Reaching natural growth: Sources of variation in plant traits between indoor and outdoor experiments

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Acknowledgements

Is the end of June 2019, almost a year before I will hopefully defence this thesis and I am starting to write this document. For obvious reasons, it reminds me my previous two theses. Is interesting to see how I started with a more technical approach during my bachelor to a more humanistic one during my master to finish somewhere in the middle during my PhD studies. These different periods have given me several tools, specially over the last three years.

Over this last period, in Switzerland and Sweden, it has been a really interesting time of my life and work. I would like to specially thanks to everybody who make it possible. At first, I would like to thank Günter Hoch and Daniel Bankeståd. Thanks for believing in my and giving me the opportunity of doing this PhD. From the Swiss side I would like to thank specially Georges Grun, for his constant help with the phytotrons. Also, the people of the botanical garden in Basel, who were always willing to help. I would also like to thank the people who make the office a nice place to be: Dr. Sarah Newberry, Maria Vorkauf, Florian Cueni and Dr. Victor Evrard. From the Swedish side I would like to thank everybody from Heliospectra who help with the different research. I would also take the opportunity to thanks my family and friends, that due distance, language barrier or simple interest, possibly will never read this. Finally, I would like to thank Yukino. Thanks for been the best partner that I could have wish and been patient enough to be at my side during this adventure with all the traveling included (75).

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General introduction

One of the main problems of indoor plant production that especially plant researchers are confronted with, is a clear difference between plants grown under indoor versus outdoor conditions. This reduce the comparability between indoor and outdoor experiments as well as the portability of findings from indoor experiments to real world conditions (Matsubara, 2018). Poorter *et al.,* (2016) suggested multiple reasons why this may occur, with major effects coming from lower light quantities, higher plant density and shorter experiment durations in indoor compared to outdoor experiments. Other sources of variation have been pointed out, including age of the plants, leaf temperature, soil temperature, soil microorganism, lack of UV light and the light quality in indoor experiments (*e.g.* Hogewoning *et al.,* 2010 b). In general, the artificial conditions in indoor growth facilities often produce higher specific leaf area, leaf nitrogen content and relative growth rate, as well as lower maximum photosynthesis, plant height and shoot dry weight, compared with outdoor experiments (Poorter *et al.,* 2016).

Light, as one of of the principal determinants of plant growth and development, is consider an important source of deviation between indoor and outdoor conditions. For example, the effect of either light quantity or quantity has been well described in plants from different species, by Arnott and Mitchell (1982). To compensate a growth limitation in plants due a possible lack of light in greenhouse or indoor growth facilities, additional lighting is well stablished in agriculture, especially in areas at higher latitudes with year-round lower levels of natural sunlight (*e.g.* Grammans *et al.,* 2018). Poorter *et al.,* (2016) suggest that an important difference between indoor and outdoor climates for plant growth is a significant lower daily light integral (DLI) radiation in indoor facilities compared with outdoor conditions. Especially in combination with a lack of light variation along the day may lead to plant growth in indoor conditions that deviates considerable from field grown plants. It was not until the development and mass production of light emitting diodes (LED) that dynamic and specifics wavelengths changes as well as fast fluctuations of light intensity became possible to be used in indoor plant growth facilities. Previous attempts in plant biological research to recreate sun-like lighting with conventional light sources used very complex and fault-prone setups (*e.g.* Thiel *et al.,* 1995) which were thus never widely used or considered for commercial plant production.

With the technical improvements in controlled environment capabilities, the use of indoor cultivation systems has increased worldwide. In indoor experiments several authors have demonstrated the positive effects of incorporating closer-to-natural environmental conditions in indoor facilities (*e.g.* Arve *et al.,* 2017, Kaiser *et al.,* 2020,), what can help without adding

higher levels of complexity to reach either closer to natural plant growth under indoor conditions and thereby increase the quality of food production to taste, smell and look more natural, attributes that are desired by consumers (Arve *et al.,* 2017)

Due to the high degree of absorption of blue (B) and red (R) light by chlorophyll, and the higher electric efficiency of LED in these spectral ranges (Overdieck, 1978), these two wavelength ranges tend to be dominating in commercial LED lamp systems (Fujiwara and Sawada, 2006). Many studies have investigated the responses of plants to different B to R ratios. These studies revealed that independent of the light intensity, a required minimum percentage of B is need for plant growth (*e.g.* Miao *et al.,* 2016), and suggestions to reproduce near to natural plant growth by correctly adjusting the B:R ratio in LED lamps has been done (Hogewoning *et al,* 2010 a), however without directly comparing indoor grown plants with an outdoor control. In the vast majority of studies related to light quality effects on plants, either low light levels (Macebo *et al.,* 2011; Hogewoning *et al.,* 2010 a; Hernandez and Kubota, 2016; Kim *et al.,* 2004; Schuerger *et al.,* 1997) or much higher than natural red to far ratios have been use (*e.g.* Bae and Cho, 2008; Hogewoning *et al.,* 2010 a; Hernandez and Kubota, 2016; Hernandez *et al.,* 2016; Kim *et al.,* 2004; Shengxin 2016; Zhen and van Iersel, 2017). However, interactions between light quantity and quality have been reported previously (Furuyama *et al.,* 2014), and modifications of the light spectra, especially in the red to far ratio, has shown to induce more natural like plant growth (Hogewoning *et al.,* 2010 b). This highlights the requirement of finding light spectral combinations in LED lighting that results in the most natural like plant growth in indoor facilities. One challenge is that different species might react differently do changes in the applied light spectrum. Tests for the effect of a light spectrum on plant performance should thus be done across different plant species (as in this thesis) in order to reveal general patterns as well as species-specific responses.

