Interactions and competition processes among tree species in young experimental mixed forests, assessed with chlorophyll fluorescence and leaf morphology

M. Pollastrini[1], V. Holland[2], W. Brüggemann[2], J. Koricheva[3], I. Jussila[3], M. Scherer-Lorenzen[4], S. Berger[4], F. Bussotti[1]

1 Department of Agricultural, Food and Environmental Sciences, University of Florence, Florence, Italy
2 Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Germany; Biodiversity and Climate Research Center, Frankfurt, Germany
3 School of Biological Sciences, Royal Holloway, University of London, London, England
4 Faculty of Biology, Geobotany and Experimental Vegetation Science, University of Freiburg, Freiburg, Germany

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Correspondence

M. Pollastrini, Department of Agricultural, Food and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, 50144, Florence, Italy.

E-mail: martina.pollastrini@unifi.it

ABSTRACT

Chlorophyll *a* fluorescence (ChlF) and leaf morphology parameters were assessed in two experimental sites in Europe (Kaltenborn, Germany and Satakunta, Finland), within a forest diversity experiment. The trees at Satakunta, planted in 1999, form a stratified canopy; while in Kaltenborn the trees are seven-years-old, with no apparent canopy connection on broadleaf species. The following ChlF parameters obtained from the measured OJIP transient curves were examined: the ratio F_V/F_M (a proxy of the maximum quantum yield, where Fv is the difference between the minimal (F_0) and the maximal (F_M) fluorescence in the dark-adapted state); Ψ_{Eo} (a proxy of the efficiency to move an electron from reduced Q_A, the secondary PSII electron acceptor into the electron transport chain); the I-P phase (a proxy of the efficiency to reduce the final acceptors beyond PSI), and PI_{tot} (the total performance index for potential energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors). At Satakunta F_V/F_M and Ψ_{Eo} values in *Betula pendula* were higher in monocultures and lower in mixed plots, maybe due to the increasing availability of light in mixed plots that can induce photoinhibition. The opposite trend was observed in *Picea abies*, which was shaded in mixed plots. At Kaltenborn F_V/F_M decreased in *Fagus sylvatica* and *P. abie*s in mixed plots. This effect was attributed to competition processes both at aboveground and belowground level. At Satakunta LMA (Leaf Mass per Area) increased in *B.pendula* leaves with increasing species richness. LA (leaf area of ten leaves) was reduced in *F. sylvatica* in the mixed plots at Kaltenborn. By upscaling the overall fluorescence response to plot level (PI_{tot_plot}), a significant positive correlation with tree diversity was found at Kaltenborn but not at Satakunta. This result may suggest that the competition/facilitation processes in mixed stands play a significant overall role in the first stages of forest establishment, but then tend to be compensated for in more mature stands.

INTRODUCTION

Biodiversity regulates several aspects of ecosystem functioning and the delivery of ecosystem services (e.g. Balvanera *et al*. 2006; Cardinale *et al.* 2011). Additionally, the ecological stability of forest ecosystems has been connected to tree diversity (Bengtsson *et al*. 2000; Thompson *et al*. 2009; Scherer-Lorenzen *et al*. 2005a). Many forest ecosystem services, such as timber production and carbon sequestration, are directly related to the growth and photosynthesis rates. A recent review (Zhang *et al*. 2012) emphasized the role of biodiversity in enhancing forest growth and the biological mechanisms and processes leading to an increased biomass production in mixed stands. This is mainly related to a more efficient exploitation of the ecological resources due to niche differentiation and complementary resource use among coexisting species (Tilman 1999; Loreau & Hector 2001), i.e. through species interactions. Such complementarity can occur aboveground within the canopy, or in the soil. For example, different timing of leaf abscission of the various species and increased decomposition rates of litter in mixed stands allow a more homogeneous release of nutrients throughout the year and enhance the biological activity of the soil (Richards *et al.* 2010). Moreover, the presence of species with symbiotic nitrogen fixation activity increases the soil fertility (Forrester *et al*. 2012; Nouvellon *et al*. 2012), representing a classical example for facilitation. Overall, competition or facilitation may be established between different tree species, consequently the performance and growth of trees may be enhanced or depressed in a species-specific way (Reiter *et al*. 2005; Lei *et al*. 2012a; b).

The quantification of the role of tree species diversity in producing ecosystem services in naturally grown forests is problematic because of the large variability of the environmental factors (Vilá *et al*. 2005). For this reason a set of experimental forests with different levels of tree diversity has been established around the world within the framework of several research programs (Scherer-Lorenzen *et al*. 2005b; Scherer-Lorenzen *et al*. 2007). In this context, experimental forests were recently planted in Europe at Kaltenborn (Germany) and Satakunta (Finland).

During the growth of a forest stand, trees establish relationships with their neighbors both at root and at canopy level, depending on different growth rates, space occupation strategies and sun/shade tolerance (Kosovits *et al*. 2005; Lei *et al*. 2012a;b; Kohyama &Takada 2012). As far as canopy processes are concerned, different height and architecture of tree species result in the formation of micro-environments with a variety of light conditions, thus allowing the appearance of shade tolerant species (Ishii & Asano 2010). A mixed forest creates varying illumination conditions which induce different photosynthetic responses in plants at both stand level and within the crown of individual trees (Ellsworth &Reich 1993; Pearcy 1999; Niinemets *et al*. 2004; Valladares & Niinemets, 2007; Niinemets 2007; Way & Pearcy, 2012; Mänd *et al*. 2013).

Plant responses to light can be efficiently measured with chlorophyll *a* fluorescence (ChlF) techniques (Adams & Demming-Adams 2004; Bruce &Vasil'ev 2004). The informative potential of ChlF analysis (Papageorgiou & Govindjee 2004) has been used for forest monitoring surveys, by applying remote sensing techniques (Rossini *et al*. 2006; Meroni *et al*. 2009), in applied forestry research (see Ball *et al*. 1994; de Carvalho *et al*. 2005; Bussotti *et al*. 2010 and citations therein) and, more in general, in forest ecology studies (see, for ex. Stylinski *et al*. 2002; Einhorn *et al*. 2004). Nevertheless, the application of ChlF in extensive terrestrial field surveys on tall trees remains problematic (Mohammed *et al*. 1995; 2003; Sampson *et al*. 2000).

