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Comparison of photosynthetic performance of *Fagus sylvatica* seedlings under natural and artificial shading

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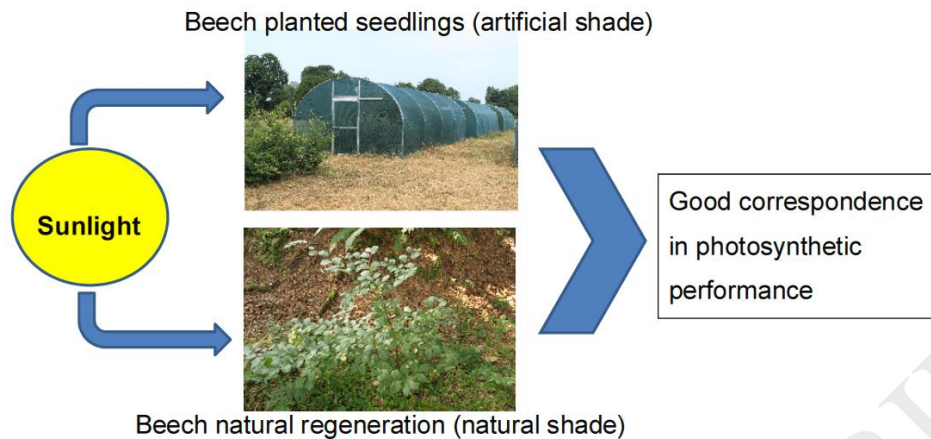
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Graphical Abstract



Highlights

- Physiological traits of beech seedlings tested under natural and artificial shade
- Increasing shade resulted in lower photosynthetic rates
- Good correspondence between natural and artificial shade results

Abstract

Commitment to sustainable forest management (alternatives to clearfelling) has led to a renewed interest in continuous cover forestry systems, which promote the control of light to produce stand benefits. Physiological performance of shade-tolerant European beech (*Fagus sylvatica* L.) in response to light availability was investigated in natural regeneration below the canopy in contrast to planted seedlings under artificial-shade conditions. Although beech seedlings had higher photosynthetic capacity with increasing light availability, they were able to maintain positive CO₂ assimilation rates under low light levels in both field and controlled conditions. Leaves of seedlings under low light had the ability to use light more efficiently (higher PSII efficiency) than those in high light, which offer some physiological explanation for the ability of beech seedlings to grow under

very low light conditions. Whilst caution is advised to interpret results from controlled to field studies, the overall general correspondence in the trend of the physiological response to light levels within beech grown below the canopy and under artificial-shade conditions suggests that it might be possible to extrapolate results from studies performed under artificial shade (nets) to field conditions. Hence, the use of nets may be an alternative way of assessing the potential physiological responses of seedlings to light availability.

Keywords: common beech; light availability; physiology; natural shading; artificial shading.

1. Introduction

European beech (*Fagus sylvatica* L.) is a dominant late successional species covering a large geographic area of Europe. Beech is an important tree in Europe, in terms of ecology and also commercial value. Although beech is not a native broadleaf species in some parts of Europe, such as Ireland and north of England, it has become widely naturalised there (Joyce *et al.*, 1998). For trees growing in a forest understory shade has been considered an important factor that limits growth and survival (Chen, 1997). Besides light availability, other resources such as water and nutrients may be also important for seedling performance in the understory (Kloppel *et al.*, 1993; Abrams and Mostoller, 1995; Walters and Reich, 1997). However, it is possible to artificially manipulate light levels in a forest. For example, canopy gaps can result from silvicultural practices (e.g., thinning), as well as from natural causes (e.g., windblow, storm

damage). Tree seedlings can respond to changes of light conditions by modifying several traits to increase light utilisation, including leaf physiology (Bazzaz, 1979). European beech is considered to be a shade-tolerant species, being able to grow under shade levels as low as 5% of full sunlight (Niinemets and Valladares, 2006).

Silvicultural methods can be successfully used to manipulate the growth of beech stands, with natural regeneration commonly being used (provided seed source is adequate) to restock the stand (Wagner *et al.*, 2010). Forest management objectives in Europe currently include sustaining multiple services and values from forests (FAO, 2010), often by using continuous cover forestry (CCF) silvicultural systems (Vítková and Ní Dhubháin, 2013), which promote the full use of natural dynamic forest processes (e.g. natural regeneration). There is also interest in reducing regeneration and management costs, while also maintaining structural and species diversity and producing high quality timber (Diaci and Kozjek, 2005). CCF promotes forest management which optimises the maintenance, conservation and use of forest ecosystems in such a way that the ecological and socio-economic functions are sustainable and profitable (Pro Silva, 2012). Therefore, where practical and appropriate, natural regeneration should be the preferred method of regenerating broadleaf stands, since it offers many benefits in terms of costs, genetics, silviculture and the environment (Joyce *et al.*, 1998; Brang *et al.*, 2014).

