Research paper

Tree diversity affects chlorophyll *a* fluorescence and other leaf traits of tree species in a boreal forest

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An assemblage of tree species with different crown properties creates heterogeneous environments at the canopy level. Changes of functional leaf traits are expected, especially those related to light interception and photosynthesis. Chlorophyll *a* fluorescence (ChlF) properties in dark-adapted leaves, specific leaf area, leaf nitrogen content (N) and carbon isotope composition ($\delta^{13}C$) were measured on *Picea abies* (L.) H.Karst., *Pinus sylvestris* L. and *Betula pendula* Roth. in monospecific and mixed boreal forests in Europe, in order to test whether they were affected by stand species richness and composition. Photosynthetic efficiency, assessed by induced emission of leaf ChlF, was positively influenced in *B. pendula* by species richness, whereas *P. abies* showed higher photosynthetic efficiency in monospecific stands. *Pinus sylvestris* had different responses when it coexisted with *P. abies* or *B. pendula*. The presence of *B. pendula*, but not of *P. abies*, in the forest had a positive effect on the efficiency of photosynthetic electron transport and N in *P. sylvestris* needles, and the photosynthetic responses were positively correlated with an increase of leaf $\delta^{13}C$. These effects on *P. sylvestris* may be related to high light availability at the canopy level due to the less dense canopy of *B. pendula*. The different light requirements of coexisting species was the most important factor affecting the distribution of foliage in the canopy, driving the physiological responses of the mixed species. Future research directions claim to enhance the informative potential of the methods to analyse the responses of pure and mixed forests to environmental factors, including a broader set of plant species' functional traits and physiological responses.

Keywords: FunDivEUROPE, mixed forest, species composition, species richness.

Introduction

Mixed forests are believed to be more productive and resilient to environmental stress factors than monocultures (Frivold and Frank 2002, Nynynen et al. 2011, Jucker et al. 2014, Ruiz-Benito et al. 2014, Drössler et al. 2015, Liang et al. 2016). This assumption, however, is not always true, since tree species composition of stands may induce different physiological responses (Rothe et al. 2003, Gayler et al. 2006, Kaitaniemi and Lintunen 2010, Grossiord et al. 2014a) because of various competition and facilitation mechanisms. Below ground competition may provoke greater drought stress (Grossiord et al. 2014b) and nutrient deficiency (Kreuzwieser and Gessler 2010) for some species. Conversely, an increase in nitrogen (N) availability can occur when N-fixing species are present in the community (Forrester et al. 2012).

Above ground competition affects canopy space filling and light availability, with consequent deficiency or excess of light interception by the crowns (Kozovits et al. 2005, Ishii and Asano 2010, Pollastrini et al. 2014, Jucker et al. 2015).

The physiological responses of trees, induced by tree diversity, are expected to be reflected in changes of functional leaf traits, with special reference to those associated with light interception and use in photosynthetic processes, as well as in the responses to drought stress (Bussotti and Pollastrini 2015a). Chlorophyll *a* fluorescence (ChlF) parameters describe the efficiency and performance of photosystems in the photosynthetic apparatus. Among the traits associated with photosynthesis, specific leaf area (SLA) and leaf N content (mass-based N) have high informative potential and can constitute a link between terrestrial and remote sensing surveys (Meroni et al. 2009, Serbin et al. 2012). Carbon isotope composition ($\delta^{13}C$), on the other hand, is a response parameter in relation to water stress (Grossiord et al. 2014a).

Chlorophyll *a* fluorescence (ChlF) analysis is used in applied sciences and in agricultural and environmental research to assess plant responses to a variety of environmental stress factors (Maxwell and Johnson 2000, Strasser et al. 2004, Murchie and Lawson 2013, Kalaji et al. 2014, Salvatori et al. 2014, Fusaro et al. 2016). Although ChlF analysis is considered a useful tool to assess the vitality of young trees in forestry and urban plantations (Bussotti et al. 2010), it is rarely used to study the photochemical properties of mature tall trees in forests or plantations (Franco et al. 2005, Bussotti and Pollastrini 2015b, Koprowski et al. 2015, Gottardini et al. 2016, Pollastrini et al. 2016a, 2016b). The portable instruments for measuring ChlF (fluorimeters) that are currently available combine manoeuvrability with rapidity of data acquisition. The use of these instruments allows the application of ChlF techniques in large-scale assessment of forest tree health and vitality.

Specific leaf area (SLA), that is, the projected leaf area per unit leaf dry mass, indicates the acclimation of leaves to irradiance: plants grown under high light intensity, generally, have thick leaves with low SLA. Thick leaves have a higher number of chloroplasts than thin leaves. Furthermore, in leaves grown at high light intensity the chloroplasts present lower and narrow thylakoidal grana stacks, with higher chlorophyll *a/b* ratio and less abundance of light-harversting complex associated with PSII complexes (Lichtenthaler et al. 1981). These structural and chemical modifications of the chloroplasts in response to light environment affect the photosynthetic capacity of leaves. Therefore, low SLA enhances photosynthetic capacity per unit leaf area (Evans and Poorter 2001). Low SLA is thought to be associated with high N content, which is a proxy for the photosynthetic capacity of leaves (Niinemets et al. 2006). However, the relationship between photosynthetic capacity of plants, SLA and N is not always clear, as shown by Gulías et al. (2003) in a comparison between endemic and non-endemic Balearic species. Foliar N is considered a proxy for chlorophyll concentration (Van den Berg and Perkins 2004) and is associated with the efficiency of the photosynthetic function.

Foliar $\delta^{13}C$ is a key parameter for exploring carbon sequestration and strategies for efficient water use of trees under water stress conditions (Farquhar et al. 1982). The increase of foliar $\delta^{13}C$ indicates a decrease in the chloroplastic CO_2 concentration, which might be due to high assimilation rates and thus a high consumption of CO_2, or to low stomatal conductance and thus restriction of resupply of CO_2 to the photosynthetic apparatus (Farquhar et al. 1982, Francey and Farquhar 1982). The parameter $\delta^{13}C$ has been shown to be affected by both the availability of water and its effect on stomatal conductance, and by radiation via its effect on photosynthesis (Gessler et al. 2001).