The survey described here represents the first experience in which ChlF techniques were used in a large scale terrestrial ecological assessment of forests, in relation to biodiversity issues. The specific aim of the present paper was to investigate the dynamics of competition and facilitation between tree species in the experimental mixed forests at Kaltenborn and Satakunta by using their ChlF properties and leaf morphology. The specific hypothesis to be tested was that the interactions between the different tree species and their physiological requirements during forest stand development and stratification -- as well as the nature of competition for space and light -- are reflected in the ChlF properties. More specifically, the heterogeneity of the canopy layer in mixed forests induces species-specific strategies for the use of light, and photoinhibition conditions,

according to the relative growth and crown interaction between the neighboring tree species. In the younger plantation, where the interaction between crowns is lacking, the competition for space, both at aboveground and belowground levels, may have a prominent importance.

MATERIAL AND METHODS

Experimental sites

The study was carried out in two experimental plantations, both included in previous projects. Kaltenborn (Thuringia, Germany) is a part of the BIOTREE experiment (Scherer-Lorenzen *et al.* 2005b, 2007), and Satakunta (Finland) belongs to the TreeDivNet platform (Scherer-Lorenzen *et al.* 2005). For details of the experimental sites see the supplementary materials.

At Kaltenborn the tree species studied were European beech (*Fagus sylvatica* L., FS), sessile oak (*Quercus petraea* Liebl., QP), Norway spruce (*Picea abies* (L.) Karst., PA) and Douglas fir (*Pseudotsuga menziesii* Franco, PM). At Satakunta we analyzed the chlorophyll fluorescence of silver birch (*Betula pendula* L., BP), European black alder (*Alnus glutinosa* (L.) Gaert., AG), Norway spruce (*Picea abies* (L.) Karst., PA), Scots pine (*Pinus sylvestris* L., PS) and Siberian larch (*Larix sibirica* Ledeb., LS). In both the experimental plantations the tree species were combined in different tree species mixtures (Table 1).

Sampling

Sampling at Kaltenborn was done on June 23-25, 2011. Eight trees per species per plot were selected, taking into account the neighboring tree species. At Satakunta sampling was done on July 11-14, 2011. Five trees per species per plot were randomly chosen. ChlF measurements were replicated on 5 different leaves per tree.

In evergreen conifers (*P. menziesii*, *P. abies* and *P. sylvestris*) the ChlF measurements were conducted on the youngest mature needles, i.e. the previous year's needles at Kaltenborn (c+1, sprouted in 2010, because the 2011 needles were not fully developed at the time of the sampling),

and current year's needles at Satakunta (c, sprouted in 2011). A preliminary survey showed that there was a significant correlation in ChlF parameters between c and c+1 needles of the species sampled at Satakunta (Table S1). In order to avoid a possible bias due to the heterogeneity of light conditions and photosynthesis within the crown (see Niinemets *et al*. 2004; Niinemets 2007), measurements were done on leaves from outer, south-exposed branches, in the upper third of the crown (sun leaves).

In field conditions, the values of many fluorescence parameters vary according to the hour of the day as an effect of sunlight exposure (Desotgiu *et al*. 2012). Strong light exposure can trigger processes of photoinhibition, which reduce the capacity to convert solar energy to electron transport (Takahashi & Murata 2008). The usual time (20-30 min) of dark-adaptation with leaf clips, prior to ChlF measurements, removes the dynamic, but not the chronic components of photoinhibition of leaves (Quich & Stitt 1989; Werner *et al*. 2002). To obtain a more complete removal of photoinhibition, leaves can either be measured at predawn or dark-adapted for a longer time (minimum 4-5 hours). Photoinhibition was removed at Kaltenborn by performing nighttime measurements directly on the crown, because plants were small enough. At Satakunta the twig sampling was performed in the morning (09:00 – 13:00) with extension loppers. Branchlets were placed in plastic bags to limit the loss of water and then stored in a dark bag at ambient temperature. Measurements were done in the late afternoon, in a darkened room at the Satakunta Environmental Research Centre (Reposaari). Before the field work, a preliminary survey was carried out to test the effectiveness of the methods applied.

Chlorophyll a fluorescence transient analysis and parameters

Direct ChlF measurements were carried out on the plants with a HandyPEA portable fluorimeter at Satakunta, and with a PocketPEA portable fluorimeter at Kaltenborn (both instruments from Hansatech Instruments, Pentney-Norfolk, UK). The fluorescence rise from the initial minimum fluorescence F_0 to the maximum fluorescence value F_M in dark-adapted samples, induced by a

saturating light pulse (intensity >3000 µmol photons $m^{-2}s^{-1}$,excitation light of650 nm), are called "fluorescence transients" (FT, direct or prompt fluorescence, Strasser *et al*. 2000, 2004, 2010; see also Stirbet and Govindjee 2011) and represent the fastphase ofChlF induction. Plotted on a logarithmic time scale, FT shows a polyphasic behaviour. The different time-steps of this polyphasic transient are labelled as: O (20-50µs), J (2 ms), I (30ms) and P (peak). The latter indicates the highest fluorescence intensity (F_M), when saturating light is used, and is generally obtainedaround 0.8s. The parameters considered in this study are:

- $F_V/F_M = [F_M-F_0]/F_M = \varphi_{Po} = TR_0/ABS$ = maximum quantum yield of PSII primary photochemistry that is measured in samples in dark-adapted state. F_V/F_M expresses the probability that an absorbed photon will be trapped by the PSII reaction center.
- $\Psi_{Eo} = ET_0/TR_0 = 1 - V_J = 1 - (F_{2ms} - F_0)/(F_M - F_0)$. Ψ_{Eo} expresses the probability that the energy of a trapped excitation is used for electron transport beyond Q_A. V_J represents the relative variable fluorescence at 2 ms (transients normalized between F_0 and F_M);
- $\Delta V_{I-P} = 1 - V_I = (F_M - F_{30ms})/(F_M - F_0)$ (I-P phase, Oukarroum *et al*. 2009). ΔV_{I-P}represents the relative contribution of the I-P phase to the fluorescence transient OJIP; it is regarded as a measure for the efficiency of electron flux through PSI to reduce the final acceptors of the electron transport chain, i.e. ferredoxin and NADP. V_I indicates the relative variable fluorescence at 30 ms (transients normalized between F_0 and F_M);
- PI_{tot} (Performance Index total). PI_{tot} is the potential for energy conservation from photons absorbed by PSII to the reduction flux (RE) of PSI end acceptors. It is a multiparametric expression that combines four parameters related to the photosynthetic activity: (1) the density of reaction centers; (2) the quantum yield of primary photochemistry; (3) the ability to feed electrons into the electron chain between PSII and PSI; (4) the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (Strasser *et al*. 2004, 2010).