Photosynthesis is a physiological process of primary importance for plants and the photosynthetic response of leaves and physiological plasticity to light availability are of great interest. Previous studies have considered the influence of light availability on the physiological responses of beech seedlings (Tognetti *et al.*

1997; Valladares *et al.* 2002; Parelle *et al.*, 2006; Balandier *et al.*, 2007; Čater and Simončič, 2009; Gardiner *et al.*, 2009; Čater, 2010). These authors reported that beech seedlings acclimate to shade, such as by decreasing maximum photosynthetic rates (Valladares *et al.*, 2002; Čater and Simončič, 2009; Gardiner *et al.*, 2009) and electron transport rates (Parelle *et al.*, 2006; Balandier *et al.*, 2007) with increasing shade levels. While most of these studies were carried out exclusively under artificial shade (Tognetti *et al.* 1997; Valladares *et al.* 2002) or natural conditions (Balandier *et al.*, 2007; Čater and Simončič, 2009; Gardiner *et al.*, 2009; Čater, 2010), only Parelle *et al.* (2006) examined beech acclimation to shade under both natural and controlled conditions. Although studies conducted under artificial shade may provide useful information on the physiological responses to light availability, such experiments may also have some drawbacks, especially if the results are to be extrapolated to field conditions. Firstly, the light conditions in the forest understory are heterogeneous, which can have important implications for most growth, physiological and morphological traits (Wayne and Bazzaz, 1993). Secondly there are confounding factors associated with measuring responses to variable light availability, such as variation in water and nutrient availability (Johnson *et al.*, 1997; Aranda *et al.*, 2002), temperatures (Küppers and Schneider, 1993), competition effects (Coll *et al.*, 2004) and other factors under a forest canopy that may also limit physiological responses. Therefore, more research is required to determine whether the physiological responses to light availability in seedlings growing under artificial shade differ from that of naturally regenerated seedlings growing under natural shade conditions in the field.

In this study, the physiological responses of beech seedlings were studied in a naturally regenerated site under different light regimes in a forest and in a shadehouse experiment. Results from both sites were examined to determine light levels that optimise the photosynthetic performance of beech seedlings. The main objective was to determine whether beech seedlings responded similarly under natural-shade as compared with artificial-shade conditions.

2. Material and methods

2.1. Shadehouse study

The study was conducted in a controlled-shade experiment located at Teagasc Ashtown Food Research Centre, D15 KN3K, Ireland (53° 22' 45" N, 6° 20' 13" W). The mean annual total rainfall in the area is 785 mm and the mean annual air temperature is 9.6 °C (all means are from the period 1999-2014). In 2014, the year this study was conducted, temperatures reached a mean temperature of 10.4 °C and the area received 885 mm of annual rainfall. Meteorological data were collected by a Weather Station located 1.93 km away from the study site (Met Éireann, Phoenix Park station). Additional details of the shadehouse study are presented in Table 1.

The experimental design was a randomised block design with split-plots: light as the whole plot factor (4 treatments) and species as subplot factors (each subplot corresponding to beech or pedunculate oak, *Quercus robur*), replicated

across 5 blocks. Seedlings were planted in each subplot at 0.5 X 0.5 m. Shade was provided with green polythene nets (Colm Warren Polyhouses Ltd., Kilmurray, Trim, Co. Meath, Ireland) erected on frames to simulate different light environments in September 2012, about one year and half after the seedlings were planted. Four different light treatments were applied in each block (one treatment per plot): full sunlight (no shade), moderate shade, medium shade and heavy shade. Each subplot comprised a total of 36 seedlings, of which 20 seedlings were used as a guard row to buffer potential 'edge effects'. Three beech seedlings from the central zone of each subplot were randomly selected and used for physiological assessment. Additional details relative to the study site and experimental design were published in Sevillano *et al.* (2016).

2.2. Field study

The field observations were conducted in Knockrath Forest, which is located in the Vale of Clara between Laragh and Ruthdrum, Co. Wicklow, Ireland (52° 57' 13" N, 6° 14' 32" W). There is a long history of forest management at Knockrath using a wide range of conifer and broadleaved species, both as pure stands and in mixtures. Since it is located adjacent to the Wicklow Mountains National Park, the Vale of Clara nature reserve, the Avonmore River (a recovering salmonid habitat) and is in an important scenic and recreational area, Knockrath Forest is in the process of conversion to CCF management which aims to achieve the multipurpose objectives, including recreation, amenity, timber production, carbon sequestration and biodiversity.