Many studies deal with the variation of morphological and photosynthetic characteristics of leaves within the canopy of a tree in relation to the light gradient (Niinemets 1998, Hallik et al. 2012, Niinemets et al. 2015). In mixed forests, the assemblage of tree species with different crown properties (light requirements, phenology, branching models and distribution of foliage) creates heterogeneous environments at the canopy level in relation to light availability, wind speed and water vapour pressure, thus affecting the photosynthesis and transpiration of trees. As a consequence, beside the position of leaves in the crown, foliar traits can be influenced by the interaction between crowns of different tree species. Few studies, however, have investigated the relationships between ChlF parameters and leaf traits in tree species in relation to stand characteristics, such as species richness and composition (Reich et al. 1992, Russell et al. 2014, Pollastrini et al. 2016b).

Our aim in this study was to test whether ChlF parameters and leaf traits of forest species associated with photosynthetic properties and water-use efficiency, this latest estimated by means of leaf $\delta^{13}C$, were affected by species diversity (species richness and/or composition) of the stands in a boreal forest. The hypothesis to be investigated is that in homogeneous forest stands (as far as soil and climate conditions are concerned), specific foliar attributes at the canopy level are influenced by the mutual or antagonistic relationships established between the crowns of the species, differing in shape, size and foliage density, that compose the stand.

Materials and methods

Sampling site and sampling procedures

The study was conducted in a boreal forest located in North Karelia (62° N, 29° E), in Finland, within the exploratory platform of the FP7 FunDivEUROPE project (Baeten et al. 2013, Jucker et al. 2014). The exploratory design included three tree species: *Betula pendula* Roth., *Picea abies* (L.) H.Karst. and *Pinus sylvestris* L., distributed in 28 plots (30 × 30 m wide), with the same number of replicates (4) for each type of tree species composition: monospecific plots (monoculture) of each species, plots with two species (*P. abies* + *P. sylvestris*; *P. abies* + *B. pendula*; *P. sylvestris* + *B. pendula*) and plots with all three

species (the detailed characteristics of the plots are reported in Table S1 available as Supplementary Data at *Tree Physiology* Online). The trees had the same age (40–50 years old), with a mean height of 12–15 m. The mean basal area (BA) of the plots was 22.70 m^2 ha^{-1} ($\pm$5.13 SD) and the mean leaf area index (LAI) was 2.77 m^2 m^{-2} ($\pm$0.770 SD). The forest stands had flat topography, with deep soils (mean 78 cm) and soil nutrient availability (mean soil C/N ratio 27.70). The elevation was between 100 and 150 m above sea level. The mean annual temperature and the mean annual precipitation of the study site were, respectively, 2.1 °C and 650 mm. The daily solar radiation was 14.907 MJ m^{-2} day^{-1} (daily average for the period April–September 2009–13, data from AGRI4CAST, http://mars.jrc.ec.europa.eu/mars). The plots were chosen to minimize the differences in soil conditions, and nutrient and water availability. According to the experimental design (Baeten et al. 2013), differences among plots were related only to the tree species composition.

In each plot, six to nine dominant and co-dominant trees were selected according to the species richness of the dominant tree species. Six trees were selected in the monocultures and three trees per species in the other species mixtures. The selection was randomly done among the dominant and co-dominant trees in each stand, that is, considering the largest diameters at breast height. South-facing branches of 40–50 cm in length were collected from the upper third of each selected tree's crown using extension loppers. The sampling was done in the first and second weeks of August 2012. After sampling, the branches were immediately placed in hermetic plastic bags, where the dark-adaptation period, necessary before taking the ChlF measurements, commenced. The branches in the plastic bags were humidified to avoid leaf dehydration. The bags were then kept at constant temperature in an insulated box until reaching the laboratory the same day.

Leaf area index of the plots was measured in the same field campaign. Five measurements of LAI in each plot were recorded early in the morning (shortly after sunrise) or late in the evening (shortly before sunset) in order to work in the presence of diffuse solar radiation. A Plant Canopy Analyzer LAI-2000 (Li-Cor, Inc., Lincoln, NE, USA) was used. The light penetration across the canopy in the stands was estimated by means of the diffuse non-interceptance index (DIFN), measured by means of LAI-2000 at the same time of the LAI measures in each plot. The DIFN indicates the fraction of sky that is not intercepted by foliage, providing also information on the canopy structure. The DIFN has a value range between zero, which corresponds to dense canopies, with a high amount of intercepted light, and one, which indicates open and/or narrow canopies, with lower light interception.

ChlF measurements

Chlorophyll *a* fluorescence measurements were done on 16 leaves for each tree using a Handy PEA fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd, Pentney, Norfolk, UK) in a dark room after 4–5 h of dark adaptation. In coniferous species, current-year needles were measured. A long dark-adaptation period was necessary to remove both dynamic and chronic leaf photoinhibition (Werner et al. 2002, Desotgiu et al. 2012) and allow the leaves to reach standard conditions after solar radiation exposure (see also Čepl et al. 2016) The effectiveness of this protocol was tested before the sampling campaign (Pollastrini et al. 2016*a*, 2016*c*). Previous studies (Pollastrini et al. 2016*a*) demonstrated that there is coherence between the ChlF parameters measured in the upper third of the canopy and the remaining foliage; therefore, the data here provided can be assumed as indicative of the photosynthetic efficiency of the whole tree.