  $PI_{tot} = (RC/ABS)\ [\varphi_{Po}/(1 - \varphi_{Po})]\ [\Psi_{Eo}/(1 - \Psi_{Eo})]\ [\delta_{Ro}/(1 - \delta_{Ro})]$

where

$RC/ABS = \varphi_{Po} (V_J/M_0)$

where $M_0 = [4 (F_{300\mu s} – F_0)/(F_M – F_0)]$

M_0 represents the initial slope of the double normalized fluorescence induction curve, and is a proxy of the net rate of PSII closure;

$\delta_{Ro} = (1 – V_I)/(1 – V_J) = (F_M – F_I)/(F_M – F_J)$. δ_{Ro} is the probability that an electron is transported from reduced PQ to the electron acceptor side of PSI.

Leaf morphology

From each sampled broadleaf tree (*B. pendula* and *A. glutinosa* at Satakunta; *F. sylvatica* and *Q. petraea* at Kaltenborn), 10 leaves from the same branch were collected and used for fluorescence measurements. Total leaf area (LA) was measured with a Li-Cor LI-3100 Area Meter (Lincoln, Nebraska, USA) and leaf dry weight (DW) was obtained after drying in an oven at 70°C (until constant weight). Leaf mass per area (LMA) was calculated as $LMA = DW\ LA^{-1}$ (mg cm^{-2}).

Data analysis

All data were tested for a normal distribution using the Kolmogorov-Smirnov test and the homogeneity of the variance was tested by the Levene test. The effects of tree species richness on the ChlF and leaf morphology parameters were analyzed by general linear models (GLM) with ‘tree species richness’ as a fixed factor and ‘tree’ as a random factor. Each species was analyzed separately. The post-hoc Tukey test was used to test the pairwise differences between the species richness levels for a given tree species. If it was not possible to use the GLM (in case of significant results of Kolmogorov-Smirnov and Levene tests, also after data transformation), we used the non parametric Kruskal-Wallis test to evaluate the difference between the species richness levels. The contrasts were performed between the means of the rank values. 5-species plots (Satakunta) and 4-species plots (Kaltenborn) were excluded from GLM analysis (in Tables 2-4) because these mixture

levels were represented only by 1 plot. Pearson's correlation coefficient was calculated to analyze the relationships between the photosynthetic performance of current and previous year needles in coniferous species at Satakunta. Linear regression was used to test the relationships between ChlF parameters and leaf morphology traits, with tree species richness expressed by the Shannon Index calculated on the basal area for each species per plot (Staddon *et al*. 1997; Spellerberg & Fedor 2003). The differences in the fluorescence parameters between the monocultures of each species were analyzed by one-way ANOVA for Kalternborn and by non-parametric statistics (Kruskal-Wallis test) for Satakunta. In order to define an indicator of PI_{tot} at plot level, we calculated (according to Bonal *et al*. 2000), PI_{tot_plot} as:

$$PI_{tot\_plot} = \frac{\sum\left(PI_{tot-spi} x BA_i\right)}{\sum BA_i}$$

where PI_{tot_spi} is the PI_{tot} of each species included in the plot and BA_i is the basal area per species. All the statistical analyses were performed with the software Statistica 7.0 (Statsoft, Tulsa OK).

RESULTS

The forest stands at the two experimental sites had very different structures in relation to their age, the competition among tree species, and the dynamic processes of growth and crown stratification. At Kaltenborn the trees were still young and canopies were not fully closed but, as shown by Lei *et al.* (2012a), there were already competitive belowground interactions among species. At Satakunta the forest was structured in different canopy layers, according to the growth rates of each species in the mixed plots, and *B. pendula* was - where present - the tallest tree species.

A preliminary analysis of the ChlF parameters considered in this study examined their variability within the crown of an individual tree (5 measurements per tree) and between the trees of a given species in an individual plot (5 trees per plot at Satakunta; 8 trees per plot at Kaltenborn).

The results (Table S2) show a very small coefficient of variation (CV=[st.dev/Mean]100) for F_v/F_M, but a large CV for PI_{tot}, both within and between trees.

The species-specific characteristics of the different tree species - obtained by comparing the monocultures - are shown in Table 2 (Satakunta) and Table 3 (Kaltenborn). At Satakunta (Table 2) *B. pendula* showed the highest values of F_v/F_M but, overall, *P. sylvestris* was the best performing tree species (higher PI_{tot}, $\Psi_{Eo}$and ΔV_{I-P}). At Kaltenborn (Table 3 the best performing tree species was *P. menziesii* (all fluorescence parameters examined were higher in this species).

The effect of tree species richness was tested with GLM analysis (Table 4) and post-hoc comparison (Tables 2, 3), whereas the role of biodiversity level (expressed by the Shannon Index) was evaluated with linear regressions (Table 5). The results show different patterns of each analyzed ChlF parameter for each tree species. At Satakunta the most sensitive parameters were F_V/F_M and the Ψ_{Eo} in *B. pendula* (both negative, decreasing as the Shannon Index increased) and *P. abies* (both positive, increasing as the Shannon Index increased). The ΔV_{I-P} was increased in mixed plots of *B. pendula*. F_V/F_M increased also in *P. sylvestris* at Satakunta, but decreased at Kaltenborn in *F. sylvatica*, *P. abies* and *P. menziesii* with increasing Shannon Index. Other significant patterns were the increasing of PI_{tot} in *P. abies* at Satakunta, and the increase of the ΔV_{I-P} and PI_{tot} in *P. menziesii* at Kaltenborn.

