

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

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17 **Keywords**

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26 **ABSTRACT**

27 Chlorophyll *a* fluorescence (ChlF) and leaf morphology parameters were assessed in two
28 experimental sites in Europe (Kaltenborn, Germany and Satakunta, Finland), within a forest
29 diversity experiment. The trees at Satakunta, planted in 1999, form a stratified canopy; while in
30 Kaltenborn the trees are seven-years-old, with no apparent canopy connection on broadleaf species.
31 The following ChlF parameters obtained from the measured OJIP transient curves were examined:
32 the ratio F_V/F_M (a proxy of the maximum quantum yield, where F_V is the difference between the
33 minimal (F_0) and the maximal (F_M) fluorescence in the dark-adapted state); Ψ_{E_0} (a proxy of the
34 efficiency to move an electron from reduced Q_A , the secondary PSII electron acceptor into the
35 electron transport chain); the I-P phase (a proxy of the efficiency to reduce the final acceptors
36 beyond PSI), and PI_{tot} (the total performance index for potential energy conservation from photons
37 absorbed by PSII to the reduction of PSI end acceptors). At Satakunta F_V/F_M and Ψ_{E_0} values in
38 *Betula pendula* were higher in monocultures and lower in mixed plots, maybe due to the increasing
39 availability of light in mixed plots that can induce photoinhibition. The opposite trend was observed
40 in *Picea abies*, which was shaded in mixed plots. At Kaltenborn F_V/F_M decreased in *Fagus sylvatica*
41 and *P. abies* in mixed plots. This effect was attributed to competition processes both at aboveground
42 and belowground level. At Satakunta LMA (Leaf Mass per Area) increased in *B.pendula* leaves with
43 increasing species richness. LA (leaf area of ten leaves) was reduced in *F. sylvatica* in the mixed
44 plots at Kaltenborn. By upscaling the overall fluorescence response to plot level (PI_{tot_plot}), a
45 significant positive correlation with tree diversity was found at Kaltenborn but not at Satakunta.
46 This result may suggest that the competition/facilitation processes in mixed stands play a significant
47 overall role in the first stages of forest establishment, but then tend to be compensated for in more
48 mature stands.

49

50 INTRODUCTION

51 Biodiversity regulates several aspects of ecosystem functioning and the delivery of ecosystem
52 services (e.g. Balvanera *et al.* 2006; Cardinale *et al.* 2011). Additionally, the ecological stability of
53 forest ecosystems has been connected to tree diversity (Bengtsson *et al.* 2000; Thompson *et al.*
54 2009; Scherer-Lorenzen *et al.* 2005a). Many forest ecosystem services, such as timber production
55 and carbon sequestration, are directly related to the growth and photosynthesis rates. A recent
56 review (Zhang *et al.* 2012) emphasized the role of biodiversity in enhancing forest growth and the
57 biological mechanisms and processes leading to an increased biomass production in mixed stands.
58 This is mainly related to a more efficient exploitation of the ecological resources due to niche
59 differentiation and complementary resource use among coexisting species (Tilman 1999; Loreau &
60 Hector 2001), i.e. through species interactions. Such complementarity can occur aboveground
61 within the canopy, or in the soil. For example, different timing of leaf abscission of the various
62 species and increased decomposition rates of litter in mixed stands allow a more homogeneous
63 release of nutrients throughout the year and enhance the biological activity of the soil (Richards *et*
64 *al.* 2010). Moreover, the presence of species with symbiotic nitrogen fixation activity increases the
65 soil fertility (Forrester *et al.* 2012; Nouvellon *et al.* 2012), representing a classical example for
66 facilitation. Overall, competition or facilitation may be established between different tree species,
67 consequently the performance and growth of trees may be enhanced or depressed in a species-
68 specific way (Reiter *et al.* 2005; Lei *et al.* 2012a; b).

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

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

84 Plant responses to light can be efficiently measured with chlorophyll *a* fluorescence (ChlF)
85 techniques (Adams & Demming-Adams 2004; Bruce & Vasil'ev 2004). The informative potential of
86 ChlF analysis (Papageorgiou & Govindjee 2004) has been used for forest monitoring surveys, by
87 applying remote sensing techniques (Rossini *et al.* 2006; Meroni *et al.* 2009), in applied forestry
88 research (see Ball *et al.* 1994; de Carvalho *et al.* 2005; Bussotti *et al.* 2010 and citations therein)
89 and, more in general, in forest ecology studies (see, for ex. Stylinski *et al.* 2002; Einhorn *et al.*
90 2004). Nevertheless, the application of ChlF in extensive terrestrial field surveys on tall trees
91 remains problematic (Mohammed *et al.* 1995; 2003; Sampson *et al.* 2000).