The emission of ChlF was induced in dark-adapted leaves through one pulse of red light (wavelength of 650 nm and intensity of 3500 μmol m^{-2} s^{-1}) for 1 s. Then, the photochemical properties of the leaves and needles of each sampled tree were assessed, analysing the fluorescence induction curves, called OJIP-ChlF transients. Plotted on a logarithmic time scale, the ChlF transient shows a polyphasic pattern (Figure S1 available as Supplementary Data at *Tree Physiology* Online). The different time steps of this polyphasic transient are labelled as O (20–50 μs), J (2 ms), I (30 ms) and P (peak). The latter indicates the highest fluorescence intensity (F_M), when all the reaction centres of photosystem II (PSII) are reduced by photosynthetic saturating light. For reviews of the theoretical background of the method used to analyse the structure and functionality of PSII (the JIP test: a quantitative analysis of the fluorescence rise kinetics at specific timesteps) and the ChlF parameters obtained from the ChlF induction curve (fast kinetics) of dark-adapted leaves, see Strasser et al. (2004) and Kalaji et al. (2014). Chlorophyll *a* fluorescence parameters used in this study were F_V/F_M, ABS/RC, K-band, Ψ_{Eo}, ΔV_{IP} and the performance indices (PI$_{ABS}$ and PI$_{TOT}$) (formulae are provided in Table S2 available as Supplementary Data at *Tree Physiology* Online). F_V/F_M is the maximum quantum yield for primary photochemistry of a dark-adapted sample; ABS/RC is the size of the antenna of PSII; K-band is the relative variable fluorescence at 300 μs, which expresses the opening of the electron transport to non-water electrons, competing, therefore, with the water donation from the oxygen-evolving system; Ψ_{Eo} is the probability of an electron to reduce the primary quinone acceptor (Q$_A$) and to move into the electron transport chain (ETC) beyond PSII; ΔV_{IP} represents the amplitude of the relative contribution of the I-to-P rise to the OJIP transient, is an indicator of the abundance of photosystem I (PSI) with respect to PSII and is related to the ETC beyond PSI (Ceppi et al. 2012). Finally, the 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 PSI end acceptors (PI$_{TOT}$). The dark fluorescence describes the 'potential' capability to do work by the photons.

Although there are no direct relationships between the capability to move electrons into the ETC and the net photosynthesis (P_N), several papers (Genty et al. 1989, Edwards and Baker 1993, Freyer et al. 1998, Maxwell and Johnson 2000) suggest that, in absence of stressful condition for plants, most of the end acceptors of electrons in the ETC feed the Calvin–Benson cycle. Chlorophyll *a* fluorescence signature is therefore used as proxy for plant growth, in terms of gross primary productivity, in remote sensing studies (Zarco-Tejada et al. 2013).

Leaf traits: nitrogen content, carbon isotope composition and SLA

Twenty fully expanded leaves and needles were sampled from the branches collected for the ChlF measurements in each sampled tree for the analysis of carbon isotope composition (δ^{13}C, ‰) and N content (mass-based N, %). After oven-drying at 60 °C for 48 h, the foliar samples of each species were finely ground.

For analysis of δ^{13}C, ~1 mg of the dried, powdered material from each sample was placed into tin capsules. The analyses were performed using an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). The isotopic composition of the sample is reported in delta notation (δ^{13}C) relative to the Vienna Pee Dee Belemnite standard. To determine the leaf N content, 2.5 mg of the dry sample was used. The samples were analysed using near-infrared spectroscopy, as described by Niederberger et al. (2015). Leaf N content and δ^{13}C at species level for each stand were calculated as average values of three to six trees per species sampled in the stand.

Specific leaf area is the one-sided area of a fresh leaf, divided by its oven-dried mass ($m^2\,kg^{-1}$). We harvested two south-facing branches from upper third of the canopy from 10 individuals per species in each plot, previously randomly selected. Then, we took 5 leaves/needles from each branch, making a total of 10 leaves per tree. Fully expanded and hardened leaves/needles were sampled. In both cases, leaves/needles were without symptoms of pathogens or herbivore attacks. We scanned them and assessed their areas using WinFOLIA and WinNEEDLE (Regent Instruments Inc., Toronto, Canada) for leaves and needles, respectively. Finally, the leaves and needles were dried in the oven at 60 °C for at least 72 h and weighed to record the dry mass. The storing, processing and measuring of SLA were conducted following Garnier et al. (2001) and Cornelissen et al. (2003).

Statistical analysis

To test the species richness and species composition effects on all leaf traits considered for each tree species separately, one-way ANOVA and the Tukey HSD post hoc test were used after the transformation of the data, when necessary to meet the assumption of the parametric statistical tests. The correlations between the leaf traits were tested with Spearman's rank correlation coefficients. The statistical analyses were performed with R (3.1.2., R Development Core Team 2014).

Results

Forest stands characteristics

Leaf area index, diffuse light non-intercepted by the canopy (DIFN) and BA of stands are reported in Table 1. Monospecific stands of *P. abies* had greater BA and LAI and less DIFN (although not significant) than monospecific stands of the other two species. *Pinus sylvestris* had higher BA, lower LAI and higher DIFN than *B. pendula* in monospecific stands. Two-species mixed stands with *B. pendula* + *P. sylvestris* and *P. abies* + *P. sylvestris* had lower LAI than *B. pendula* + *P. abies* mixed stands and mixed stands with three species. In *B. pendula* + *P. abies* stands, the DIFN (less amount of light intercepted by foliage) was higher than in *P. abies* + *P. sylvestris* stands. Furthermore, in mixed stands *B. pendula* + *P. sylvestris* BA was lower than in mixed stands *B. pendula* + *P. abies* and in stands with all three species.

ChlF parameters

The effects of the species richness level and species composition on ChlF parameters and on the other leaf traits analysed for each tree species are summarized in Table 1. *Betula pendula* (Table 1A) was higher performing in mixed plots: the parameters F_V/F_M, Ψ_{Eo}, ΔV_{IP} and PIs were significantly higher in two-species mixed plots than in monoculture. *Picea abies* (Table 1B) was lower performing in stands with high diversity (three species mixed): PI_{ABS} and PI_{TOT} were lower in *P. abies* trees in mixed stands than in monospecific stands; K-band and ABS/RC, on the other hand, were higher in mixed stands. The response of *P. sylvestris* (Table 1C) to species richness and species composition was more complex. In mixtures with *B. pendula*, *P. sylvestris* had significantly higher K-band, ABS/RC and ΔV_{IP} than in mixtures with *P. abies*. The values of the ChlF parameters of *P. sylvestris* mixed with *P. abies* were similar to those observed in monocultures, whereas the ChlF parameters of *P. sylvestris* mixed with *B. pendula* had similar values to those observed in stands with three species.

The electron transport efficiency in the photosynthetic processes of the tree species in mixed stands is shown in Figure 1, where the V_{OP} curves, that is, the comparison between double-normalized OJIP-ChlF transients (normalization per F_O and F_M) are shown (panels A–C). Positive peaks in the curves indicate the slowing of electron transport at the points K, J and I in mixed stands with respect to monocultures; negative peaks (dips) indicate faster electron transport. Negative peaks can be observed in *B. pendula* growing in mixed stands. It is remarkable that *P. sylvestris* in the two-species mixed stands with *B. pendula* showed a positive peak at the K point and a negative peak at the J and I points. The K-band in *P. sylvestris* mixed with *B. pendula* is shown in Figure 1D.