Among the ChlF parameters, PI_{tot} was upscaled in order to obtain an average value (see Materials and Methods) representative of the overall "plant fitness" of each plot – PI_{tot_plot}. Fig. 1 ranks the plots assessed at Satakunta (A) and Kaltenborn (B), whereas Fig. 2 expresses the linear regression between the Shannon Index and PI_{tot_plot} in the experimental forests of Satakunta (A) and Kaltenborn (B). While at Satakunta no relationship was found between the investigated variables, at Kaltenborn a positive correlation was found (Table S1, Pearson's coefficient $r = 0.597$; $p = 0.001$).

At Satakunta, leaf mass per area (LMA) increased with increasing tree species richness in *B. pendula* (Table 6), but not in *A. glutinosa*. No significant trend for LMA was detected on *Q. petraea*

and *F. sylvatica* at Kaltenborn (Table 6), but in *F. sylvatica* the leaf area (LA) decreased with increased tree species richness.

DISCUSSION AND CONCLUSION

The survey presented in this paper took into account different tree species co-occurring in experimental plots with different levels of tree species richness. A first result was to highlight specific ChlF properties of the different tree species examined. These properties are not directly connected to growth, but may reflect the strategies to cope with stress and carbon allocation (Marshall *et al*. 2011). In general, conifers performed better than broadleaf species, with higher levels of PI_{tot}. The parameters which revealed the most evident responses connected to species diversity were F_v/F_M and theΨ_{Eo}. F_V/F_M expresses the maximum quantum yield of PSII primary photochemistry. It is well known that this parameter is only scarcely responsive to the action of several stress factors, such as drought stress (Cornic & Fresnau 2002), but it is very sensitive to the light environment and especially to excess light (Adams & Demmig-Adams 2004). Leaves grown in high light conditions (sun leaves) are efficient in dissipating energy as heat (Ballottari *et al*. 2007). This phenomenon is partly due to photoinhibition, which involves the deactivation and turnover of the protein D1 in PSII (Ohira *et al*. 2004). The concomitant decrease of the capacity to trap solar energy and to feed the electron transport chain is considered a down regulation mechanism (Adir *et al*. 2003; Cui *et al*. 2003; Stroch *et al*. 2008). In fact it reduces the flow of electrons within the electron transport chain when the reduction potential, originating from high light intensity, is too high for the needs of the photosynthesis processes and cannot be utilized for metabolism (Lu *et al*. 2001; Ogaya *et al*. 2011).

At Satakunta the pattern of F_V/F_M and Ψ_{Eo}in *B. pendula* (decreasing when the level of tree species richness increases) is consistent with a gradient of diffusion and availability of light. *B. pendula* monocultures form a continuous canopy layer: the upper leaves sampled in this survey may be shaded by lateral branches of the same tree or of neighboring trees. With increasing tree species

richness, the canopy structure is more irregular because of the different growth rates and growth forms of the different tree species. Kaitaniemi and Lintunen (2010) reported that in *B. pendula* the increase in height was accelerated by competition with *L. sibirica* and *P. sylvestris* in mixed experimental forest stands in Finland. In *L. sibirica*, on the other hand, the average height increment was reduced by competition with *B. pendula* in the same study. These differences in growth create a large heterogeneity of light availability at the canopy level. Because of an increasing admixture of smaller trees, the top leaves of *B. pendula* are exposed to increasing average light intensities. Unlike *B. pendula*, at Satakunta the F_V/F_M and $\Psi_{Eo}$of *P. abies* increased with increasing species richness. This species has a lower growth potential in height than *B. pendula*, *L. sibirica* and *P. sylvestris*. Consequently in mixed stands *P. abies* was found under the canopies of the tallest tree species, with lower light availability and absence of photoinhibitory conditions. In both tree species, *B. pendula* and *P. abies*, the CV of F_V/F_M increased with decreasing values of F_V/F_M and Ψ_{Eo}(Table 2), confirming the relevance of the heterogeneity of light environments in mixed plots.

It was expected that the heterogeneity of luminous environments would also be reflected in the behavior of theΔV_{I-P} . The ΔV_{I-P} is considered to be sensitive to the light environment, although in the opposite way from F_V/F_M. Sun leaves have a lower capacity to trap electrons (low F_V/F_M) and a greater capacity to reduce the final acceptors of electrons beyond PSI (Cascio *et al*. 2010; Desotgiu *et al*. 2012). Nevertheless, no relation was found between ΔV_{I-P} and light availability in the mixed plots at Satakunta.

At Kaltenborn F_V/F_M values decreased in *F. sylvatica* with increasing tree species richness. This pattern is apparently in contrast to the availability of light in the different mixture conditions. In fact, the monoculture of *F. sylvatica* is made up of small trees isolated from each other and exposed to full sunlight at midday. This pattern can be compared to the competition processes in the first phases of establishment in a mixed forest stand. Many studies have shown that in its juvenile stage *P. abies* is very competitive in relation to *F. sylvatica* both aboveground (Kozovits *et al*. 2005; Reiter *et al*. 2005; Gayler *et al*. 2006) and belowground (Bolte & Villanueva 2006). A possible

307 explanation for decreasing F_V/F_M ratios of *F. sylvatica* with increasing species competition could be
308 competition for soil resources, i.e. water and/or nutrients, provided that the other three species are
309 more competitive. Nitrogen is known to depress F_V/F_M in *F. sylvatica* (Percival *et al*. 2008),
310 whereas the effect of water shortage is more questionable (Tognetti *et al*. 1995). The competition
311 processes in the mixed plots of Kaltenborn may be very variable and the specific competitiveness of
312 neighboring tree species may depend on physical distance. Another point that should be considered
313 is that - in relation to tree size and plot structure - the different species are not only competing for
314 water and/or nutrients with each other, they are also competing with the understory, primarily in the
315 monocultures. It can be assumed that the competition with the herbal layer for the smaller trees, like
316 *Q. petraea* and particularly *F. sylvatica,* was much stronger than for e.g. *P. menziesii,* which
317 displaced the understory more or less completely.