In principal, lamps with multi-channel LEDs enable the application of lighting that can mimic close to natural light quality and intensities changes during plant cultivation in indoor growth facilities (Bula *et al.,* 1991). However, although the newest generation of LED lighting systems are equipped with 4 or more individually controllable spectral channels, growth facilities generally do not apply dynamic and natural changes in the light spectra on a standard base. The knowledge about the changes in light quality related to the solar elevation angle, latitude, as well as the presence or absence of clouds (*e.g.* Smit, 1982; Goldberg *et al.,* 1977) has been so far reported mainly from an atmosphere-physical point of view, and has not been transferred to actual lighting systems used for plant culture in greenhouses or growth chambers. Additionally, it has been shown that light quality effects on plants can interact with other

environmental factors, like temperature (*e.g.* Chiang *et al.,* 2018). This highlights the importance of understanding the role of the light quality variation on plant development, especially in order to correctly predict the effect of climate crisis on plants from indoor experiments.

Although it is known that the fluctuation of environmental factors has an effect on plant phenology and development, it is common practice to apply static environmental conditions in indoor experiments. Fixed day and night time climates may be oversimplified reductions of natural conditions and may lead to plant growth significantly deviating from field grown plants (Poorter *et al.,* 2016). Especially, it is well-known that random and daily fluctuations of temperature and light, can affect plant performance in both positive and negative ways (*e.g.* Myster and Moe,1995; Kaiser *et al.,* 2015; Kaiser *et al.,* 2018). Several studies have measured the effect of light or temperature variations on plant performance under semi-controlled and controlled conditions, but again, simultaneous comparisons with outdoor grown plants are rare in the literature and normally just *Arabidopsis thaliana* has been used (*e.g.* Vialet-Chabrand *et al.,* 2017; Annunziata *et al.,* 2017; Annunziata *et al.,* 2018). Nevertheless, from these studies it could be derived that changes in light quantity along the day may induce lower biomass but also higher maxium photosynthesis, especially per unit of leaf mass (Vialet-Chabrand *et al.,* 2017), even though fast fluctuations in light intensity have been shown to reduce photosynthesis and productivity in the long term (Kaiser *et al.,* 2018). Additionally, these studies have shown more evidence of the difference of plants grown under totally fixed climatic conditions compared with semi-controlled environments (*i.e.* greenhouses), highlighting the necessity of a better knowledge for a minimum requirement of environmental fluctuations for natural like growth in indoor experiments.

To investigate more closer the potential causes for the differences in plant performance between indoor and outdoor plant experiments, and to enable more natural-like plant growth in indoor facilities, a joint project had been stablish between the University of Basel (Basel, Switzerland) and Heliospectra A.B. (Gothenburg, Sweden) within the research consortia PlantHUB (European industrial doctoral programme (EID) funded by the H2020 PROGRAMME Marie Curie Actions- People), coordinated and managed by the Zurich-Basel plant science center. The project consisted of 18 months of basic research at the University of Basel, followed by 18 months of applied research, software development and documentation at Heliospectra A.B.

As a result of this collaboration, the present thesis aims to identify how climatic conditions (especially, light quality and fluctuation of light intensity, temperature and air

humidity) need to be adjusted in growth chambers in order to reach the most natural like plant growth under indoor conditions. To avoid documentation about only species-specific reactions, several species from different functional plant types were always used. The work on this thesis was divided in 5 main modules that aimed to:

1) Understand and quantify the natural light quality changes along the day and along a whole season, assess the effect of cloudiness on the natural light spectrum, and correlated these findings to previous studies on light quality effects in trees (Chapter 1)

2) Investigate which light spectral combination of LED-lights can induce the most natural-like growth in plants grown in indoor chambers with constant climatic conditions (Chapter 2)

3) Identify the minimal degree of environmental fluctuations (of light, temperature and air humidity) necessary to reach natural-like growth in indoor grown plants (Chapter 3)

4) Understand the effect of asynchrony environmental fluctuations in indoor growth chambers, were potential interaction and/or synergies may occur depending of the degree of variability of each environmental variable (Chapter 4)

5) Test possible applications of light fluctuations to improve crop quality and develop software applications for optimized light control of multi-wavelengths LED assimilation lamps (Chapter 5 and Appendix)

Chapter 1: Latitude and weather influences on sun light quality and the relationship to tree growth

In this study, continuous field measurements of the natural changes of the spectral composition of sunlight over a full year at a mid-latitudinal site (47° N) are presented. In a first step, these field measurements of the sunlight spectrum were analyzed and summarized to be easily applicable for LED lighting systems in plant growth chambers. In a second step, the data were combined with an analysis of studies investigating the effect of light quality on growth of tree seedlings of different latitudinal origin. The study thereby focuses on the comprehensive effects of sun light quality changes due to weather conditions and time, excluding further, smaller-scale modifications of the light spectra due to the presence of ‘green shade’ below a canopy. The correlation between wavelength-specific light quantity requirements of tree seedlings from different latitude origins and the natural availability of these wavelengths due to geographical, annual, and diurnal changes, at their respective origin was investigated. Significant correlation would indicate ecotypic adaptations of tree populations to the specific spectral light quality and dynamics at their site of origin.