Table 1. Descriptive statistics for forest stand parameters (LAI, diffuse light non-interceptance and BA), ChlF parameters (see abbreviations in Table S2 available as Supplementary Data at *Tree Physiology* Online) and leaf traits (SLA, N and δ^{13}C) (mean ± standard deviation) of each tree species in relation to species richness level (1-sp = one species; 2-sp = two species; 3-sp = three species) and tree species composition (PA = *P. abies*; PS = *P. sylvestris*; BP = *B. pendula*) of the stands.

	1-sp	2-sp	2-sp	3-sp	ANOVA	
	BP	BP + PA	BP + PS	BP + PA + PS	Richness	Composition
(A) *Betula pendula*						
Forest stand parameters						
LAI (m^2 m^{-2})	2.33 ± 0.44a	3.2 ± 0.90a	2.24 ± 0.32a	3.34 ± 0.46a	0.146	**0.023**
Diffuse non-interceptance	0.19 ± 0.07a	0.27 ± 0.02a	0.14 ± 0.06a	0.16 ± 0.09a	0.734	0.127
BA (m^2 ha^{-1})	15.6 ± 1.0b	22.52 ± 5.0a	19.7 ± 1.1ab	25.4 ± 4.1a	**0.003**	**0.005**
Prop. BA (%) [1]	100	28	25	13		
ChlF parameters						
ABS/RC	1.97 ± 0.29a	1.88 ± 0.28a	2.02 ± 0.24a	2.08 ± 0.21a	0.405	0.162
F_V/F_M	0.77 ± 0.04b	0.79 ± 0.03a	0.8 ± 0.02a	0.78 ± 0.03ab	**0.022**	0.051
K-band	0.38 ± 0.04a	0.37 ± 0.05a	0.4 ± 0.04a	0.41 ± 0.03a	0.138	0.105
Ψ_{Eo}	0.5 ± 0.07b	0.56 ± 0.07ab	0.58 ± 0.05a	0.55 ± 0.08ab	**0.003**	**0.008**
ΔV_{IP}	0.18 ± 0.04b	0.22 ± 0.05a	0.23 ± 0.04a	0.21 ± 0.06ab	**0.003**	0.813
PI_{ABS}	22.3 ± 12.7a	30.5 ± 11.4a	30.2 ± 8.9a	25 ± 12.0a	0.061	0.132
PI_{TOT}	13.2 ± 8.9b	20.5 ± 9.6a	19.6 ± 5.6a	16.5 ± 7.9ab	**0.028**	0.066
Leaf traits						
SLA (m^2 kg^{-1})	14.9 ± 0.61a	14.12 ± 0.36b	13.99 ± 0.30b	13.88 ± 0.36b	**0.02**	0.81
N (%)	2.38 ± 0.15a	2.57 ± 0.26a	2.3 ± 0.28a	2.55 ± 0.08a	0.605	0.292
δ^{13}C (‰)	−29 ± 0.24a	−29.9 ± 0.96a	−29.2 ± 0.46a	−29.2 ± 0.52a	0.567	0.389
(B) *Picea abies*						
Forest stand parameters						
LAI (m^2 m^{-2})	3.61 ± 0.40a	2.71 ± 0.55a	3.26 ± 0.99a	3.34 ± 0.46a	0.299	0.296
Diffuse non-interceptance	0.20 ± 0.12a	0.10 ± 0.03a	0.27 ± 0.03a	0.17 ± 0.09a	0.882	0.098
BA (m^2 ha^{-1})	28.8 ± 2.2a	26.06 ± 1.3a	22.5 ± 5.0a	25.4 ± 4.1a	0.166	0.151
Prop. BA (%) [1]	100	60	72	38		
ChlF parameters						
ABS/RC	1.62 ± 0.14b	1.67 ± 0.15b	1.7 ± 0.19ab	1.86 ± 0.18a	**<0.001**	**0.033**
F_V/F_M	0.82 ± 0.02a	0.81 ± 0.01a	0.81 ± 0.02a	0.8 ± 0.02a	0.099	0.182
K-band	0.34 ± 0.02b	0.34 ± 0.03b	0.35 ± 0.04b	0.37 ± 0.03a	**0.001**	0.054
Ψ_{Eo}	0.57 ± 0.04a	0.56 ± 0.04a	0.58 ± 0.05a	0.55 ± 0.05a	0.231	0.323
ΔV_{IP}	0.22 ± 0.02a	0.21 ± 0.02a	0.22 ± 0.04a	0.21 ± 0.04a	0.466	0.533
PI_{ABS}	43.4 ± 11.4a	36.8 ± 8.9ab	41.7 ± 12.5ab	30.5 ± 7.6b	**0.005**	**0.009**
PI_{TOT}	27.4 ± 9.0a	23 ± 6.5ab	26.9 ± 10.0ab	19.1 ± 7.6b	**0.018**	**0.036**
Leaf traits						
SLA (m^2 kg^{-1})	2.99 ± 0.32a	2.92 ± 0.09a	3.01 ± 0.14a	3.12 ± 0.28a	0.49	0.355
N (%)	1.15 ± 0.10a	1.12 ± 0.07a	1.09 ± 0.09a	1.15 ± 0.07a	0.455	0.689
δ^{13}C (‰)	−28 ± 0.11a	−28.3 ± 0.18a	−28.3 ± 0.57a	−28 ± 0.34a	0.322	0.559
(C) *Pinus sylvestris*						
Forest stand parameters						
LAI (m^2 m^{-2})	1.94 ± 0.33b	2.71 ± 0.55ab	2.24 ± 0.32b	3.34 ± 0.46a	**0.002**	**0.003**
Diffuse non-interceptance	0.20 ± 0.04a	0.11 ± 0.04a	0.15 ± 0.06a	0.17 ± 0.09a	0.347	0.466
BA (m^2 ha^{-1})	20.6 ± 5.1a	26 ± 1.3a	19.7 ± 1.15a	25.4 ± 4.1a	0.288	**0.046**
Prop. BA (%) [1]	100	40	75	49		
ChlF parameters						
ABS/RC	1.7 ± 0.15b	1.72 ± 0.18b	2 ± 0.25a	1.83 ± 0.18a	**0.023**	**0.009**
F_V/F_M	0.82 ± 0.02a	0.81 ± 0.02a	0.81 ± 0.02a	0.81 ± 0.02a	0.218	0.288
K-band	0.35 ± 0.03b	0.35 ± 0.03b	0.41 ± 0.04a	0.38 ± 0.03a	**0.042**	**0.002**
Ψ_{Eo}	0.63 ± 0.04a	0.61 ± 0.06a	0.64 ± 0.06a	0.62 ± 0.04a	0.936	0.393
ΔV_{IP}	0.32 ± 0.05ab	0.3 ± 0.04b	0.37 ± 0.05a	0.35 ± 0.06a	0.21	**0.008**
PI_{ABS}	52.6 ± 12.2a	48.7 ± 16.3a	47.3 ± 13.5a	46.7 ± 12.2a	0.3175	0.488
PI_{TOT}	58.7 ± 20.4a	49.8 ± 20.7a	67.6 ± 24.8a	66.6 ± 26.1a	0.722	0.221