The soil type is an acid brown earth. Mean temperatures range from 5.7 °C in January to 15.8 °C in July, with a mean annual temperature of 10.2 °C, based

on data obtained for the nearby Glenealy weather station (Met Éireann, Glenealy), located 13 km from the site (all means are from the period 1999-2014). The region receives 1213 mm in average annual precipitation. Additional details of the field site are presented in Table 1.

The stand is composed of beech, Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Scots pine (*Pinus sylvestris* L.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Norway spruce (*Picea abies* (L.) Karst.). The site has abundant natural regeneration of beech seedlings. The plantation age is mixed and the mature beech from which the regeneration arises is estimated to be approximately 110 years old, but the trees vary in age. Beech regeneration is of mixed age but generally from 1 to 15 years old.

Five small research plots of 3 m radius were laid down to cover a range of different light regimes, from closed canopy to open gaps. Five beech seedlings 150–200 cm in height were randomly selected in each plot and used for physiological measurements. The selected seedlings were approximately the same size as those used in the shadehouse study.

2.3. Light availability

Light availability in each plot was evaluated using the method described in Parent and Messier (1996) and verified for mixed-species stands with irregular canopies (Messier and Parent, 1997; Gendron *et al.*, 1998). These authors showed that an

instantaneous measurement of the percentage of above-canopy PAR (400–700 nm) taken under overcast sky conditions provides an accurate estimate of the mean daily percentage of PAR reaching a location in the understory (%PAR). Incident above-canopy PAR (PAR_a) was estimated by installing a point quantum sensor (LI-190SA, LI-COR Inc., Lincoln, Nebraska) on a tripod in a large adjacent open gap for the field study or outside the shadehouses for the controlled-shade experiment. The quantum sensor was located as far as possible from the mature stand or the shadehouses to minimise any interaction. A second line quantum sensor (LI-191SA, LI-COR Inc., Lincoln, Nebraska) was used to measure received PAR of seedlings in the understory or below the nets (PAR_u) in each plot. Both sensors were linked to a datalogger (LI-1400, LI-COR Inc., Lincoln, Nebraska). The datalogger was set to record PAR every second over a 0.5 min period. Measurements were carried out in each research plot on overcast days in September 2014. The time of each measurement was recorded and %PAR was calculated as $(PAR_u/PAR_a) \times 100$, where PAR values were recorded at the same time. To cross calibrate both sensors, PAR readings were recorded using both sensors in the open area before measurements. The ratio between the point and line quantum sensor was calculated to correct line quantum sensor readings. Percentages of PAR reaching beech seedlings in the field and in the shadehouses are presented in Table 2.

2.3. Physiological measurements

CO_2 assimilation rate (A , $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$) and transpiration rate (E , $\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$), expressed on a leaf area basis, were measured in September 2014 with a portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, Nebraska, USA) on

previously tagged beech seedlings. Measurements were carried out on fully expanded, non-senescent and healthy leaves from the upper terminal shoot of each seedling and leaves were kept as close to their natural position as possible during measurements. In each plot, leaf gas exchange measurements were recorded under ambient conditions of air temperature, humidity and PAR, with the reference CO₂ concentration maintained at 400 $\mu\text{mol mol}^{-1}$. Gas exchange measurements were also carried out at common light levels in all plots: 1500 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. These PAR values were used because data collected during gas exchange measurements in the shadehouse experiment revealed that photosynthesis of beech seedlings was saturated at around 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and differences in photosynthesis became apparent at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. While conducting these measurements, CO₂ concentration was kept at 400 $\mu\text{mol mol}^{-1}$, block temperature was set to 25°C and relative humidity was around 50%. Values were recorded after short adaptation when CO₂ exchange had remained stable. The ratio of A to E and A to g_s were calculated to determine instantaneous (A/E , $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$) and intrinsic (A/g_s , $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$) water use efficiency, respectively.

Chlorophyll fluorescence was measured simultaneously with gas exchange under ambient conditions using the portable LI-6400XT equipped with a leaf chamber fluorometer LI-6400-40 (LI-COR Inc., Lincoln, Nebraska, USA). Under known light conditions, the steady-state level of fluorescence (F'), the maximum fluorescence (F'_m) and the minimal fluorescence (F'_o) were estimated according to common protocols for fluorescence analysis at a known light intensity (Murchie and Lawson, 2013). F'_o and F'_m were determined by applying a dark and a saturating pulse to a light-adapted leaf, respectively. The operating efficiency of

photosystem II (Φ_{PSII}), PSII maximum efficiency (F'_v/F'_m) and photochemical quenching (qP) were calculated as $(F'_m - F)/F'_m$, $(F'_m - F'_o)/F'_m$ and $(F'_m - F)/(F'_m - F'_o)$, respectively. The photosynthetic electron transport rate (ETR, $\mu\text{mol (e}^-) \text{ m}^{-2} \text{ s}^{-1}$) was calculated as $\Phi_{\text{PSII}} \times f \times I \times \alpha_{\text{leaf}}$, where f is the fraction of absorbed quanta that is used by PSII, I is the incident PAR and α_{leaf} is the leaf absorptance (LI-COR, 2011). f was assumed to be 0.5 (Laisk and Loreto, 1996) and the average value of α_{leaf} for green leaves of 0.84 was used (Björkman and Demmig, 1987).