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

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

104

105 **MATERIAL AND METHODS**

106 Experimental sites

107 The study was carried out in two experimental plantations, both included in previous projects.

108 Kaltenborn (Thuringia, Germany) is a part of the BIOTREE experiment (Scherer-Lorenzen *et al.*
109 2005b, 2007), and Satakunta (Finland) belongs to the TreeDivNet platform (Scherer-Lorenzen *et al.*
110 2005). For details of the experimental sites see the supplementary materials.

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

118

119 Sampling

120 Sampling at Kaltenborn was done on June 23-25, 2011. Eight trees per species per plot were
121 selected, taking into account the neighboring tree species. At Satakunta sampling was done on July
122 11-14, 2011. Five trees per species per plot were randomly chosen. ChlF measurements were
123 replicated on 5 different leaves per tree.

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

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

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

147

148 Chlorophyll a fluorescence transient analysis and parameters

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

153 saturating light pulse (intensity $>3000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, excitation light of 650 nm), are called
 154 “fluorescence transients” (FT, direct or prompt fluorescence, Strasser *et al.* 2000, 2004, 2010; see
 155 also Stirbet and Govindjee 2011) and represent the fast phase of ChlF induction. Plotted on a
 156 logarithmic time scale, FT shows a polyphasic behaviour. The different time-steps of this
 157 polyphasic transient are labelled as: O (20-50 μs), J (2 ms), I (30ms) and P (peak). The latter
 158 indicates the highest fluorescence intensity (F_M), when saturating light is used, and is generally
 159 obtained around 0.8s. The parameters considered in this study are:

- 160 - $F_V/F_M = [F_M - F_0]/F_M = \phi_{P_0} = TR_0/ABS =$ maximum quantum yield of PSII primary
 161 photochemistry that is measured in samples in dark-adapted state. F_V/F_M expresses the
 162 probability that an absorbed photon will be trapped by the PSII reaction center.
- 163 - $\Psi_{E_0} = ET_0/TR_0 = 1 - V_J = 1 - (F_{2\text{ms}} - F_0)/(F_M - F_0)$. Ψ_{E_0} expresses the probability that the
 164 energy of a trapped excitation is used for electron transport beyond Q_A . V_J represents the
 165 relative variable fluorescence at 2 ms (transients normalized between F_0 and F_M);
- 166 - $\Delta V_{I-P} = 1 - V_I = (F_M - F_{30\text{ms}})/(F_M - F_0)$ (I-P phase, Oukarroum *et al.* 2009). ΔV_{I-P} represents
 167 the relative contribution of the I-P phase to the fluorescence transient OJIP; it is regarded as
 168 a measure for the efficiency of electron flux through PSI to reduce the final acceptors of the
 169 electron transport chain, i.e. ferredoxin and NADP. V_I indicates the relative variable
 170 fluorescence at 30 ms (transients normalized between F_0 and F_M);
- 171 - PI_{tot} (Performance Index total). PI_{tot} is the potential for energy conservation from photons
 172 absorbed by PSII to the reduction flux (RE) of PSI end acceptors. It is a multiparametric
 173 expression that combines four parameters related to the photosynthetic activity: (1) the
 174 density of reaction centers; (2) the quantum yield of primary photochemistry; (3) the ability
 175 to feed electrons into the electron chain between PSII and PSI; (4) the efficiency with which
 176 an electron can move from the reduced intersystem electron acceptors to the PSI end
 177 electron acceptors (Strasser *et al.* 2004, 2010).

178 $PI_{\text{tot}} = (RC/ABS) [\phi_{P_0}/(1 - \phi_{P_0})] [\Psi_{E_0}/(1 - \Psi_{E_0})] [\delta_{R_0}/(1 - \delta_{R_0})]$

179 where

$$180 \quad RC/ABS = \phi_{Po} (V_J/M_0)$$

$$181 \quad \text{where } M_0 = [4 (F_{300\mu s} - F_0)/(F_M - F_0)]$$

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

184 $\delta_{Ro} = (1 - V_I)/(1 - V_J) = (F_M - F_I)/(F_M - F_J)$. δ_{Ro} is the probability that an electron is
185 transported from reduced PQ to the electron acceptor side of PSI.