(Continued)

Table 1. (Continued)

	1-sp	2-sp	2-sp	3-sp	ANOVA	
	BP	BP + PA	BP + PS	BP + PA + PS	Richness	Composition
Leaf traits						
SLA (m^2 kg^{-1})	3.61 ± 0.07a	3.69 ± 0.06a	3.55 ± 0.12a	3.63 ± 0.07a	0.954	0.2
N (%)	1.08 ± 0.10b	1.2 ± 0.03b	1.21 ± 0.08b	1.35 ± 0.09a	**0.002**	**0.044**
$\delta^{13}C$ (‰)	−28 ± 0.22a	−28.1 ± 0.25a	−27.7 ± 0.33a	−27.6 ± 0.39a	0.102	0.253

For SLA, N and $\delta^{13}C$, we used data at stand level ($n = 4$ per each species richness level and per each species composition type); for ChlF parameters, the data were at tree level ($n = 12$ per each species richness level and per each species composition type). Different letters on the row indicate significant differences ($P < 0.05$) between species richness levels and species composition. P values (one-way ANOVA) of the effect of species richness and composition on forest stand parameter, ChlF parameters and leaf traits are indicated. Significance for $P < 0.05$ is indicated in bold.
[1] Proportion (%) of the BA of a given species in the different species composition of the stand.
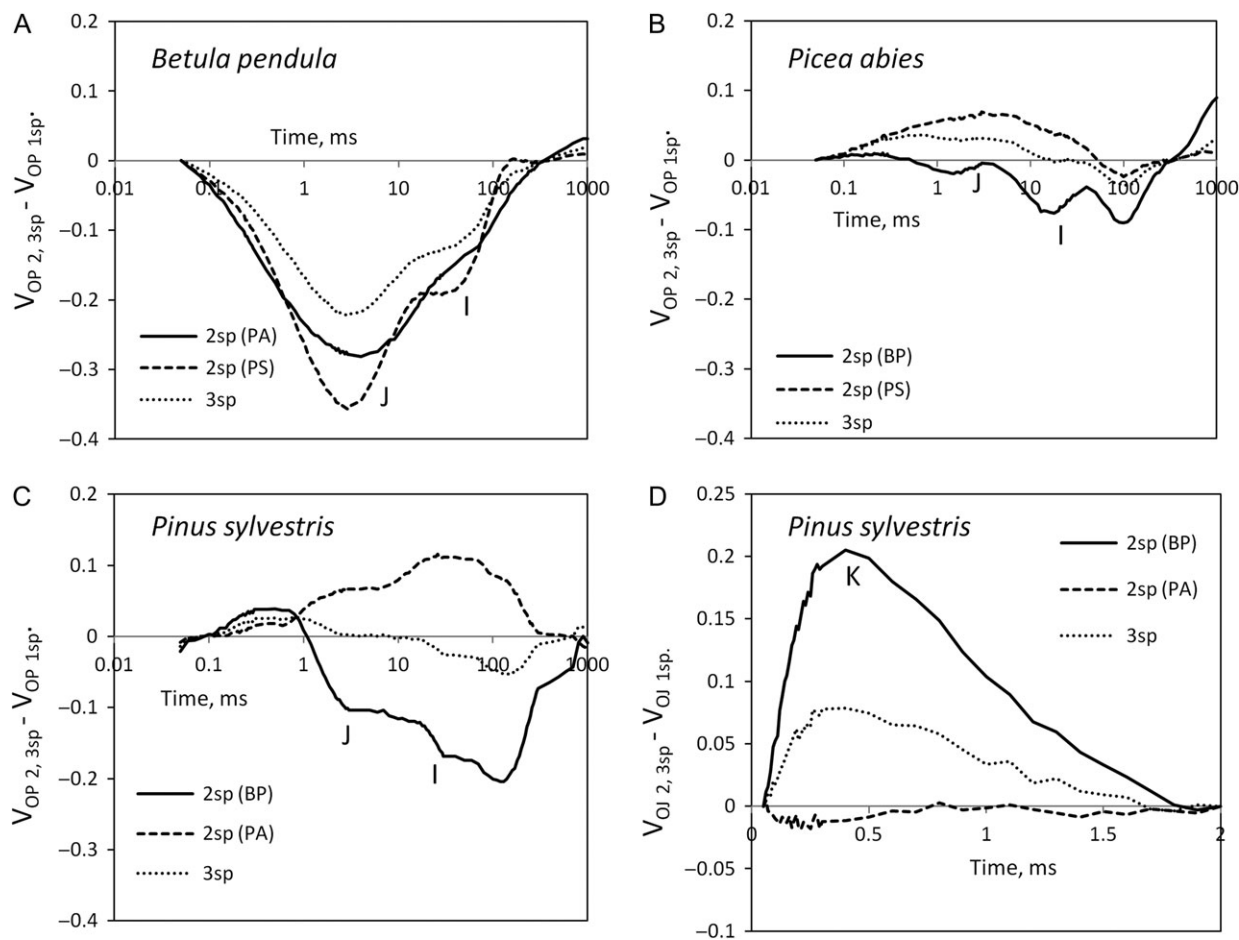