2.4. Phenotypic plasticity

Plasticity of physiological traits for beech seedlings were calculated based on the phenotypic plasticity index, PI_v (Valladares *et al.*, 2006). This index, ranging from zero to one, is the difference between the minimum and the maximum mean values of a trait divided by the maximum mean value (Valladares *et al.*, 2000). This index allows changes in traits expressed in different units to be compared. Mean physiological plasticity was the average plasticity value for all physiological traits.

2.5. Data analysis

All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Physiological responses were analysed using the MIXED procedure of SAS with light availability considered a fixed effect. Regression analysis was used to determine if a trend was detectable and nominal light availability explained most of the variation in the responses. The light availability was treated as a

quantitative variable that explained the photosynthetic response. A linear regression as a function of available light was fitted for each physiological trait. All tests for significance were conducted at $p < 0.05$.

Additionally, Pearson correlations were carried out to identify linear relationships between A and ETR at ambient conditions.

3. Results

3.1. Leaf gas exchange and chlorophyll fluorescence

The photosynthetic capacity and stomatal conductance of beech seedlings at saturating light (A_{1500} and g_{s1500} , respectively) were significantly influenced by light availability in both field and shadehouse studies (Table 3). In contrast, water use efficiency at saturating light was not affected by light levels (Table 3). In both experimental situations A_{1500} and g_{s1500} increased with increasing light availability (Table 3), and beech seedlings exhibited the lowest A_{1500} and g_{s1500} under low light conditions (Fig. 1A and 1B).

Light available in the field or shadehouse study did not influence photosynthetic rate, stomatal conductance or water use efficiency of beech seedlings at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Table 3).

At ambient PAR, beech seedlings exhibited similar trends for photosynthetic rate (A_{amb}) and ETR, with values for both variables increasing as light levels increased in both studies (Table 3; Fig. 1C and 1G). A_{amb} was significantly and positively correlated with ETR (Fig. 2). In contrast, Φ_{PSII} , F'_v/F'_m and qP decreased with increasing light availability (Table 3; Fig. 1D, 1E and 1F).

The responses (slopes) of most physiological traits to light availability were greater for seedlings grown in the shadehouse experiment than those in the field, with the exception of A_{1500} and g_{s1500} (Table 3). Overall, a similar response was found in the physiological responses to light availability of beech seedlings grown in the field and shadehouse study (Table 3, Fig. 1). However, the regression line for seedlings in the field was clearly under the values of the shadehouse study for photosynthetic rates and ETR (Fig. 1A, 1C and 1G). In contrast, the efficiency of the PSII was lower in the shadehouse study than in the field (Fig. 1D, 1E and 1F).

3.2. Phenotypic plasticity

Physiological plasticity of beech seedlings in response to light availability was greater under ambient PAR than under controlled conditions (500 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) (Table 4). In response to the controlled light conditions applied (1500 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), beech seedlings had greater phenotypic plasticity at saturating light than at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4). The variables ranged from those with higher plasticity index (e.g., photosynthetic rate, stomatal conductance and electron transport rate) to those with lower plasticity values (e.g., most chlorophyll fluorescence variables) (Table 4). Water use efficiency had variable plasticity depending on the PAR conditions used during measurements, i.e. high plasticity index under ambient PAR but far lower plasticity values under 500 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Table 4).

Averaging the plasticity index for all variables showed a value 27% higher in the field than in the shadehouse study.

4. Discussion

Overall, the trend in the physiological responses (photosynthetic capacity, water use efficiency and chlorophyll fluorescence) to light availability observed in this study was similar in the field and under artificial-shade conditions. Same physiological pattern in response to light was observed for seedlings in the field and in the shadehouse experiment under fixed (500 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) and ambient PAR.

In both sites (field and shadehouse study), the increase of photosynthetic capacity with increasing light agrees with the results reported in the literature under field conditions in planted (Balandier *et al.*, 2007; Gardiner *et al.*, 2009) and naturally regenerated (Parelle *et al.*, 2006; Čater and Simončič, 2009; Čater, 2010) beech seedlings.