Chapter 2: Reaching natural growth: Light quality effects on plant performance in indoor growth facilities.

In this study, which is the first in a series of experiments in walk- in phytotrons, the effects of different wavelength combinations in LED lighting on plant growth and physiology in seven different plant species from different plant functional types was investigated. The results were compared against field-grown plants of the same species. Treatments of different proportions of blue and red light were applied, were mean environmental conditions (photoperiod, total radiation, red to far red ratio and day/night temperature and air humidity) from the field trial control were used in order to assess, which wavelength combinations result in the less extreme (*i.e.* most natural-like) plant performance in the phytotrons. Different plant traits and physiological parameters, including photosynthesis under a standardized light and the respective growing light, biomass productivity, SLA and leaf pigmentation, were measured in each treatment after 35 days of growth under the respective growth light. Especially, I hypothesized that applying steady, average climatic conditions would lead to plant growth that deviates most from natural growth, but that light spectra similar to the natural sunlight lead to more natural-like growth compared with light spectra that deviate significantly form the natural blue: red ratio.

Chapter 3: Reaching natural growth: The significance of light and temperature fluctuations on plant performance in indoor growth facilities

As a second step of our series of experiments in walk-in phytotrons, the effects of fluctuating light, temperature and humidity in an indoor environment on plant performance was investigated. The same seven plant species from different functional plant types used in Chapter 2 were grown outdoors during summer and spring. Following these field trials, the same species were grown in indoor growth chambers under different scenarios of climate complexity in terms of fluctuation: 1) fixed night and day conditions, 2) daily sinusoidal changes corresponding to the mean daily fluctuations measured in the respective field trials and 3) variable conditions tracking the exact climate records from the field trials. Productivity-, gas exchange- and leaf pigment-traits were measured in all plants at the end of the experiments. It is hypothesized that applying steady, average climatic conditions would lead to plant growth that deviates most from natural growth, while the application of real fluctuations of temperature, humidity and light will produce plants that show similar performance to field grown plants.

Chapter 4: Reaching natural growth: Effect of asynchronous light and temperature fluctuations on plant traits in indoor growth facilities

In this third experiment in walk-in phytotrons, it was investigated the effects of asynchronous variations of environmental factors on plant growth. The application of un-synchronized climatic variations is a common practice today, e.g. in commercial greenhouses which aim to keep continuous levels of light. Again, the same seven plant species as used in chapter 2 and 3 were grown indoors under full-factorial combinations of either fixed or variable conditions of air temperature and light intensity. The results were also compared with field grown plants of the same species. The same set of plant traits as in chapter 2 and 3 were measured at the end of the experiment: Productivity-, gas exchange- and leaf pigment-traits. The main hypothesis was that under totally fluctuating conditions a lower biomass would be reached due the stress of changing environmental conditions together with a more efficient photosynthesis, where light dynamics play a secondary role compared with temperature dynamics. A second hypothesis was that asynchronous fluctuations of one of the two environmental factors will lead to stress responses in plants.

Supplementary studies and applications:

Chapter 5: Exploring the potential of applying variable light conditions to improve crop quality and production

Using the acquired knowledge from the previous chapters, two additionally experiments were performed at the Heliospectra labs to study the commercial feasibility of using dynamic light quality and/or quantity to increase crop production and quality in indoor facilities. It was hypothesized that 1) increasing the percentage of blue light over the morning could help to a faster stomatal opening that could contribute to a higher productivity under optimal growth conditions and 2) that fluctuations of light may help to increase the plant's shelf-life (*i.e.* the durability of fresh crops after harvest) without affecting the total biomass production.

Appendix: LED controlling software for near-natural plant growth and a Complete LED lighting system for optimized light conditions

As a result of the different experiments performed at the University of Basel and at Heliospectra, a set of different tools were designed to facilitate the automatization of the light conditions during the experiments and allow for more dynamic environmental conditions, specially from a lighting point of view. Using these tools two different deliverables are presented in chapter 6:

A LED controlling software for near-natural plant growth and a complete LED lighting system for optimized light conditions. Additionally, it is present how these deliverables can be used in future experiments or transferred to other facilities, as has been already successfully done in the case of the terraXCube experimental facility at EURAC (Bolzano, Italy).

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Chapter 1
Latitude and Weather Influences on Sun Light Quality and the Relationship to Tree Growth

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Abstract: Natural changes in photoperiod, light quantity, and quality play a key role in plant signaling, enabling daily and seasonal adjustment of growth and development. Growing concern about the global climate crisis together with scattered reports about the interactive effects of temperature and light parameters on plants necessitates more detailed information about these effects. Furthermore, the actual light emitting diode (LED) lighting technology allows mimicking of light climate scenarios more similar to natural conditions, but to fully exploit this in plant cultivation, easy-to-apply knowledge about the natural variation in light quantity and spectral distribution is required. Here, we aimed to provide detailed information about short and long-term variation in the natural light climate, by recording the light quantity and quality at an open site in Switzerland every minute for a whole year, and to analyze its relationship to a set of previous tree seedling growth experiments. Changes in the spectral composition as a function of solar elevation angle and weather conditions were analyzed. At a solar elevation angle lower than 20°, the weather conditions have a significant effect on the proportions of blue (B) and red (R) light, whereas the proportion of green (G) light is almost constant. At a low solar elevation, the red to far red (R:FR) ratio fluctuates between 0.8 in cloudy conditions and 1.3 on sunny days. As the duration of periods with low solar angles increases with increasing latitude, an analysis of previous experiments on tree seedlings shows that the effect of the R:FR ratio correlates with the responses of plants from different latitudes to light quality. We suggest an evolutionary adaptation where growth in seedlings of selected tree species from high latitudes is more dependent on detection of light quantity of specific light qualities than in such seedlings originating from lower latitudes.