Figure 1. V_{OP} curves of tree species in different species richness level and composition of the stand (A–C). V_{OP} curves were calculated after the normalization of the OJIP-ChlF transient emission between F_O, assumed equal to 0 and F_M assumed equal to 1. For each time-point between 0 and 1, we calculated the relative variable fluorescence (V_t) with the equation: $V_t = (F_t − F_O)/(F_M − F_O)$, where F_t is the fluorescence intensity at any time t, F_O is minimum fluorescence emission and F_M is maximal fluorescence emission. (D) V_{OJ} curve was calculated after normalization of the OJIP-ChlF transient emission between F_O, assumed equal to 0 and F_J, assumed equal to 1. V_{OJ} curve evidences the K-band (K). In brackets is indicated the name of the tree species included in the species mixture. In (A–C) the V_{OP} curves of *Betula pendula* (BP), *Picea abies* (PA) and *Pinus sylvestris* (PS), respectively, in mixed stands (2- and 3-species, 2, 3sp) in comparison with monocultures (1sp). J and I indicate the time-step of the fluorescence transient at 2 and 30 ms, respectively. In (D) the V_{OJ} curves of *P. sylvestris*, showing the effect of the tree species richness on the electron transport efficiency at the beginning of the electron transport process (K-band). Straight lines ('0' on the y-axis) represent the electron transport efficiency of *P. sylvestris* in monoculture, assumed as reference.

Leaf traits: nitrogen content, carbon isotope composition and SLA

In *B. pendula* trees, SLA was significantly higher in those grown in monospecific stands than those in mixed stands (Table 1A). Leaf N content and $\delta^{13}C$ in *B. pendula* were not affected by stand species richness and species composition. In *P. abies*, SLA, N and $\delta^{13}C$ had similar values in trees grown in monospecific and mixed stands (Table 1B), whereas in *P. sylvestris*, N was higher in three-species mixed stands than in two-species mixed and monospecific stands (Table 1C).

In *B. pendula*, N showed a positive significant correlation with F_V/F_M and a negative one with ABS/RC and K-band; in *P. abies*, N was negatively correlated with Ψ_{Eo}, ΔV_{IP}, PI_{ABS} and PI_{TOT} (Table S3 available as Supplementary Data at *Tree Physiology* Online); $\delta^{13}C$ was positively correlated with ΔV_{IP} in *P. sylvestris* and negatively with Ψ_{Eo}, ΔV_{IP}, PI_{ABS} and PI_{TOT} in *P. abies* (Table S3 available as Supplementary Data at *Tree Physiology* Online). Moreover, in the two coniferous species $\delta^{13}C$ was positively correlated with N, while in *B. pendula* $\delta^{13}C$ resulted negatively correlated to SLA (Table S3 available as Supplementary Data at *Tree Physiology* Online).

Discussion

After removing the dynamic and chronic photoinhibition of leaves, by means of their long dark-adaptation period, we can accept that ChlF responses of leaves reflect the structural and functional properties of the photosynthetic apparatus and its strategy of acclimation to environmental factors during the whole period of leaf development (Pollastrini et al. 2016*a*, 2016*c*).

This study showed that in a forest ecosystem with homogenous climate and soil conditions, the leaf traits of tree species in mixed stands are affected by the species richness and specific composition of the stands. In *P. sylvestris* and, partially in *B. pendula*, the changes in ChlF parameters and leaf traits detected in the boreal forest were driven by species composition of the stand rather than the number of species mixed (species richness).

Pinus sylvestris showed two distinct patterns in response to stand composition. Mixed with *B. pendula* and in the three-species mixed stands, we observed an increase of the K-band, ABS/RC and ΔV_{IP} with respect to the mixture with *P. abies* and in monocultures. In previous works, it has been shown that the ecological conditions that provoke an increase of ΔV_{IP} values are related to mineral nutrition, with special reference to N availability (Nikiforou and Manetas 2011) and to high solar radiation (Cascio et al. 2010). Heat stress (Srivastava et al. 1997) and high light (Desotgiu et al. 2012), on the other hand, are known to be able to increase the relative fluorescence emission at 300 μs (K-band). In our study, the enhanced ΔV_{IP}, combined with the high value of K-band, suggests the role of solar radiation as the driver of these photochemical responses. Both *P. sylvestris* and

B. pendula are more light-demanding species than *P. abies*, and in their mixed stands LAI had low values, allowing the penetration of sunlight through the canopies, as highlighted by higher values of DIFN (although not significant). The low density of the canopy of *B. pendula* leads to better exploitation of solar energy by *P. sylvestris*.

Besides, the different phenology of the two species induces longer sun exposure of *P. sylvestris* foliage in spring, when *B. pendula* leaves have not sprouted yet. In *P. sylvestris*, $\delta^{13}C$ was positively related to ΔV_{IP}. An increment of ΔV_{IP} combined with an increase of leaf $\delta^{13}C$, in conditions of mild water stress, was observed before by Pollastrini et al. (2010) and may indicate an enhanced requirement of photosynthetic efficiency for better carbon exploitation when stomata are closed (Francey and Farquhar 1982, Keitel et al. 2003). The higher light availability in *P. sylvestris* + *B. pendula* stands (lower LAI) may affect the increase of $\delta^{13}C$ in *P. sylvestris* needles in two ways: through stomatal closure induced by drier conditions in the canopy (with a high vapour pressure deficit between the inside and outside of the canopy), with a consequent decrease of stomatal conductance of water vapour (gs); or through the increase of the rates of photosynthesis (Pn), as consequences of higher radiation interception (Gessler et al. 2001). Both processes, which cause the decrease of CO_2 concentration in the leaf (increase of Pn or decrease of gs) and in the needles of *P. sylvestris*, are influenced by the radiation intercepted by the trees and then by the species composition of the stands. The positive relationship between needle $\delta^{13}C$ and N content found in *P. sylvestris* suggests an increase of Pn, driven by an increase of photosynthetic enzymes and then of N amount, rather than a decrease of gs. Finally, the greater N content in leaves of *P. sylvestris* growing in more diverse stands may be related to the rates of litter decomposition of different types of leaves (Augusto et al. 2015). As is known, the plant diversity affects indirectly the decomposition and, consequently, the mineralization rate and the N cycling in the soil, via changes in understory environmental conditions (Scherer-Lorenzen 2008).