The objective of this study was to compare the physiological performance in response to light availability of seedlings grown in the field (natural light gradient) and in a controlled-shade environment (shade levels obtained artificially with nets). In fact, there was correspondence in the trend of the physiological responses to shade of beech seedlings grown under controlled and field conditions. Similarly, Parelle *et al.* (2006) reported that maximum carboxylation rates and ETR decreased with shade in natural regeneration (field conditions) and potted saplings (controlled) of beech. These findings suggest that studies conducted under artificial shade (using nets) could be used to investigate the

physiological response (gas exchange and chlorophyll fluorescence) of seedlings to light availability. However, lower photosynthetic rates and higher PSII efficiencies were found in the field than in the shadehouses. The degree of plasticity was slightly higher for seedlings grown in the field than in the shadehouses, which could be due to the fact that seedlings in the field experienced lower light availability in the densest shade. In contrast to the results of this study, Wayne and Bazzaz (1993) found that maximum net photosynthesis of two birch species (*Betula populifolia* and *B. alleghaniensis*) was higher in gap (heterogeneous light) than in shadehouse (uniform light) environments. One possible reason for this difference in our study is that seedlings growing in the gaps were considered to have received 100% PAR, given that it was not possible to find an area close to the stand that received full sunlight, whereas the control seedlings in the shadehouse experiment received full sunlight. Therefore, PAR values were always much lower in the field than in the controlled experiment at similar %PAR. In fact, PAR absolute values during the physiological measurements were lower in the field than in the shadehouse experiment, while other environmental parameters that could affect physiological performance (such as vapour pressure deficit, relative humidity or leaf temperature) showed similar values. Although these changes could be mainly attributed to different PAR absolute values, the fact that shadehouses did not alter light quality and the light regime provided by the nets is more uniformly than that found in the forest understory should also be considered. Forest canopies might also alter light quality in the understory (Holmes, 1981) and, therefore, these different photosynthetic values between the field and controlled conditions could have also been due to changes in light quality. The downside of the nets used in this study

is that while they may reduce overall irradiance in a similar way to a forest canopy, they might not have an equivalent effect on the quality of transmitted light.

In addition to light, there are also other factors that might contribute to explain the difference observed between field and shadehouse seedlings. One hypothesis may be that seedlings in the field and the shadehouses were at different stages of development (plants with different ages). Although several studies have reported a reduction of photosynthesis and stomatal conductance with tree age (Fredericksen *et al.*, 1996; Niinemets, 2012), exceptions to the general trends are also found in the literature (Bond, 2000). Since tree size usually increases with tree age and it is not easy to identify or separate variation by age from variation by size (Bond, 2000), we tried to minimise the age/size effect on the physiological responses by choosing seedlings of similar size. That difference between the field and the shadehouse study could also be attributed to the rainfall received in both sites (Table 1). For example, Robson *et al.* (2009) reported that drought stress adversely affected photosynthetic performance of beech seedlings in the understory, but there was no evidence of water stress in the current study. In fact, shadehouse seedlings received less rainfall than seedlings the field, so, if water availability was a major issue, shadehouse seedlings should have showed lower photosynthetic rates than field seedlings, according to previous studies (Tognetti *et al.*, 1995; Robson *et al.*, 2009). It is known that beech populations (different provenances) might differ in their photosynthetic performance in response to light availability (Tognetti *et al.*, 1997, 1998). Although beech seedlings showed a similar pattern in response to changing light conditions, provenances from northern Italy were found to be more

susceptible to photoinhibition than provenances from southern Italy and, therefore, southern provenances showed generally greater maximum photosynthetic rates than northern provenances (Tognetti *et al.*, 1997, 1998). Unfortunately, it was not possible to find out provenance details of the field study in the current work, although it is likely that plant material was sourced from British provenances (Huss *et al.*, 2016). Therefore, we cannot state if seedling provenance could be a factor to explain lower photosynthetic rates and greater PSII efficiencies in the field than in the shadehouses. Other parameters that should be considered when studying physiological performance of beech seedlings grown on different sites are soil conditions (Johnson *et al.*, 1997; Pröll *et al.*, 2016) and vegetative competition (Fotelli *et al.*, 2001; Coll *et al.*, 2004). For example, soil water content, which can be greatly reduced when grass competition is an issue (Coll *et al.*, 2004), might affect ecophysiological performance of beech seedlings limiting photosynthetic rate and stomatal conductance (Pröll *et al.*, 2016). In both sites (field and shadehouses), vegetation competition was low and soils were good ones with no sign of nutrient deficiency, so unlikely to be an issue for the photosynthetic performance of beech seedlings.

