keenan TF, Hallik L. 2015. A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types. *New Phytologist* **205**: 973–993.

Niinemets Ü, Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. *Ecological Monographs* **74**: 521–547.

Paillotin G. 1976. Movement of excitations in the photosynthesis domain of photosystem II complex. *Journal of Theoretical Biology* **58**: 237–252.

Papageorgiou GC, Govindjee eds. 2004. *Advances in photosynthesis and respiration series. Chlorophyll fluorescence: a signature of photosynthesis.* Dordrecht, the Netherlands: Springer.

Peguero-Pina JJ, Sancho-Knapik D, Morales F, Flexas J, Gil-Pelegrín E. 2009. Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. *Functional Plant Biology* **36**: 453–462.

Peñuelas J, Filella I, Gamon JA. 1995. Assessment of photosynthetic radiation use efficiency with spectral reflectance. *New Phytologist* **131**: 291–296.

Pollastrini M, Holland V, Brüggemann W, Koricheva J, Jussila I, Scherer-Lorenzen M, Berger S, Bussotti F. 2014. Interactions and competition processes among tree species in young experimental mixed forests, assessed with chlorophyll fluorescence and leaf morphology. *Plant Biology* **16**: 323–331.

R Core Team. 2014. *R: a language and environment for statistical computing. v.3.1.2.* Vienna, Austria: R Foundation for Statistical Computing. URL http://www.R-project.org/ [accessed 22 January 2016].

Ribeiro RV, Souza GM, Oliveira RF, Machado EC. 2005. Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. *Revista Brasileira de Botânica* **28**: 149–161.

Rousseau C, Belin E, Bove E, Rousseau D, Fabre F, Berruyer R, Guillaumes J, Manceau C, Jacques M-A, Boureau T. 2013. High throughput quantitative phenotyping of plant resistance using chlorophyll fluorescence image analysis. *Plant Methods* **9**: 1–17.

Rozema J, Boelen P, Blokker P. 2005. Depletion of stratospheric ozone over the Antarctic and Arctic: responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview. *Environmental Pollution* **137**: 428–442.

Salvatori E, Fusaro L, Gottardini E, Pollastrini M, Goltsev V, Strasser RJ, Bussotti F. 2014. Plant stress analysis: application of prompt, delayed chlorophyll fluorescence and 820 nm modulated reflectance. Insights from independent experiments. *Plant Physiology and Biochemistry* **85**: 105–113.

Sánchez-Gómez D, Valladares F, Zavala MA. 2006. Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species. *Tree Physiology* **26**: 1425–1433.

Sardans J, Janssens IA, Alonso R, Veresoglou SD, Rilig MC, Sanders TGM, Carnicer J, Filella J, Farré-Armengol G, Peñuelas J. 2015. Foliar elemental composition of European forest tree species associated with evolutionary traits and present environmental and competitive conditions. *Global Ecology and Biogeography* **24**: 240–255.

Serbin SP, Dillaway DN, Kruger EL, Townsend PA. 2012. Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of Experimental Botany* **63**: 489–502.

Shannon CE. 1948. A mathematical theory of communication. *The Bell System Technical Journal* **27**: 379–423.

Sobrado MA. 2008. Leaf and photosynthetic characteristics of pioneer and forest species in tropical montane habitats. *Photosynthetica* **46**: 604–610.

Sokal RR, Rohlf FJ. 1995. *Biometry: the principles and practice of statistics in biological research*, 3rd edn. New York, NY, USA: WH Freeman.

Sperlich D, Chang CT, Peñuelas J, Gracia C, Sabaté S. 2015. Seasonal variability of foliar photosynthetic and morphological traits and drought impacts in a Mediterranean mixed forest. *Tree Physiology* **35**: 501–520.

Strasser RJ, Srivastava A, Tsimilli-Michael M. 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P, eds. *Probing photosynthesis: mechanisms, regulation and adaptation*. London, UK: Taylor & Francis, 445–483.

Strasser RJ, Tsimilli-Michael M, Srivastava A. 2004. Analysis of the fluorescence transient. In: Papageorgiou GC, Govindjee, eds. *Advances in photosynthesis and respiration series. Chlorophyll fluorescence: a signature of photosynthesis.* Dordrecht, the Netherlands: Springer, 321–362.

Swenson NG, Enquist BJ. 2007. Ecological and evolutionary determinants of a key plant functional trait: wood density and its communitywide variation across latitude and elevation. *American Journal of Botany* **94**: 451–459.