In *B. pendula*, the low photochemical efficiency observed in monospecific stands confirms the better conditions for growth and productivity of this species in mixed forests (Hynynen et al. 2010). In *B. pendula* trees, the SLA values were highest in monospecific stands and it was negatively correlated with ChlF parameters and $\delta^{13}C$, pointing out the non-optimal conditions for growth in pure stands.

In *P. abies*, photosynthetic efficiency declined in mixed stands. The negative correlations between the $\delta^{13}C$ and ChlF parameters may be related to the below ground competition for water available in the soil (Grossiord et al. 2014*b*), but we cannot exclude the influence of high light on the increase of $\delta^{13}C$. Leaf area index was lower (albeit not significantly) in mixed stands of *P. abies* than in the monospecific stands. In conditions of more light availability, generally, the net photosynthesis of trees is high, with high N content that can drive an increase of

leaf $\delta^{13}C$ (Gessler et al. 2001). No significant changes in the photochemical properties (ChlF parameters) were found in *P. abies* needles when this species coexists with either *P. sylvestris* or *B. pendula*. In fact, *P. abies* is a competitive species for above ground space (Kozovits et al. 2005), is shade-tolerant, with low sensitivity to the effects of neighbouring trees.

An overall evaluation of the leaf traits presented in this study suggests different responses of the light-demanding species (*B. pendula* and *P. sylvestris*) and the shade-tolerant tree species (*P. abies*) to stand composition. The first group of species was positively influenced by tree species composition for the exploitation of solar radiation. In the shade-tolerant species, on the other hand, the leaf traits were affected by species richness but not by species composition of the stands.

Finally, the spectral composition of the light, beside the intensity, plays a relevant role in morphogenesis of leaves, affecting their morphological and physiological characteristics (Xu et al. 2009). Leaves absorb more red than far-red light and, therefore, in stands with more dense canopies, and within the crowns of trees, is greater the ratio far-red/red light at forest floor and within the canopies (Jordan 1969), inhibiting growth and functioning (Ballaré et al. 1987).

Conclusions

The combined analysis of selected ChlF parameters and leaf traits may help to explore the photosynthetic responses of tree species to forest tree diversity. In this way, we can individuate the most suitable species composition and, consequently, the structure of stands to minimize the impacts of stressful environments and climate change on forests.

Future research directions claim to enhance the informative potential of the methods to analyse tree responses to environment, including a broader set of plant traits, such as those related to growth, species assemblage and ecological requirements.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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References

Augusto L, De Schrijver A, Vesterdal L, Smolander A, Prescott C, Ranger J (2015) Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biol Rev 90:444–466.

Baeten L, Verheyen K, Wirth C et al. (2013) A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. Perspect Plant Ecol Evol Syst 15:281–291.

Ballaré CL, Sánchez RA, Scopel AL, Casal JJ, Ghersa CM (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. Plant Cell Environ 10:551–557.

Bussotti F, Desotgiu R, Pollastrini M, Cascio C (2010) The JIP test: A tool to screen the capacity of plant adaptation to climate change. Scand J For Res 25:43–50.

Bussotti F, Pollastrini M (2015*a*) Evaluation of leaf features in forest trees: methods, techniques, obtainable information and limits. Ecol Ind 52:219–230.

Bussotti F, Pollastrini M (2015*b*) Do tree species richness, stand structure and ecological factors affect the photosynthetic efficiency in European forests? Web Ecol 15:39–41.

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. Environ Exp Bot 68:188–197.

Čepl J, Holá D, Stejskal J et al. (2016) Genetic variability and heritability of chlorophyll *a* fluorescence parameters in Scots pine (*Pinus sylvestris* L.). Tree Physiol 36:883–895.

Ceppi MG, Oukarroum A, Cicek 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. Physiol Plant 144:277–288.

Cornelissen JHC, Lavorel S, Garnier E et al. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Austr J Bot 51:335–380.

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 Biosys 146:206–216.

Drössler L, Övergaard R, Ekö PM, Gemmel P, Böhlenius H (2015) Early development of pure and mixed tree species plantations in Snogeholm, southern Sweden. Scand J For Res 30:304–316.

Edwards GE, Baker NR (1993) Can CO_2 assimilation in maize leaves be predictive accurately from chlorophyll fluorescence analysis? Photosynth Res 37:89–102.

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 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 Struct Funct 26:1203–1213.

Francey JR, Farquhar GD (1982) An explanation of $^{13}C/^{12}C$ in tree rings. Nature 297:28–31.

Franco AC, Duarte HM, Gessler A, de Mattos EA, Nahm M, Rennenberg H, Ribeiro KT, Scarano FR, Lüttge U (2005) In situ measurements of

carbon and nitrogen distribution and composition, photochemical efficiency and stable isotope ratios in *Araucaria angustifolia*. Trees Struct Funct 19:422–430.

Freyer MJ, Andrews JR, Oxborough K, Blowers DA, Baker NR (1998) Relationship between CO_2 assimilation, photosynthetic electron transport and active oxygen metabolism in leaves of maize in the field during periods of low temperature. Plant Physiol 108:761–767.

Frivold LH, Frank J (2002) Growth of mixed birch-coniferous stands in relation to pure coniferous stands at similar sites in South-eastern Norway. Scand J For Res 17:139–149.

Fusaro L, Gerosa G, Salvatori E, Marzuoli R, Monga R, Kuzminsky E, Angelaccio C, Quarato D, Fares S (2016) Early and late adjustments of the photosynthetic traits and stomatal density in *Quercus ilex* L. grown in an ozone-enriched environment. Plant Biol 18:13–21.

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 Biol 8:503–514.

Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. Funct Ecol 15:688–695.

Genty B, Briantais JM, Baker NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92.

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 Phytol 150: 653–664.

Gulías J, Flexas J, Mus M, Cifre J, Lefi E, Medrano H (2003) Relationship between maximum leaf photosynthesis, nitrogen content and specific leaf area in Balearic endemic and non-endemic Mediterranean species. Ann Bot 92:215–222.