Overall, this study showed a general correspondence in the trend of the physiological performance in response to light availability between beech seedlings grown under natural and artificial shade. We suggest that the lower photosynthetic rates and greater PSII efficiencies observed in field than in shadehouse seedlings (regardless of %PAR) are mainly due to lower PAR absolute values in the field, since PAR play a crucial role in gas exchange and chlorophyll fluorescence measurements. Therefore, our results support the use of nets (shadehouses) to study the impact of light availability on physiological

responses of tree seedlings. Controlled-shade studies allow the effects of light to be separated from other effects and reduce confounding effects due to climatic and edaphic factors and competition.

5. Conclusions

The results from this study confirm that light levels strongly affected physiological responses of beech seedlings in the field and shadehouse study. Although beech seedlings displayed greater photosynthetic performance in high light than in low light conditions, plants were able to photosynthesise efficiently in a range of light conditions down to 28% of full light and 14% of the light found in the open gap in the shadehouse and field study, respectively. Compared with the measurements in the shade-controlled study, natural regeneration of beech exhibited a similar trend in physiology with increasing light levels. Further research is needed, however, to confirm tree responses in relation to light environments under both controlled and field conditions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Figures captions

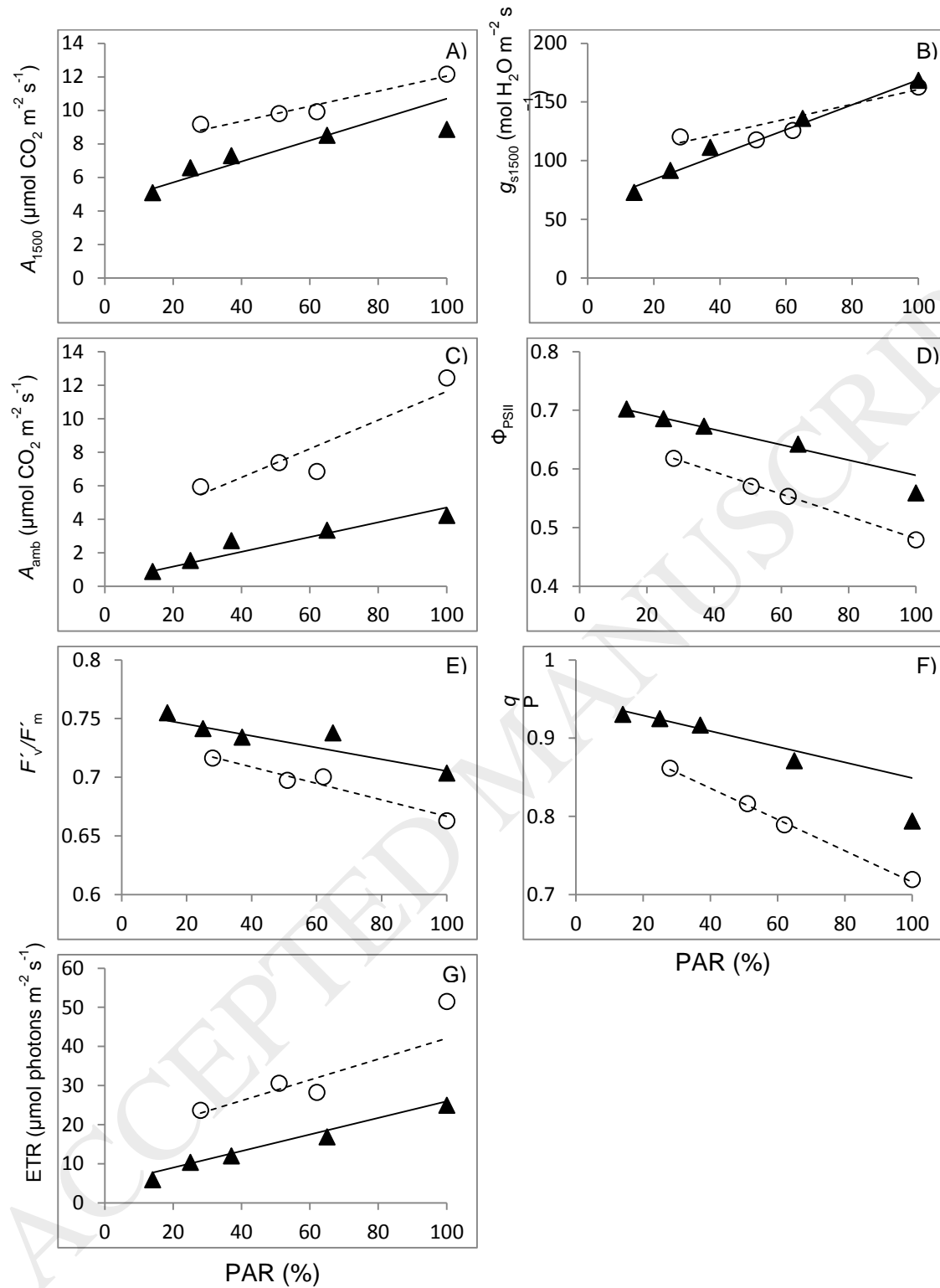


Fig. 1. Photosynthetic rate at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A), stomatal conductance at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (B), photosynthetic rate at ambient PAR (C), PSII operating efficiency (D), PSII maximum efficiency (E), photochemical quenching (F) and electron transport rate (G) as a function of light availability in the field (solid triangles and continuous lines) and shadehouse study (open circles and dotted lines). Regression lines represent fitted equations and symbols are the mean of the observed data.

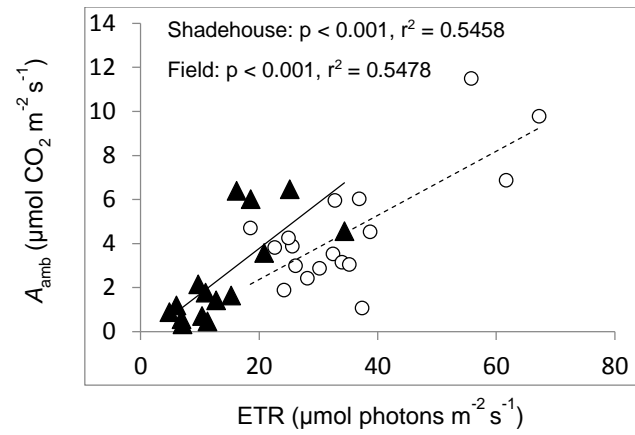


Fig. 2. Relationship between photosynthetic rate (A_{amb}) and electron transport rate (ETR) for beech seedlings in the field (solid triangles and continuous line) and shadehouse study (open circles and dotted line).

Tables

Table 1. Characteristics of the site and plant material in the field and shadehouse study.