Keywords: light quantity; light quality; spectrometer; shoot elongation; tree seedlings

1. Introduction

Light is one of the main environmental signals affecting plant biology, with multiple physiological responses being controlled by changes in light quantity, quality, and photoperiod

(Garner *et al.,* 1923, Robertson *et al.,* 1966, Smith*,* 1982). Although the effects of the natural daily variation in light quality on plants have not been quantified in situ, it could be shown experimentally by using artificial lighting of defined wavelength ranges, that specific developmental processes in plants are differently affected by different fractions of the sunlight spectrum (Terashima *et al.,* 2009, Hogewoning *et al.,* 2010, Jenkins, 2014, Zhen *et al.,* 2017). Punctual measurements comparing sun light spectral composition at different solar elevation angles showed a lower fraction of blue (B, 400–500 nm) and red (R, 600–700 nm) light and a higher fraction of green (G, 500–600 nm) light in the middle of the day than at sunset (Smith, 1982). Smith (1982) quantified the effect of the weather conditions on light quality at high solar elevation angles and showed that clouds and dust cover have a small effect on the light spectra, mainly affecting the light in the B and R ranges, not unlike the changes in the spectral composition of sun light that occur when passing through a plant canopy. Yet, detailed information about the dynamic changes in these light qualities, especially with respect to their potential impact on plant biology, have so far not been reported, although there is substantial knowledge about static light quality effects on gas-exchange and other plant physiological processes (Overdieck, 1978, Furuyama et al., 2014, Hernandez and Kubota, 2016).

At the short wavelength end of the sun spectra, effects of ultraviolet (UV) light on plants (e.g., shoot elongation, production of UV-protecting secondary compounds) and the associated UV-B receptors (UVR8) and UV-A-blue light receptors, have been well described in plants (Jenkins, 2014). Interestingly, the signaling effects of UV light on plants is reduced at higher radiation, implying that UV as a plant signal may be most important during twilight. The next section of the light spectrum, the B light, which is mainly sensed by cryptochromes, phototropins, and other blue light-UV-A receptors, affects, for example, stomatal opening and plant phototropism. High percentages of B light have been shown to affect plant morphology (Hogewoning *et al.,* 2010). Although chlorophyll, as the central plant pigment of the photosynthetic light reaction, absorbs mainly B and R light, G light has also been shown to contribute to photosynthesis and to be especially important at lower canopy levels (i.e., the so-called ‘green shade’) and at deeper levels of the leaves (Terashima *et al.,* 2009). At the longer wavelength end of the visible sun spectra, R and far-red (FR) light have important signal functions for plants. The ratio between red to far red (R:FR) is sensed by the phytochrome system and changes in the R:FR ratio can influence important physiological processes like growth, germination, and flowering. In addition, FR has an important role in optimizing photosynthesis upon combined action of the PSII and PSI, increasing the photosynthetic efficacy (Zhen *et al.,* 2017).

Recent developments in light emitting diode (LED) lighting systems potentially enable the mimicking of more natural light quality changes during plant cultivation in indoor growth facilities (Bula *et al.,* 1991). Due to the high degree of absorption of B and R light by photosynthesis-related pigments and higher electric efficiency (Overdieck, 1978), these two wavelength ranges tend to be dominating in commercial LED lamp systems. However, the knowledge about the changes in light quality related to the solar elevation angle, latitude, time of the day, and the day of year, as well as the weather in general (Smith , 1982, Goldberg and Klein, 1977) has been so far reported mainly from an atmosphere-physical approach, and has not been transferred to actual lighting systems used for plant culture in greenhouses or growth chambers.

Changes of light quality in the morning and evening hours may be an especially important plant signal at higher latitudes where twilight conditions persist for a substantial period. Several studies have shown how different ecotypes of tree species react differently to R or FR light treatments as day extension, and it has been hypothesized that this could be due to adaptations to the light quality at the end of the day at their site of origin (Clapham *et al.,* 1998, Mølmann *et al.,* 2006, Opseth *et al.,* 2016). Additionally, it has been shown that light quality can interact with other environmental factors, like temperature, where higher temperatures have shown to reduce the promoting effect of FR light on growth (Chiang *et al.,* 2018). Understanding the role of the light quality variation in plants is a crucial factor to predict the effect of the currently rising temperatures, especially in marginal areas such as those close to the latitudinal range limits of trees.