318 Leaf morphology supports the importance of the distribution of light at the canopy level at
319 Satakunta. Sunlight is a very powerful factor able to determine foliar morphology, and results in an
320 increase of leaf mass per area (LMA) in sun exposed leaves (Bussotti 2008). In this study LMA of
321 *B. pendula* reflected a gradient of available light intensity, indeed *B. pendula* crowns have sun
322 leaves in the highest mixture plots. A very different dynamic was observed at Kaltenborn, where the
323 leaf area of *F. sylvatica* was reduced in highly mixed plots without changes of LMA, suggesting a
324 worsening of the growth conditions in these plots with the admixture of the (faster growing)
325 coniferous species, not connected to light availability.

326 The differences and the trends highlighted for F_V/F_M and $\Psi_{E_o}$with biodiversity were no
327 longer significant at Satakunta with PI_{tot}, thus suggesting a compensation between the different
328 photosynthetic processes described by the various parameters. The overall results from the two sites
329 reveal in general greater PI_{tot} values at Kaltenborn than at Satakunta. The authors consider that it is
330 not possible to compare the two sites because they were assessed on different months of the year,
331 and using two different instruments. It is possible, however, to compare the trends of fluorescence

parameters and behaviour of the different species across the levels of species richness and combination within each plot.

The analysis of the Performance Index total upscaled to plot level (PI_{tot_plot}) suggests an effect of the species composition (Fig.1) in the overall photosynthetic efficiency, but the lack of replicates for each kind of mixture does not allow for a statistical verification of this point. This survey was designed to evaluate the role of tree diversity "per se" rather than the neighboring tree species effect. A positive response to tree diversity on PI_{tot_plot} was found at Kaltenborn but not at Satakunta (Fig. 1). This behavior may suggest that the overall role of the competition/facilitation processes in mixed stands is detectable in the early stages of forest establishment, but then tends to be compensated for in more mature stands.

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Table 1.

Experimental design of Satakunta and Kalternborn plantation. Number of total and sampled plots and number of sampled trees.

	Total number of plots				No. trees
SATAKUNTA	mono	2-sp	3-sp	5-sp	
Betula pendula	1	3	4	1	*45*
Alnus glutinosa	1	2	3	1	*35*
Picea abies	1	4	3	1	*44*
Pinus sylvestris	1	3	4	1	*43*
Larix sibirica	1	2	4	1	*40*
No. sampled plots	*5*	*7*	*6*	*1*	
KALTENBORN	mono	2-sp	3-sp	4-sp	
Fagus sylvatica	1	3	3	1	*63*
Quercus petraea	1	3	3	1	*57*
Picea abies	1	3	3	1	*64*
Psedotsuga menziesii	1	3	3	1	*60*
No. sampled plots	*4*	*6*	*4*	*1*	

Table 2.

Satakunta. Fluorescence parameters per each species in the different tree species richness level (mean ± standard error; CV = [standard deviation/mean]*100). Number of trees per each species and site is indicated in Table 1. 5-species plot was excluded from this analysis. Uppercase letters indicate significant differences between different species for $p<0.05$ (only in monocultures, on the column). Lowercase letters indicate significant differences for $p<0.05$ (within the same species) between different levels of tree-species richness (on the column). Tukey test was applied. The parameters F_v/F_M, Ψ_{Eo}, ΔV_{I-P} are expressed in a-dimensional ratios. PI_{tot} is in a.u.

		Fv/Fm			Ψ_{Eo}			ΔV_{I-P}			PI_{tot}		
Specie	mixture	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV
B.pendula	monocolture	0.836	± 0.001 a, A	0.008	0.627	± 0.005 a, AB	0.029	0.165	± 0.015 b, B	0.177	17.808	± 2.66 a, AB	0.321
	2 sp-	0.815	± 0.004 b	0.013	0.615	± 0.011 a	0.041	0.255	± 0.017 a	0.137	29.346	± 3.092 a	0.261
	3 sp-	0.809	± 0.003 b	0.016	0.558	± 0.010 b	0.063	0.232	± 0.007 a	0.142	22.365	± 1.786 a	0.320
A.glutinosa	monocolture	0.792	± 0.006 a, B	0.017	0.554	± 0.021 a, AB	0.052	0.243	± 0.029 a, AB	0.113	21.795	± 6.451 a, AB	0.319
	2 sp-	0.781	± 0.005 a	0.015	0.558	± 0.012 a	0.034	0.276	± 0.009 a	0.158	24.505	± 2.968 a	0.379
	3 sp-	0.794	± 0.004 a	0.016	0.583	± 0.016 a	0.056	0.262	± 0.024 a	0.130	25.368	± 5.125 a	0.325
P.abies	monocolture	0.786	± 0.009 b, B	0.024	0.557	± 0.017 b, AB	0.055	0.233	± 0.018 a, AB	0.090	18.801	± 3.297 a, AB	0.308
	2 sp-	0.817	± 0.003 a	0.012	0.630	± 0.007 a	0.045	0.225	± 0.009 a	0.125	26.874	± 2.507 a	0.335
	3 sp-	0.822	± 0.002 a	0.012	0.624	± 0.011 a	0.047	0.213	± 0.012 a	0.134	26.267	± 3.272 a	0.392
L.sibirica	monocolture	0.797	± 0.003 a, AB	0.037	0.548	± 0.009 a, B	0.106	0.201	± 0.007 a, AB	0.155	13.302	± 1.171 a, B	0.336
	2 sp-	0.798	± 0.007 a	0.020	0.543	± 0.027 a	0.085	0.200	± 0.006 a	0.143	14.836	± 1.598 a	0.348
	3 sp-	0.798	± 0.005 a	0.028	0.559	± 0.018 a	0.106	0.196	± 0.008 a	0.170	15.657	± 1.529 a	0.495
P.sylvestris	monocolture	0.830	± 0.004 a, AB	0.012	0.632	± 0.008 c, A	0.046	0.255	± 0.005 a, A	0.102	33.668	± 3.003 a, A	0.428
	2 sp-	0.835	± 0.001 a	0.008	0.687	± 0.005 a	0.031	0.288	± 0.007 a	0.102	44.481	± 3.307 a	0.287
	3 sp-	0.834	± 0.001 a	0.009	0.665	± 0.005 b	0.040	0.266	± 0.006 a	0.078	38.918	± 2.717 a	0.273

F_v/F_M: maximum quantum yield of PSII primary photochemistry, with $Fv = F_M - F_0$, where F_0 is the initial minimum fluorescence and F_M the maximum fluorescence; Ψ_{Eo}: efficiency of an electron to move from reduced Q_A, the secondary PSII electron acceptor into the electron transport chain; ΔV_{I-P}: the efficiency to reduce the final acceptors beyond the PSI; PI_{tot}, the total performance index for (potential) energy conservation from photons absorbed by PSII to the reduction flux of PSI end acceptors.