Characteristic	Field study	Shadehouse study
Elevation (m ASL)	115	40
Temperature (°C) ^a	13.6 (14.5)	13.6 (14)
Rainfall (mm) ^a	486 (34.3)	336 (15.5)
Soil	Deep well drained acid mineral	Shallow well drained basic mineral
Nutrient availability	No sign of nutrient deficiency	No sign of nutrient deficiency
Vegetation competition	Low	Low
Provenance	Unknown ^b	Cirinceste Region 404, United Kingdom
Age (years)	≈5-10	6
Plant height (cm)	150-200	150-200
Root collar diameter (mm)	21-28	21-28

^a Mean values for 2014 growing season. Values in brackets are for the month in which physiological measurements were conducted.

^b Beech are naturally regenerated and there is no record of the parent tree provenances. It is likely that most of the beech planted in Ireland previous to 1930 was sourced from British provenances although French and Belgium provenances are a distinct possibility (Huss *et al.*, 2016).

Table 2. Mean values and ranges (minimum and maximum values) of the PAR measurements from the field and shadehouse study.

Canopy type	Field	Shadehouses	
	PAR (%)	Light treatment	PAR (%)
Open gap	100	Control (full light)	100
Moderate shade	65 (63.1–65.2)	Moderate shade	62 (61.8–64.3)
Medium shade	37 (36.3–38.3)	Medium shade	51 (49.6–51.8)
Heavy shade	25 (24.9–25.4)	Heavy shade	28 (27.2–31.1)
Very heavy shade	14 (12.9–14.5)		

Table 3. Regression equations used to model the dynamics of different physiological traits as function of percentage of light availability found in the field (natural) and shadehouse study (artificial) for beech seedlings ($y = a + b \times \text{PAR}$). Given are the estimates (a and b), standard errors (SE_a and SE_b) and p -values (p_a and p_b) for the coefficients and the correlation coefficient (r^2) of the regression equations for some physiological traits at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1), 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (2) and ambient PAR (3). Traits: A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$): photosynthetic rate; g_s ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$): stomatal conductance; (A/g_s) ($\mu\text{mol CO}_2/\text{mol H}_2\text{O}$): intrinsic water use efficiency; (A/E) ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$): instantaneous water use efficiency; Φ_{PSII} : PSII operating efficiency; F'_v/F'_m : PSII maximum efficiency; qP : photochemical quenching; ETR ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$): electron transport rate. Values in bold indicate a significant effect of light availability on the physiological trait ($p < 0.05$).