In the current study, we present detailed, easy-to-apply, and continuous field measurements of the natural changes of the spectral composition of sunlight over a full year at a mid-latitudinal site (47° N). This data was then combined with an analysis of studies investigating the effect of light quality on growth of tree seedlings of different latitudinal origin. Our study thereby focuses on the comprehensive effects of sun light quality changes due to weather conditions and time of the year, excluding further, smaller-scale modifications of the light spectra due to the presence of ‘green shade’ below a canopy. Here, we investigated the correlation between wavelength-specific light quantity requirements of tree seedlings from different latitude origins and the natural availability of these wavelengths due to geographical, annual, and diurnal changes, at their respective origin. Such a correlation would indicate ecotypic adaptations of tree populations to the specific spectral light quality and dynamics at their original site.

2. Materials and Methods

2.1. Light Spectra Recordings

A USB2000+XR1-ES spectrometer (25 μm entrance slit, range 200–1000 nm, 1.5 nm resolution, Ocean Optics Inc., Largo, Florida, USA) was installed twelve meters above ground level at the Botanical garden of the University of Basel (257 m AMSL, 47° 33′ 30.3′’ N, 7° 34′ 52.4′’ E, Basel, Switzerland) to acquire the light spectrum during a chronological year under a ensured shadow-free environment with minimalized light reflection from buildings, surface water bodies, or vegetation in the surrounding areas. Light spectra from 200–1000 nm were recorded every minute from 21 February 2018 to 21 February 2019 using a single board computer (Raspberry pi 2, Cambridge, UK) allowing dynamic change in the integration time, reducing the electric noise in the measurements through the use of 75–85% of the saturation point of the equipment. The optical fiber was installed at a 90° angle relative to the horizon. A cosine corrector made of Spectralon was used to capture environmental light coming from 180° (CC-3-UV-S, Ocean Optics Inc.). The cosine corrector was replaced every three months. The spectrometer was additionally equipped with a fan to avoid heat accumulation on hot days and was calibrated with a calibration lamp (HL-3 plus, Ocean Optics Inc.), once before mounting, and then every 3 months during the measurement year. The calibration lamp was warmed up for 15 min before the calibrations that were performed using a boxcar width of 2 wavelengths every 6 nm in both directions and the average of 5 measurements for each curve.

2.2. Light Energy Calculations

To acquire an initial dark library, the spectrometer was set in darkness at 20 °C, and a dark spectrum was recorded for integration times between 100 ms and 10 s every 100 ms. For each light measurement, the corresponding or interpolated dark spectrum was removed from the raw measurement in the corresponding integration time. The remaining count was multiplied with the corresponding calibration file of the calibration lamp. The resultant count was then divided by the area (m^2) of the cosine corrector (1.19×10^{-5} m^2) and then divided by the integration time of each measurement (s). Additionally, the energy in each particular wavelength was calculated multiplying each specific frequency by the Planck constant. To obtain the photon flux of each wavelength as μmol photons m^{-2} s^{-1}, the resultant was divided by the Avogadro number and the pre-calculated energy of each wavelength (Plank, 1900).

2.3. Light Quantity and Quality Proportions

To simplify the results from a biological and practical point of view, from each measurement three proportions were separately calculated from the visible light spectra: The percentage of blue (B), green (G), and red (R). For the calculation of B, G, and R, the light spectrum as μmol photons m^{-2} s^{-1} was integrated from 400 to 700 nm to obtain the total photosynthetic photon flux density (PPFD). Furthermore, every 100 nm between 400 and 700 nm, the proportions of B, G, and R where calculated. The B proportion corresponded to the percentage of photons from 400 to 500 nm compared with the total PPFD, G from 500 to 600 nm and R from 600 to 700 nm, respectively. Additionally, the red to far red (R:FR) ratio was also calculated. This was done through the division of the sum of photons (as μmol m^{-2} s^{-1}) between 655 and 665 nm and the sum of photons between 725 and 735 nm, respectively (Sager and Smith, 1988). For the analysis of the weather conditions on the light spectra through the day, the sunniest and the most cloud-covered day of each month were selected from the recorded data (n = 12). After this, a locally estimated scatterplot smoothing (LOESS) regression was fitted for both weather conditions (i.e., clear sky and overcast).

2.4. Solar Elevation Angle Calculation

To quantify the average effect of the weather and remove the effect of the time of the day and day length through the year, the collected sun spectral data were analyzed as a function of the solar elevation angle. For each measurement throughout the observation year, the solar elevation angle was calculated based on the geographic position and time of the day and the day of year using the solar position calculator available online (NOAA, 2018) and confirmed through the OCE R package based on the NASA-provided Fortran program, using equations from "The Astronomical Almanac".

2.5. Literature Review

To relate our light quality measurements to potential effects on growth of tree seedlings from the boreal/temperate zone, we conducted a literature search and performed an analysis on a set of published experiments that investigated the effect of light quality on seedling growth of selected tree species from different latitudes: The conifers *Pinus sylvestris* L., *Picea abies* (L.) H.Karst, and *Abies lasiocarpa* (Hook.) Nuttall, as well as the deciduous *Betula pendula* Roth. (more information in Supplementary Table S1). We exclusively choose studies that (1) were conducted under similar controlled conditions, (2) treated tree seedlings with different R:FR ratio light, (3) made quantitative growth measurements on potted seedlings of trees, (4) ran the