186

187 Leaf morphology

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

193

194 Data analysis

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

205 levels were represented only by 1 plot. Pearson's correlation coefficient was calculated to analyze
206 the relationships between the photosynthetic performance of current and previous year needles in
207 coniferous species at Satakunta. Linear regression was used to test the relationships between ChlF
208 parameters and leaf morphology traits, with tree species richness expressed by the Shannon Index
209 calculated on the basal area for each species per plot (Staddon *et al.* 1997; Spellerberg & Fedor
210 2003). The differences in the fluorescence parameters between the monocultures of each species
211 were analyzed by one-way ANOVA for Kalternborn and by non-parametric statistics (Kruskal-Wallis
212 test) for Satakunta. In order to define an indicator of PI_{tot} at plot level, we calculated (according to
213 Bonal *et al.* 2000), PI_{tot_plot} as:

214

$$215 \quad PI_{tot_plot} = \frac{\sum (PI_{tot_spi} \times BA_i)}{\sum BA_i}$$

216

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

219

220 **RESULTS**

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

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

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

232 The species-specific characteristics of the different tree species - obtained by comparing the
233 monocultures - are shown in Table 2 (Satakunta) and Table 3 (Kaltenborn). At Satakunta (Table 2)
234 *B. pendula* showed the highest values of F_V/F_M but, overall, *P. sylvestris* was the best performing
235 tree species (higher PI_{tot} , Ψ_{E0} and ΔV_{I-P}). At Kaltenborn (Table 3) the best performing tree species
236 was *P. menziesii* (all fluorescence parameters examined were higher in this species).

237 The effect of tree species richness was tested with GLM analysis (Table 4) and post-hoc
238 comparison (Tables 2, 3), whereas the role of biodiversity level (expressed by the Shannon Index)
239 was evaluated with linear regressions (Table 5). The results show different patterns of each analyzed
240 ChlF parameter for each tree species. At Satakunta the most sensitive parameters were F_V/F_M and
241 the Ψ_{E0} in *B. pendula* (both negative, decreasing as the Shannon Index increased) and *P. abies* (both
242 positive, increasing as the Shannon Index increased). The ΔV_{I-P} was increased in mixed plots of *B.*
243 *pendula*. F_V/F_M increased also in *P. sylvestris* at Satakunta, but decreased at Kaltenborn in *F.*
244 *sylvatica*, *P. abies* and *P. menziesii* with increasing Shannon Index. Other significant patterns were
245 the increasing of PI_{tot} in *P. abies* at Satakunta, and the increase of the ΔV_{I-P} and PI_{tot} in *P. menziesii*
246 at Kaltenborn.

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

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

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

257

258 **DISCUSSION AND CONCLUSION**

259 The survey presented in this paper took into account different tree species co-occurring in
260 experimental plots with different levels of tree species richness. A first result was to highlight
261 specific ChlF properties of the different tree species examined. These properties are not directly
262 connected to growth, but may reflect the strategies to cope with stress and carbon allocation
263 (Marshall *et al.* 2011). In general, conifers performed better than broadleaf species, with higher
264 levels of PI_{tot} . The parameters which revealed the most evident responses connected to species
265 diversity were F_v/F_M and the Ψ_{Eo} . F_v/F_M expresses the maximum quantum yield of PSII primary
266 photochemistry. It is well known that this parameter is only scarcely responsive to the action of
267 several stress factors, such as drought stress (Cornic & Fresnau 2002), but it is very sensitive to the
268 light environment and especially to excess light (Adams & Demmig-Adams 2004). Leaves grown in
269 high light conditions (sun leaves) are efficient in dissipating energy as heat (Ballottari *et al.* 2007).
270 This phenomenon is partly due to photoinhibition, which involves the deactivation and turnover of
271 the protein D1 in PSII (Ohira *et al.* 2004). The concomitant decrease of the capacity to trap solar
272 energy and to feed the electron transport chain is considered a down regulation mechanism (Adir *et*
273 *al.* 2003; Cui *et al.* 2003; Stroch *et al.* 2008). In fact it reduces the flow of electrons within the
274 electron transport chain when the reduction potential, originating from high light intensity, is too
275 high for the needs of the photosynthesis processes and cannot be utilized for metabolism (Lu *et al.*
276 2001; Ogaya *et al.* 2011).