Table 3.

Kaltenborn. Fluorescence parameters per each species in the different tree species richness level (mean ± standard error; CV = [standard deviation/mean] *100). Number of trees per each species and site is indicated in Table 1. 4-species plot was excluded from this analysis. Uppercase letters indicate significant differences between different species for $p<0.05$ (only in monocultures, on the column). Lowercase letters indicate significant differences for $p<0.05$ (within the same species) between different levels of tree-species richness (on the column). Tukey test was applied. Explanation of parameters in Table 2.

		Fv/Fm			Ψ_{Eo}			ΔV_{I-P}			PI_{tot}		
Specie	mixture	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV
F.sylvatica	monocolture	0.779	± 0.004 a, B	0.018	0.479	± 0.026 a, B	0.156	0.193	± 0.012 a, B	0.177	31.840	± 5.882 a, B	0.523
	2 sp-	0.758	± 0.005 a	0.038	0.506	± 0.015 a	0.147	0.187	± 0.007 a	0.187	30.373	± 4.465 a	0.705
	3 sp-	0.743	± 0.009 a	0.062	0.487	± 0.025 a	0.253	0.190	± 0.008 a	0.231	32.663	± 4.250 a	0.637
Q.petraea	monocolture	0.782	± 0.007 a, B	0.028	0.524	± 0.035 bc, B	0.192	0.237	± 0.015 a, AB	0.184	56.284	± 11.784 bc, AB	0.592
	2 sp-	0.799	± 0.004 a	0.029	0.620	± 0.013 a	0.099	0.265	± 0.008 a	0.265	93.801	± 6.962 a	0.348
	3 sp-	0.788	± 0.005 a	0.031	0.550	± 0.017 c	0.138	0.246	± 0.009 a	0.164	63.249	± 7.209 c	0.497
P.abies	monocolture	0.790	± 0.005 ab, AB	0.018	0.559	± 0.007 b, AB	0.038	0.249	± 0.005 a, A	0.059	46.959	± 3.100 a, AB	0.187
	2 sp-	0.805	± 0.006 a	0.038	0.614	± 0.012 a	0.103	0.263	± 0.010 a	0.263	85.574	± 9.327 a	0.819
	3 sp-	0.780	± 0.005 b	0.036	0.597	± 0.011 ab	0.093	0.275	± 0.007 a	0.131	74.169	± 8.615 a	0.557
P.menziesii	monocolture	0.809	± 0.008 a, A	0.030	0.632	± 0.016 a, A	0.074	0.245	± 0.012 a, A	0.148	74.787	± 13.872 a, A	0.525
	2 sp-	0.796	± 0.005 a	0.033	0.571	± 0.014 a	0.125	0.226	± 0.007 a	0.226	51.666	± 6.652 a	0.631
	3 sp-	0.791	± 0.006 a	0.035	0.613	± 0.016 a	0.123	0.255	± 0.010 a	0.178	73.946	± 9.751 a	0.590

Table 4.

GLM analysis for the effect of tree species richness on the fluorescence parameters. The 5-species plot (Satakunta) and 4-species plot (Kaltenborn) were excluded from this analysis. Levels of the significance are indicated for P<0.05; P<0.01 and P<0.001. Values are reported for each specie at two experimental site. Explanation of parameters in Table 2.

	F_v/F_M	Ψ_{Eo}	$\Delta V_{I\text{-}P}$	PI_{tot}
Satakunta				
B.pendula	<0.01	<0.001	<0.01	<0.05
A.glutinosa	-	-	-	-
P.abies	<0.01	<0.01	-	-
P.sylvestris	-	<0.001	<0.05	-
L.sibirica	-	-	-	-
Kaltenborn				
F.sylvatica	<0.01	-	-	-
Q.petraea	-	<0.01	-	<0.01
P.abies	<0.001	<0.05	-	-
P.menziesii	-	<0.01	<0.001	<0.001

Table 5.

Results of the linear regression of fluorescence parameters in relation to tree species diversity calculated with the Shannon Index. In bold the values of regression with $p \leq 0.05$. Number of trees per each species and site is indicated in Table 1. Explication of parameters in Table 2.

Species	Parameter	slope	intercept	r^2	p value
SATAKUNTA					
B.pendula	F_V/F_M	-0.024	0.828	0.230	**0.009**
	Ψ_{Eo}	-0.094	0.640	0.353	**0.000**
	$\Delta V_{I\text{-}P}$	0.011	0.225	0.006	0.607
	PI_{tot}	-3.510	26.266	0.020	0.357
A.glutinosa	F_V/F_M	0.007	0.784	0.028	0.335
	Ψ_{Eo}	0.008	0.556	0.004	0.713
	$\Delta V_{I\text{-}P}$	0.010	0.253	0.004	0.726
	PI_{tot}	5.501	20.106	0.025	0.367
P.abies	F_V/F_M	0.025	0.798	0.331	**0.000**
	Ψ_{Eo}	0.038	0.591	0.131	**0.015**
	$\Delta V_{I\text{-}P}$	0.002	0.219	0.000	0.894
	PI_{tot}	8.883	19.201	0.107	**0.030**
P.sylvestris	F_V/F_M	0.007	0.830	0.103	**0.034**
	Ψ_{Eo}	0.020	0.656	0.082	0.059
	$\Delta V_{I\text{-}P}$	-0.002	0.274	0.001	0.849
	PI_{tot}	4.330	37.585	0.020	0.357
L.sibirica	F_V/F_M	0.002	0.798	0.001	0.823
	Ψ_{Eo}	-0.033	0.571	0.029	0.294
	$\Delta V_{I\text{-}P}$	-0.002	0.197	0.001	0.849
	PI_{tot}	-0.324	15.008	0.001	0.888
KALTENBORN					
F.sylvatica	F_V/F_M	-0.050	0.782	0.212	**0.000**
	Ψ_{Eo}	-0.019	0.503	0.005	0.585
	$\Delta V_{I\text{-}P}$	0.000	0.189	0.000	0.990
	PI_{tot}	-2.216	31.988	0.002	0.750
Q.petraea	F_V/F_M	-0.003	0.798	0.001	0.778
	Ψ_{Eo}	0.036	0.560	0.019	0.298
	$\Delta V_{I\text{-}P}$	0.020	0.242	0.025	0.244
	PI_{tot}	17.849	67.374	0.027	0.219
P.abies	F_V/F_M	-0.031	0.809	0.122	**0.005**
	Ψ_{Eo}	0.027	0.583	0.030	0.174
	$\Delta V_{I\text{-}P}$	0.018	0.254	0.025	0.215
	PI_{tot}	7.493	68.098	0.005	0.602
P.menziesii	F_V/F_M	-0.018	0.807	0.065	**0.050**
	Ψ_{Eo}	0.026	0.588	0.018	0.308
	$\Delta V_{I\text{-}P}$	0.047	0.218	0.125	**0.006**
	PI_{tot}	38.91	-47.34	0.085	**0.024**