experiments for at least one month (i.e., between 35 and 50 days) and/or (5) used different tree populations of different latitudinal origins (Clapham *et al.,* 2002, Tsegay, 2005, Mølmann *et al.,* 2006, , Aas 2015, Chiang, 2016, Opseth *et al.,* 2016, Chiang *et al.,* 2018). The treatments corresponded to day extensions with different R:FR ratios and main light periods of 9–12 h with similar light quantities (W m^{-2}) during the day and day extension/night treatment. Growth was measured at the end of the experiments as the distance between the soil and apical bud (shoot elongation) or the elongation of the needles, depending on the study. To quantitatively compare the results among the experiments, the measured growth parameters (i.e., either needle elongation or shoot elongation), were analyzed by considering only the effect size, i.e., growth relative to the average growth under pure R light day extensions and, if the experiment included more than one ecotype, the average growth of the most southern ecotype investigated under pure R light day extensions. For the analysis, the effect of the different light quality treatments and the population origin on the measured growth variables was analyzed through forward selection and backward elimination on a single dataset, where both variables were included in a two-way analysis of variance (ANOVA) with light quality and latitudinal origin as fixed factors. All analyses were performed using R 3.6 (R core team, 2019).

3. Results

3.1. Light Quality Changes Throughout the Day

Our field radiation measurements under different weather conditions and time of the day showed a reduction of the blue (B) light proportion and an increase of the green (G), red (R), and far red (FR) light proportion from sunrise and sunset to the middle of the day (Figure 1A–D). The analysis of multiple days with either clear or overcast conditions throughout the year revealed quality changes induced by the weather conditions that can be of similar magnitude as the diurnal effects of the solar elevation angle on the B and R fraction of the spectrum (Figure 2). In the middle of the day, the presence of clouds increased the B fraction, depending on the cloud cover density and height, with a simultaneous reduction of the R fraction. Weather conditions had no significant effect on values on the R:FR ratio in the middle of the day.

3.2. Effect of Weather on Light Quality Changes at Low Solar Elevations Angles

The effect of the weather conditions on the light quality was significantly stronger at lower solar elevation angles. At solar angels below 20°, overcast conditions led to a significantly