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

281 richness, the canopy structure is more irregular because of the different growth rates and growth
282 forms of the different tree species. Kaitaniemi and Lintunen (2010) reported that in *B. pendula* the
283 increase in height was accelerated by competition with *L. sibirica* and *P. sylvestris* in mixed
284 experimental forest stands in Finland. In *L. sibirica*, on the other hand, the average height increment
285 was reduced by competition with *B. pendula* in the same study. These differences in growth create a
286 large heterogeneity of light availability at the canopy level. Because of an increasing admixture of
287 smaller trees, the top leaves of *B. pendula* are exposed to increasing average light intensities. Unlike
288 *B. pendula*, at Satakunta the F_V/F_M and Ψ_{E_0} of *P. abies* increased with increasing species richness.
289 This species has a lower growth potential in height than *B. pendula*, *L. sibirica* and *P. sylvestris*.
290 Consequently in mixed stands *P. abies* was found under the canopies of the tallest tree species, with
291 lower light availability and absence of photoinhibitory conditions. In both tree species, *B. pendula*
292 and *P. abies*, the CV of F_V/F_M increased with decreasing values of F_V/F_M and Ψ_{E_0} (Table 2),
293 confirming the relevance of the heterogeneity of light environments in mixed plots.

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

300 At Kaltenborn F_V/F_M values decreased in *F. sylvatica* with increasing tree species richness.
301 This pattern is apparently in contrast to the availability of light in the different mixture conditions.
302 In fact, the monoculture of *F. sylvatica* is made up of small trees isolated from each other and
303 exposed to full sunlight at midday. This pattern can be compared to the competition processes in the
304 first phases of establishment in a mixed forest stand. Many studies have shown that in its juvenile
305 stage *P. abies* is very competitive in relation to *F. sylvatica* both aboveground (Kozovits *et al.* 2005;
306 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 Ψ_{E_0} 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

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

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

342

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Table 1.

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

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

Table 2.

Satakunta. Fluorescence parameters per each species in the different tree species richness level (mean \pm 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.

Specie	mixture	F_v/F_M			Ψ_{Eo}			ΔV_{I-P}			PI_{tot}		
		mean	err.st	CV	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV
<i>B.pendula</i>	monoculture	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
<i>A.glutinosa</i>	monoculture	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
<i>P.abies</i>	monoculture	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
<i>L.sibirica</i>	monoculture	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
<i>P.sylvestris</i>	monoculture	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 $F_v = F_M - F_0$, 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 \pm 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.

Specie	mixture	Fv/Fm			Ψ_{E_0}			$\Delta V_{I,p}$			PI _{tot}		
		mean	err.st	CV	mean	err.st	CV	mean	err.st	CV	mean	err.st	CV
<i>F.sylvatica</i>	monoculture	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
<i>Q.petraea</i>	monoculture	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
<i>P.abies</i>	monoculture	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
<i>P.menziesii</i>	monoculture	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}	ΔV_{I-P}	PI_{tot}
Satakunta				
<i>B.pendula</i>	<0.01	<0.001	<0.01	<0.05
<i>A.glutinosa</i>	-	-	-	-
<i>P.abies</i>	<0.01	<0.01	-	-
<i>P.sylvestris</i>	-	<0.001	<0.05	-
<i>L.sibirica</i>	-	-	-	-
Kaltenborn				
<i>F.sylvatica</i>	<0.01	-	-	-
<i>Q.petraea</i>	-	<0.01	-	<0.01
<i>P.abies</i>	<0.001	<0.05	-	-
<i>P.menziesii</i>	-	<0.01	<0.001	<0.001