Table 6.

Results of the linear regression of foliar morphology parameters in relation to tree species diversity calculated with the Shannon Index.

	Leaf Mass per Area			Leaf Area		
	r	r^2	p	r	r^2	p
Satakunta						
B. pendula	0.570	0.324	<0.001	-0.010	0.000	>0.05
A. glutinosa	-0.113	0.013	>0.05	0.105	0.011	>0.05
Kaltenborn						
F. sylvatica	-0.094	0.008	>0.05	-0.451	0.203	<0.001
Q. petraea	0.064	0.004	>0.05	-0.176	0.031	>0.05

FIG.1

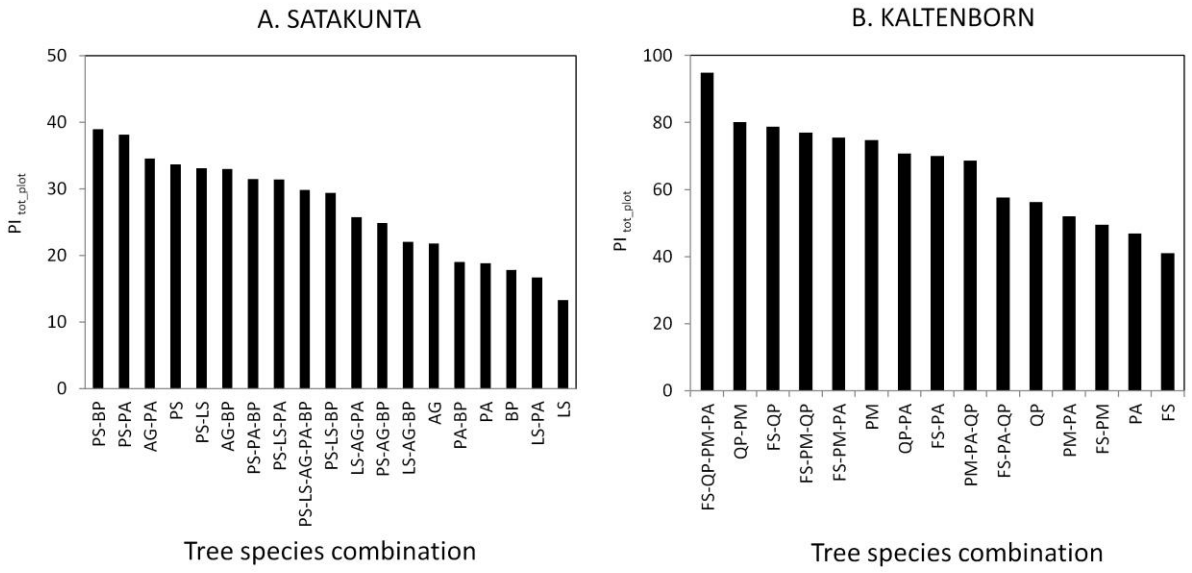

Fig.1. Rank of the PI_{tot} at plot level (PI_{tot_plot}) in relation to tree species combination in Satakunta (A) and Kaltenborn (B). BP – *Betula pendula*; AG – *Alnus glutinosa*; LS – *Larix sibirica*; PA – *Picea abies*; PS – *Pinus sylvestris;* FS – *Fagus sylvatica*; PM – *Pseudotsuga menziesii*; QP – *Quercus petraea*.

FIG.2

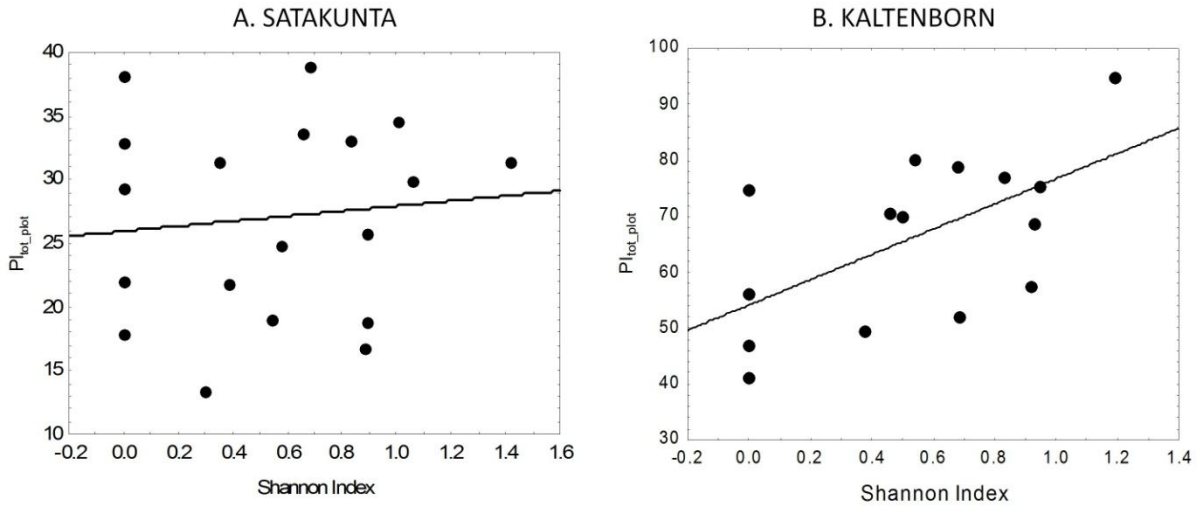

Fig. 2. Correlations between the PI_{tot_plot} and Shannon Index in the two experimental forests (A. Satakunta: r=0.110; r^2=0.012; p>0.05, not significant; B. Kaltenborn: r=0.597; r^2=0.357; p<0.05, significant).