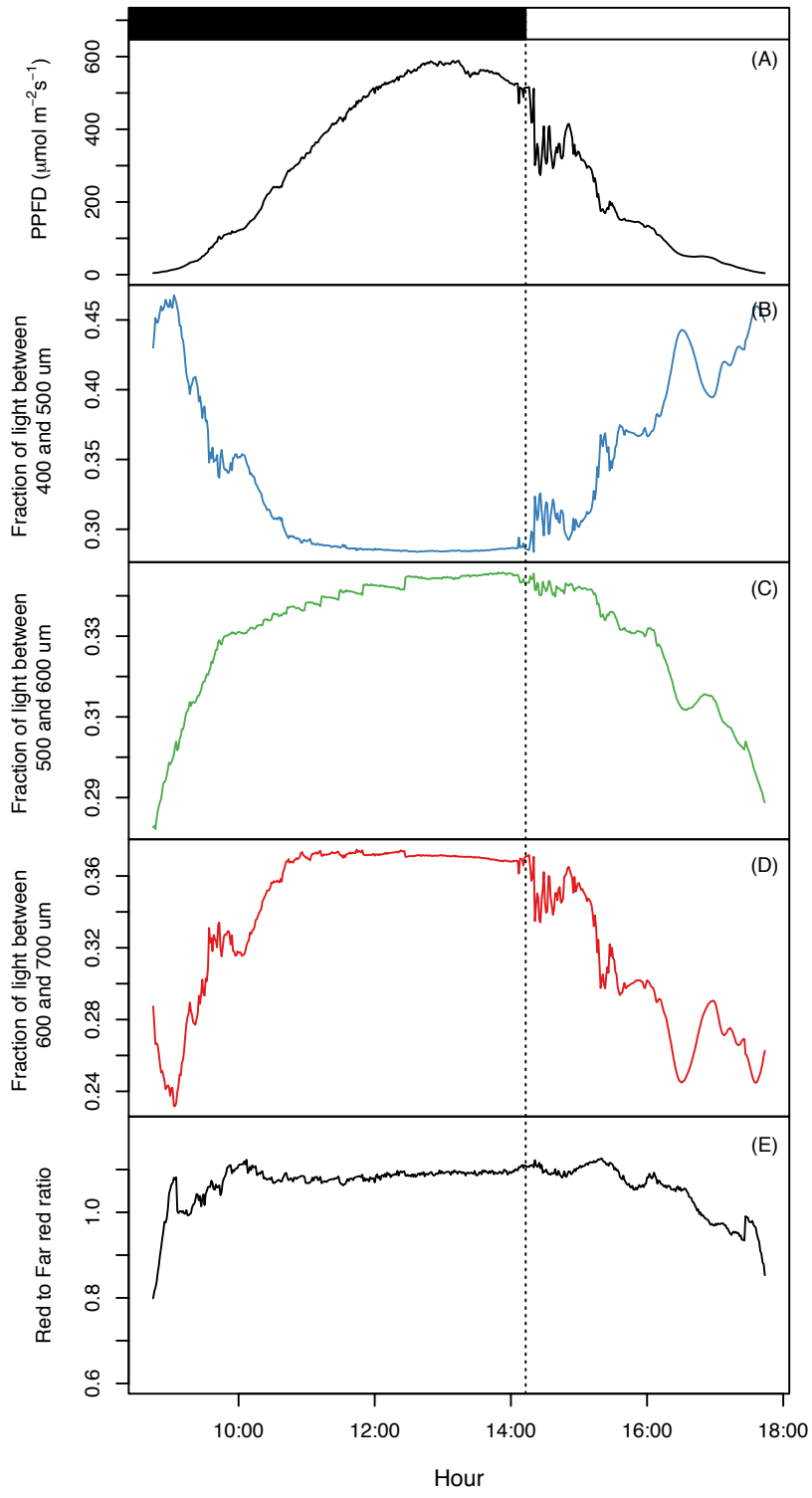

Figure 1. Changes in light quantity and quality as fraction of the photosynthetic photon flux density (PPFD) during a diurnal course. (**A**) Total PPFD; (**B**) blue light fraction (from 400 to 500 nm); (**C**) green light fraction (from 500 to 600 nm); (**D**) red light fraction (from 600 to 700 nm). (**E**) Red to far red (R:FR) ratio. The values are from a single, representative day with varying weather conditions with clear sky conditions until 14:15 (left hand side of the dotted vertical line) and partially overcast conditions during afternoon and evening (right hand side of the dotted vertical line). The data were recorded on 25 November 2018.

lower proportion of B light and a higher proportion of R light, while the effect was much weaker for G light (Figure 2). At solar elevation angles below 1°, close to 37 % of the incoming PPFD consisted of B light, while G light and R light accounted for 31 and 30% of the PPFD, respectively, independently of the weather conditions. During a clear sky after sunrise and before sunset (sun angles between 5 and 8°), an average of 40, 33, and 28% of the light was coming from B, G, and R light, whereas under cloudy conditions at the same solar elevation angles, the values for these light qualities were 34, 32, and 34%, respectively (Figure 2). A strong effect of low solar elevation angles was also found on the R:FR ratio. At clear sky conditions, the average R:FR ratio at 10° of solar elevation angle was 1.2, while it was close to 1.0 on cloudy days, and decreased strongly at solar angles <10° (Figure 2).

The inflection points calculated as the maximum value of the first derivate of each curve, for B, R light, and R:FR ratio under clear sky conditions was at a solar elevation angle of 13° for B and R and 14° for the R:FR ratio. Light quality quickly approached very stable values at solar elevation angles higher than 20°. As stated above, at solar elevation angles beyond 20°, only moderate effects of cloud cover on any wavelength fraction were found, with a small increase of the fraction of B light, on average, from 27% to 29% and a small reduction of the fraction of R from 38% to 36% at solar angles between 20 and 60° (Figure 2). The G light fraction, on the other hand, reached values close to 35% independently of the weather conditions, with its inflection point close to 10°. Finally, the R:FR ratio did not differ significantly between sunny and overcast days at solar elevations angles between 20° and 50° and stayed at a constant average value of 1.1, while at solar angles >50°, the R:FR ratio was even slightly higher on overcast days compared to days without cloud cover (Figure 2. More detailed values in supplementary Table S2).

3.3. Latitude Effects: Duration of Modified Light Quality and its Effect on Seedlings of Selected Tree Species

At higher latitudes, the period of daytime under modified sun light spectrum (i.e., solar angle below 20°) is significantly longer compared with lower latitudes, showing an exponential increase at higher latitudes. For example, at 30° N the maximum daily twilight period is reached as a single peak in mid-winter (Day 356) and does not exceed 5 h per day, while it shifts to the beginning of spring (Day 53) and the end of autumn (Day 292) at 60° N with a daily maximum duration of over 9 h (Figure 3).