1 **Table 5.**

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

5

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

6

7

8 **Table 6.**

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

11

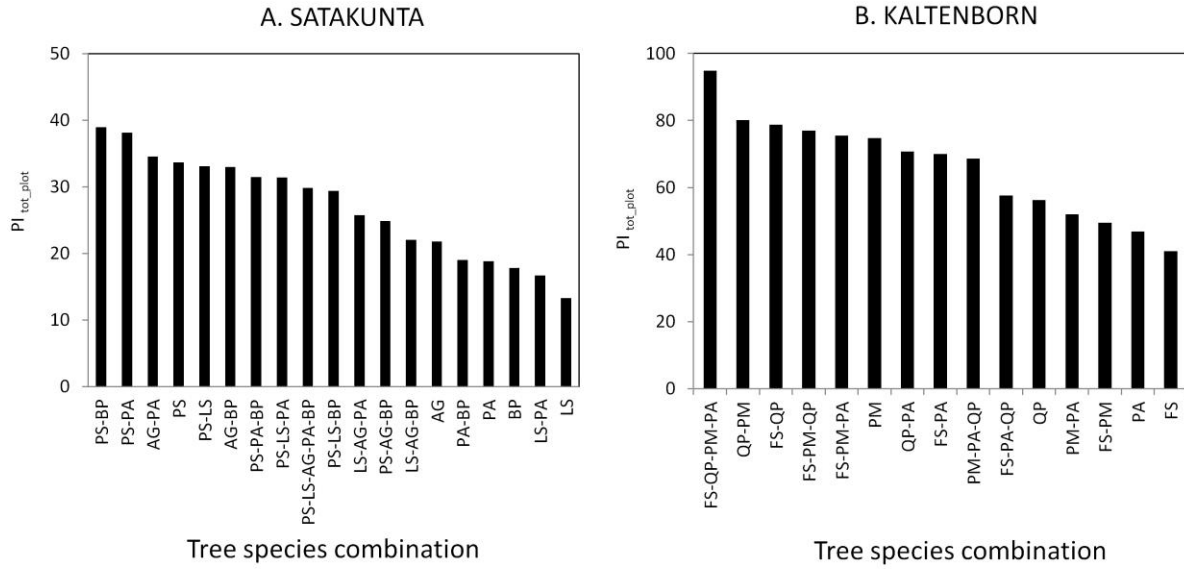
12

	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

13

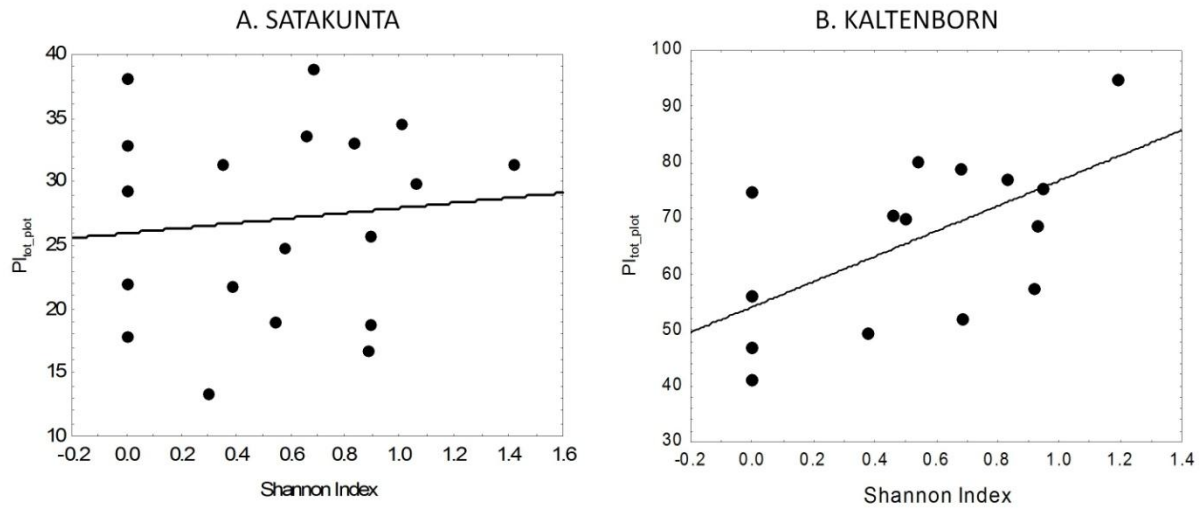
14

FIG.1



15
 16 **Fig.1.** Rank of the PI_{tot} at plot level (PI_{tot_plot}) in relation to tree species combination in Satakunta
 17 (A) and Kaltenborn (B). BP – *Betula pendula*; AG – *Alnus glutinosa*; LS – *Larix sibirica*; PA –
 18 *Picea abies*; PS – *Pinus sylvestris*; FS – *Fagus sylvatica*; PM – *Pseudotsuga menziesii*; QP –
 19 *Quercus petraea*.
 20