Tsimilli-Michael M, Strasser RJ. 2008a. Experimental resolution and theoretical complexity determine the amount of information extractable from the chlorophyll fluorescence transient OJIP. In: Allen JF, Gantt E, Golbeck JH, Osmond B, eds. *Photosynthesis: energy from the Sun*. 14th International Congress on Photosynthesis, Glasgow 2007. Dordrecht, the Netherlands: Springer, 697–701.

Tsimilli-Michael M, Strasser RJ. 2008b. *In vivo* assessment of stress impact on plants' vitality: applications in detecting and evaluating the beneficial role of mycorrhization on host plants. In: Varma A, ed. *Mycorrhiza: state of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics, vol. 3*. Dordrecht, the Netherlands: Springer, 679–703.

Tsonev T, Velikova V, Georgieva K, Hyde PF, Jones HG. 2003. Low temperature enhances photosynthetic down-regulation in French bean (*Phaseolus vulgaris* L.) plants. *Annals of Botany* **91**: 343–352.

Urban O, Košvancová M, Marek MV, Lichtenthaler HK. 2007. Induction of photosynthesis and importance of limitations during the induction phase in sun and shade leaves of five ecologically contrasting tree species from the temperate zone. *Tree Physiology* **27**: 1207–1215.

Valladares F, Sánchez-Gómez D. 2006. Ecophysiological traits associated with drought in Mediterranean tree seedlings: individual responses versus interspecific trends in eleven species. *Plant Biology* **8**: 688–697.

Walters RG. 2005. Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany* **56**: 435–447.

Weng J-H, Liao T-S, Hwang M-Y, Chung C-C, Lin C-P, Chu C-H. 2006. Seasonal variation in photosystem II efficiency and photochemical reflectance index of evergreen trees and perennial grasses growing at low and high elevations in subtropical Taiwan. *Tree Physiology* **26**: 1097–1104.

Wilhelm C, Wirth C. 2015. Physiodiversity – new tools allow physiologist to embrace biodiversity and reconstruct the evolutionary of "physiologies"? *Journal of Plant Physiology* **172**: 1–3.

Yang X, Tang J, Mustard JF, Lee J-E, Rossini M, Joiner J, Munger JW, Kornfeld A, Richardson AD. 2015. Solar-induced chlorophyll fluorescence that correlates with canopy photosynthesis on diurnal and seasonal scales in a temperate deciduous forest. *Geophysical Research Letters* **42**: 2977–2987.

Zanne AE, Lopez-Gonzalez G, Coomes DA, Ilic J, Jansen S, Lewis SL, Miller RB, Swenson NG, Wiemann MC, Chave J. 2009. *Data from: towards a worldwide wood economics spectrum.* Dryad Digital Repository. [WWW document] URL http://dx.doi.org/10.5061/dryad.234 [accessed 10 January 2016].

Zarco-Tejada PJ, Catalina A, González MR, Martín P. 2013. Relationships between net photosynthesis and steady-state chlorophyll fluorescence retrieved from airborne hyperspectral imagery. *Remote Sensing of Environment* **136**: 247–258.

Zhao X, Li Y, Zheng M, Bian X, Liu M, Sun Y, Jiang J, Wang F, Li S, Cui Y *et al.* 2015. Comparative analysis of growth and photosynthetic characteristics of (*Populus simonii* × *P. nigra*) × (*P. nigra* × *P. simonii*) hybrid clones of different ploidies. *PLoS ONE* **10**: e0119259.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Map of the sampling sites.

Fig. S2 Relationships between PI_{ABS} and Shannon index.

Fig. S3 PCA analysis of ChlF parameters and functional traits in coniferous and broadleaf species.

Table S1 List of chlorophyll *a* fluorescence (ChlF) parameters

Table S2 Chlorophyll *a* fluorescence (ChlF) parameters in conifers and broadleaf species

Table S3 Output of linear models for F_V/F_M and ΔV_{IP} and correlation between F_V/F_M and ΔV_{IP} and environmental factors

Table S4 Effects of species and mixture on PI_{ABS}

Table S5 Effects of species and mixture on PI_{TOT}

Table S6 Principal component analysis (PCA) of the chlorophyll *a* fluorescence (ChlF) parameters

Table S7 Principal component analysis (PCA) of the chlorophyll *a* fluorescence (ChlF) parameters for *Pinus sylvestris*, *Picea abies* and *Quercus* sp. pl.

Table S8 Variability of the chlorophyll *a* fluorescence (ChlF) parameters per site and per species

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