Trait		Regression model coefficients					r^2	
		a	SE_a	p_a	b	SE_b		p_b
1) PAR=1500								
A_{1500}	Natural	4.4469	0.3295	<0.001	0.0625	0.0093	<0.001	0.964
	Artificial	7.5546	0.7574	<0.001	0.0448	0.0096	<0.001	0.970
g_{s1500}	Natural	0.0627	0.0073	<0.001	0.0011	0.0001	<0.001	0.980
	Artificial	0.0978	0.0121	<0.001	0.0006	0.0002	<0.001	0.968
$(A/g_s)_{1500}$	Natural	72.1950	5.8651	<0.001	-0.1017	0.1048	0.342	0.996
	Artificial	98.2158	11.1102	<0.001	-0.2615	0.1258	0.052	0.976
$(A/E)_{1500}$	Natural	3.6771	0.2891	<0.001	-0.0018	0.0054	0.744	0.930
	Artificial	4.7177	0.4708	<0.001	-0.0095	0.0056	0.104	0.939
2) PAR=500								
A_{500}	Natural	4.1500	0.3504	<0.001	0.0174	0.0098	0.088	0.972
	Artificial	6.9937	1.1057	<0.001	0.0193	0.0160	0.243	0.986
g_{s500}	Natural	0.0954	0.0104	<0.001	0.0004	0.0002	0.077	0.953
	Artificial	0.0984	0.0205	<0.001	0.0005	0.0003	0.094	0.942
$(A/g_s)_{500}$	Natural	45.1668	4.8633	<0.001	0.0155	0.1001	0.878	0.998
	Artificial	67.9504	5.2965	<0.001	-0.0315	0.0896	0.729	0.988
$(A/E)_{500}$	Natural	3.3920	0.3406	<0.001	0.0043	0.0075	0.572	0.954
	Artificial	4.3551	0.3143	<0.001	0.0008	0.0058	0.899	0.926
3) Ambient PAR								
A_{amb}	Natural	0.2973	0.2796	0.307	0.0441	0.0129	0.005	0.975
	Artificial	3.0659	0.7923	0.001	0.0857	0.0133	<0.001	0.978
g_{samb}	Natural	0.0939	0.0105	<0.001	0.0004	0.0003	0.318	0.912
	Artificial	0.1160	0.0109	<0.001	-0.0003	0.0002	0.279	0.953
$(A/g_s)_{\text{amb}}$	Natural	11.7150	3.379	0.004	0.2419	0.1371	0.101	0.999
	Artificial	17.4680	11.2507	0.138	0.3967	0.1905	0.052	0.999
$(A/E)_{\text{amb}}$	Natural	1.4846	0.4023	0.003	0.0297	0.0162	0.090	0.981
	Artificial	3.5543	2.6480	0.196	0.0429	0.0454	0.357	0.996
Φ_{PSII}	Natural	0.7193	0.0080	<0.001	-0.0013	0.0002	<0.001	0.973
	Artificial	0.6713	0.0354	<0.001	-0.0019	0.0006	0.004	0.846
F'_v/F'_m	Natural	0.7553	0.0030	<0.001	-0.0005	0.0001	<0.001	0.992
	Artificial	0.7368	0.0161	<0.001	-0.0007	0.0003	0.028	0.953
qP	Natural	0.9492	0.0096	<0.001	-0.0010	0.0003	0.005	0.959
	Artificial	0.9161	0.0355	<0.001	-0.0020	0.0006	0.003	0.834
ETR	Natural	4.7756	1.1392	0.001	0.2118	0.0423	0.000	0.992
	Artificial	15.4786	5.1528	0.009	0.2659	0.0997	0.018	0.997

Table 4. Plasticity index in response to different light availabilities of beech seedlings in the field (natural) and shadehouse study (artificial) for the leaf gas exchange and chlorophyll fluorescence variables studied during 2014. Variables are arranged by PAR conditions used during measurements.

Condition	Variable	Plasticity index	
		Natural	Artificial
PAR = 1500	A_{1500}	0.40	0.25
	g_{s-1500}	0.52	0.28
	$(A/g_s)_{1500}$	0.20	0.30
	$(A/E)_{1500}$	0.11	0.26
	Mean	0.31	0.27
PAR = 500	A_{500}	0.30	0.26
	g_{s-500}	0.25	0.27
	$(A/g_s)_{500}$	0.09	0.10
	$(A/E)_{500}$	0.15	0.06
	Mean	0.20	0.17
Ambient PAR	A_{amb}	0.79	0.52
	g_{s-amb}	0.60	0.31
	$(A/g_s)_{amb}$	0.67	0.47
	$(A/E)_{amb}$	0.63	0.40
	Φ_{PSII}	0.20	0.22
	F'_v/F'_m	0.07	0.07
	qP	0.15	0.17
	ETR	0.76	0.54
	Mean	0.48	0.34
Total mean	0.33	0.26	