FIG.2



22

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

26

27 SUPPLEMENTARY MATERIAL

28 Description of experimental sites

29 The experimental site at Kaltenborn (Thuringia, Germany, 10°13'E, 50°47'N; elevation 32 m asl)
30 was planted in winter 2003/2004 as part of the BIOTREE experiment (Scherer-Lorenzen *et al.*
31 2005b, 2007). The soil is acid arenosol on sandstone bedrock. The climate is Sub-Atlantic with a
32 mean annual temperature of 7.8°C and mean annual precipitation of 650 mm. Until 1975 the site
33 was used as cropland and then converted to grassland. Saplings of four tree species were planted in
34 plots of 1, 2, 3, and 4-species mixtures. The desired species mixture was achieved by patch planting
35 (patches of 8x8 m). The species planted were: European beech (*Fagus sylvatica* L., FS), sessile oak
36 (*Quercus petraea* Liebl., QP), Norway spruce (*Picea abies* (L.) Karst., PA) and Douglas fir
37 (*Pseudotsuga menziesii* Franco, PM). The present study was carried out in 15 plots, representing
38 different tree species richness levels and species combinations (Table 1). *P. menziesii* was the tallest
39 tree species (3-4 m height), followed by *P. abies*. Among the deciduous broadleaved species *Q.*
40 *petraea* trees were higher (2-3 m) than *F. sylvatica* (1-2 m). Unlike conifers, the broadleaved trees
41 stand separately without crown interaction.

42 The Satakunta forest diversity experiment (Finland, 61°N, 22°E, elevation 20-50 m asl) was
43 established in spring 1999 on three clear-cut areas about 1.5 ha each; it belongs to the TreeDivNet
44 platform (Scherer-Lorenzen *et al.* 2005). The sites are within the boreal coniferous forest, where the
45 soil is podzolic and the climate is Subarctic, with cold winters and no dry season. The mean annual
46 temperature is 5.0°C and the mean annual precipitation is 585 mm. Each experimental area included
47 38 plots (each 20m x 20m), which were randomly allocated to 19 treatments; the plots represent
48 monocultures as well as mixtures of up to five tree species. The species used in the experiment
49 were: silver birch (*Betula pendula* L., BP), European black alder (*Alnus glutinosa* (L.) Gaert., AG),
50 Norway spruce (*Picea abies* (L.) Karst., PA), Scots pine (*Pinus sylvestris* L., PS) and Siberian larch
51 (*Larix sibirica* Ledeb., LS). This study was carried out in 19 plots, representing different levels of
52 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

58 **Table S1**

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

62 F_v/F_M : maximum quantum yield of PSII primary photochemistry; Ψ_{E_0} : efficiency of an electron to
 63 move from reduced Q_A , the secondary PSII electron acceptor, into the electron transport chain; ΔV_{I-P}
 64 : the efficiency to reduce the final acceptors beyond the PSI; PI_{tot} , the total performance index for
 65 (potential) energy conservation from photons absorbed by PSII to the reduction flux of PSI end
 66 acceptors.

67

		<i>P. abies</i>	<i>P. sylvestris</i>
F_v/F_M	r	0.79	0.40
	p	<0.001	<0.05
	r^2	0.62	0.16
Ψ_{E_0}	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

68

69 **Table S2**

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

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

77

	F _v /F _M		Ψ _{Eo}		ΔV _{L-P}		PI _{tot}	
	A.Trees	B.Plot	A.Trees	B.Plot	A.Trees	B.Plot	A.Trees	B.Plot
Satakunta								
<i>B.pendula</i>	1.75	1.62	5.75	7.08	16.27	14.94	32.01	30.43
<i>A.glutinosa</i>	1.67	1.69	5.55	7.79	12.35	18.89	32.76	50.98
<i>P.abies</i>	1.24	1.27	4.57	5.15	12.84	13.27	35.24	33.02
<i>P.sylvestris</i>	0.90	0.78	3.85	3.21	9.05	9.33	30.74	27.48
<i>L.sibirica</i>	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								
<i>F.sylvatica</i>	3.54	3.72	15.14	18.11	15.04	18.51	47.96	54.54
<i>Q.petraea</i>	2.11	2.36	10.04	13.97	13.57	16.69	41.36	46.25
<i>P.abies</i>	3.18	2.66	9.83	8.39	17.85	13.43	57.62	51.02
<i>P.menziesii</i>	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

78

79