SUPPLEMENTARY MATERIAL

Description of experimental sites

The experimental site at Kaltenborn (Thuringia, Germany, 10°13'E, 50°47'N; elevation 32 m asl) was planted in winter 2003/2004 as part of the BIOTREE experiment (Scherer-Lorenzen *et al.* 2005b, 2007). The soil is acid arenosol on sandstone bedrock. The climate is Sub-Atlantic with a mean annual temperature of 7.8°C and mean annual precipitation of 650 mm. Until 1975 the site was used as cropland and then converted to grassland. Saplings of four tree species were planted in plots of 1, 2, 3, and 4-species mixtures. The desired species mixture was achieved by patch planting (patches of 8x8 m). The species planted were: European beech (*Fagus sylvatica* L., FS), sessile oak (*Quercus petraea* Liebl., QP), Norway spruce (*Picea abies* (L.) Karst., PA) and Douglas fir (*Pseudotsuga menziesii* Franco, PM). The present study was carried out in 15 plots, representing different tree species richness levels and species combinations (Table 1). *P. menziesii* was the tallest tree species (3-4 m height), followed by *P. abies*. Among the deciduous broadleaved species *Q. petraea* trees were higher (2-3 m) than *F. sylvatica* (1-2 m). Unlike conifers, the broadleaved trees stand separately without crown interaction.

The Satakunta forest diversity experiment (Finland, 61°N, 22°E, elevation 20-50 m asl) was established in spring 1999 on three clear-cut areas about 1.5 ha each; it belongs to the TreeDivNet platform (Scherer-Lorenzen *et al.* 2005). The sites are within the boreal coniferous forest, where the soil is podzolic and the climate is Subartic, with cold winters and no dry season. The mean annual temperature is 5.0°C and the mean annual precipitation is 585 mm. Each experimental area included 38 plots (each 20m x 20m), which were randomly allocated to 19 treatments; the plots represent monocultures as well as mixtures of up to five tree species. The species used in the experiment were: silver birch (*Betula pendula* L., BP), European black alder (*Alnus glutinosa* (L.) Gaert., AG), Norway spruce (*Picea abies* (L.) Karst., PA), Scots pine (*Pinus sylvestris* L., PS) and Siberian larch (*Larix sibirica* Ledeb., LS). This study was carried out in 19 plots, representing different levels of tree species richness and different species combinations (Table 1). *B. pendula* was the tallest tree

53 species (10-12 m) and reached the dominant canopy layer in all plots in which it was present. *P.*
54 *sylvestris* and *L. sibirica* (8-10 m height) occupied the intermediate layer, whereas *P. abies* (3-5 m
55 height), growing in the lower layer of the forest, was the dominated species. Finally, *A. glutinosa*
56 grew mainly as a shrub.
57

Table S1

Pearson's coefficient of correlation (r); significance level (p) and coefficient of determination (r^2) between the fluorescence parameters of current year (c) and previous year (c+1) needles in *P. abies* (A) and *P. sylvestris* (B) at Satakunta.

F_v/F_M: maximum quantum yield of PSII primary photochemistry;Ψ_{Eo}: efficiency of an electron to move from reduced Q_A, the secondary PSII electron acceptor, into the electron transport chain;ΔV_{I-P} : the efficiency to reduce the final acceptors beyond the PSI; PI_{tot}, the total performance index for (potential) energy conservation from photons absorbed by PSII to the reduction flux of PSI end acceptors.

		P. abies	*P. sylvestris*
F_v/F_M	r	0.79	0.40
	p	*<0.001*	*<0.05*
	r^2	0.62	0.16
Ψ_{Eo}	r	0.68	0.66
	p	*<0.001*	*<0.001*
	r^2	0.47	0.44
ΔV_{I-P}	r	0.76	0.73
	p	*<0.001*	*<0.001*
	r^2	0.58	0.54
PI_{tot}	r	0.50	0.62
	p	<0.01	<0.001
	r^2	0.25	0.39

Table S2

Coefficient of variation (CV= [st.dev./mean]*100) of selected fluorescence parameters for each tree species at the two experimental sites of Satakunta and Kaltenborn.

A.Trees – Average CV of the different replication within a same tree (number of leaves replicated per tree = 5; number of trees per species is indicated in Table 1); B. plot – Average CV of the different trees within a same plot (number of trees replicated per plot = 5 at Satakunta and 8 at Kaltenborn; number of plots per species is indicated in Table 1). Explanation of parameters in Table S1.

	F_v/F_M		Ψ_{Eo}		$\Delta V_{I\text{-}P}$		PI_{tot}	
	A.Trees	B.Plot	A.Trees	B.Plot	A.Trees	B.Plot	A.Trees	B.Plot
Satakunta								
B.pendula	1.75	1.62	5.75	7.08	16.27	14.94	32.01	30.43
A.glutinosa	1.67	1.69	5.55	7.79	12.35	18.89	32.76	50.98
P.abies	1.24	1.27	4.57	5.15	12.84	13.27	35.24	33.02
P.sylvestris	0.90	0.78	3.85	3.21	9.05	9.33	30.74	27.48
L.sibirica	2.86	2.47	10.76	11.52	16.03	18.41	28.36	33.62
All species	1.68	1.57	6.10	6.95	13.31	14.97	31.82	35.11
Kaltenborn								
F.sylvatica	3.54	3.72	15.14	18.11	15.04	18.51	47.96	54.54
Q.petraea	2.11	2.36	10.04	13.97	13.57	16.69	41.36	46.25
P.abies	3.18	2.66	9.83	8.39	17.85	13.43	57.62	51.02
P.menziesii	3.48	3.21	9.92	7.99	18.80	12.56	57.63	48.19
All species	3.08	2.99	11.23	12.12	16.31	15.30	51.14	50.00