Gottardini E, Cristofolini F, Cristofori A, Camin F, Calderisi M, Ferretti M (2016) Consistent response of crown transparency, shoot growth and leaf traits on Norway spruce (*Picea abies* (L.) H. Karst.) trees along an elevation gradient in northern Italy. Ecol Ind 60:1041–1044.

Grossiord C, Granier A, Ratcliffe S et al. (2014*a*) Tree diversity does not always improve resistance of forest ecosystems to drought. Proc Natl Acad Sci USA 11:14812–14815.

Grossiord C, Gessler A, Granier A, Pollastrini M, Bussotti F, Bonal D (2014*b*) Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. For Ecol Manage 318:54–61.

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 Biol 14:88–99.

Hynynen J, Niemistö P, Viherä-Aarnio A, Brunner A, Hein S, Velling P (2010) Silviculture of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) in northern Europe. Forestry 83:103–119.

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. Ecol Res 25:715–722.

Jordan CF (1969) Derivation of leaf area index from quality of light on the forest floor. Ecology 50:663.

Jucker T, Bouriaud O, Avacaritei D, Coomes DA (2014) Stabilizing effects of diversity on aboveground wood production in forest ecosystems: linking patterns and processes. Ecol Lett 17:1560–1569.

Jucker T, Bouriaud O, Coomes DA (2015) Crown plasticity enables trees to optimize canopy packing in mixed-species forests. Funct Ecol 29: 1078–1086.

Kaitaniemi P, Lintunen A (2010) Neighbor identity and competition influence tree growth in Scots pine, Siberian larch and silver birch. Ann For Sci 67:604–611.

Kalaji HM, Schansker G, Ladle RJ et al. (2014) Frequently asked questions about in vivo chlorophyll fluorescence: pratical issues. Photos Res 122:121–158.

Keitel C, Adams M, Holst T, Matzarakis A, Mayer H, Rennenberg H, Gessler A (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). Plant Cell Environ 26:1157–1168.

Koprowski M, Robertson I, Wils THG, Kalaji HM (2015) The application of potato starch effluent causes a reduction in the photosynthetic efficiency and growth of Scots pine (*Pinus sylvestris* L.). Trees Struct Funct 29:1471–1481.

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 Phytol 167: 181–196.

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

Liang J, Crowther TW, Picard N et al. (2016) Positive biodiversity–productivity relationship predominant in global forests. Science 354: aaf8957.

Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf U (1981) Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynth Res 2:115–141.

Maxwell C, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51:659–668.

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 Sens Environ 113:2037–2051.

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 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 (1998) Adjustment of foliage structure and function to a canopy light gradient in two co-existing deciduous trees. Variability in leaf inclination angles in relation to petiole morphology. Trees 12: 446–451.

Niinemets Ü, Portsmuth A, Tobias M (2006) Leaf size modifies support biomass distribution among stems, petioles and mid-ribs in temperate plants. New Phytol 171:91–104.

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 Phytol 205: 973–993.

Nikiforou C, Manetas Y (2011) Inherent nitrogen deficiency in *Pistacia lentiscus* preferentially affects photosystem I: a seasonal field study. Funct Plant Biol 38:848–855.

Nynynen J, Repola J, Mielikäinen K (2011) The effects of species mixture on the growth and yield of mid-rotation mixed stands of Scots pine and silver birch. For Ecol Manage 262:1174–1183.

Pollastrini M, Desotgiu R, Cascio C, Bussotti F, Cherubini P, Saurer M, Gerosa G, Marzuoli R (2010) Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements. Trees Struct Funct 24:695–704.

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 Biol 16:323–331.

Pollastrini M, Holland V, Brüggemann W, Bussotti F (2016*a*) Chlorophyll *a* fluorescence analysis in forests. Ann Bot 6:23–37.

Pollastrini M, Feducci M, Bonal D et al. (2016*b*) Physiological significance of forest tree defoliation: results from a survey in a mixed forest in Tuscany (central Italy). For Ecol Manage 361:170–178.

Pollastrini M, Holland V, Brüggemann W, Bruelheide H, Dănilă IC, Jaroszewicz B, Valladares F, Bussotti F (2016*c*) Taxonomic and ecological relevance of the chlorophyll *a* fluorescence signature of tree species in mixed European forests. New Phytol 212:51–65.

R Development Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ (22 February 2016, date last accessed).

Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecol Monogr 62:365–392.

Rothe A, Ewald J, Hibbs DE (2003) Do admixed broadleaves improve foliar nutrient status of conifer tree crops? For Ecol Manage 172:327–338.

Ruiz-Benito P, Gómez-Aparicio L, Paquette A, Messier C, Kattge J, Zavala M (2014) Diversity increases carbon storage and tree productivity in Spanish forests. Glob Ecol Biogeogr 23:311–322.

Russell MB, Woodall CW, D'Amato AW, Domke GM, Saatchi SS (2014) Beyond mean functional traits: Influence of functional trait profiles on forest structure, production, and mortality across the eastern US. For Ecol Manage 328:1–9.

Salvatori E, Fusaro L, Gottardini E, Pollastrini M, Goltsev V, Strasser RJ, Bussotti F (2014) Application of prompt, delayed chlorophyll fluorescence and 820 nm modulated reflectance. Insights from independent experiments. Plant Physiol Biochem 85:105–113.

Scherer-Lorenzen M (2008) Functional diversity affects decomposition processes in experimental grassland. Funct Ecol 22:547–555.

Serbin SP, Dillaway DN, Kruger EL, Towsend PA (2012) Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. J Exp Bot 63:489–502.

Srivastava A, Guissé B, Greppin H, Strasser RJ (1997) Regulation of antenna structure and electron transport in Photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll *a* fluorescence transient: OKJIP. Biochim Biophys Acta 1320:95–106.

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govindjee (eds) Advances in photosynthesis and respiration series. Chlorophyll *a* fluorescence signature of photosynthesis. Springer, Dordrecht, The Netherlands, pp 322–358.

Van de Berg AK, Perkins TD (2004) Evaluation of a portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (*Acer saccharum* Marsh.) leaves. For Ecol Manage 200:113–117.

Werner C, Correia O, Beyschlag W (2002) Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem. Funct Plant Biol 29:999–1011.

Xu F, Guo W, Xu W, Wei Y, Wang R (2009) Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves? Prog Nat Sci 19:1789–1798.

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 Sens Environ 136:247–258.