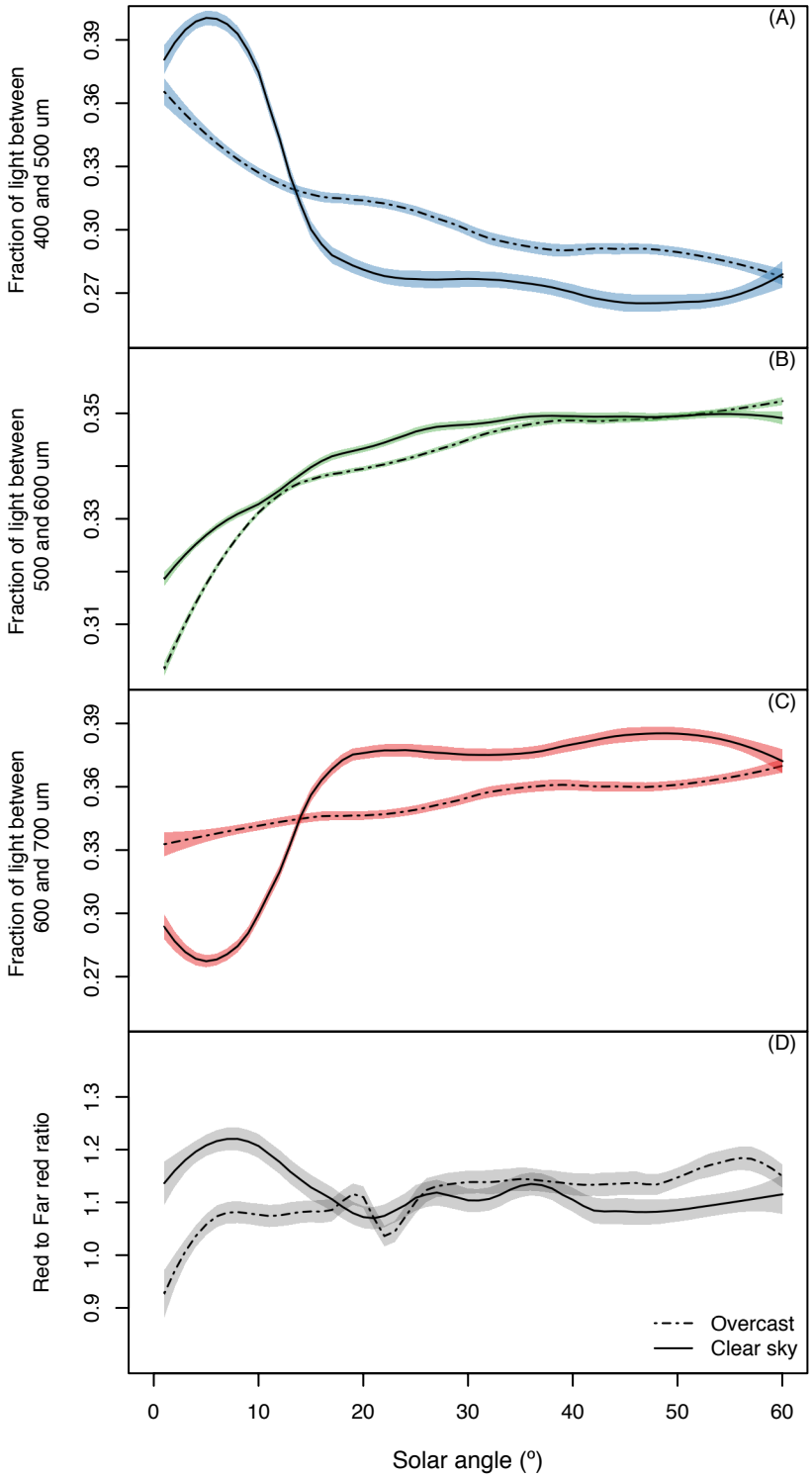

Figure 2. Changes in light quality as a fraction of the photosynthetic photon flux density (PPFD) depending of cloudiness (full line: Clear sky, dotted line: Overcast conditions) and the solar elevation angel. (**A**) Blue light fraction (from 400 to 500 nm), (**B**) green light fraction (from 500 to 600 nm), (**C**) red light fraction (from 600 to 700 nm). (**D**) Red to far red (R:FR) ratio. The lines represent the mean value of one day of each weather condition per month (n = 12; see methods for detail). Shaded areas correspond to the standard error of a locally estimated scatterplot smoothing (LOESS) fitted model.

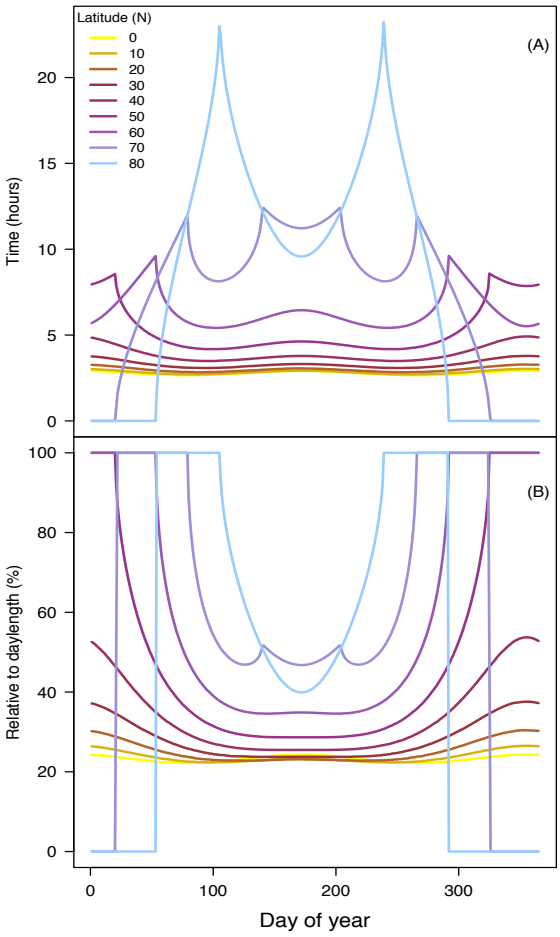

Figure *3.* Estimated day length duration (**A**) and percentage relative to the total day length (**B**) of solar elevation angles between 0 and 20° for different latitudes.

For the seedlings of the selected tree species included in our analysis, both the light quality treatments and the latitudinal origin of the population significantly affected growth ($P_{value} < 2.2e^{-16}$ and 0.02, respectively). However, no interaction between these two factors was found ($P_{value} = 0.4$). The latitude effect is best described (fitted) as a quadratic effect (Figure 4A). Plants from higher latitudes had lower shoot elongation under the same light quality than southern ecotypes. In all studies higher R:FR ratios led to decreasing growth (Figure 4B). Additionally, the difference between the effects of the R and FR light treatments was more or less constant across trees from different latitudinal origin. For the light treatments, the best fit was a linear function after a logarithmic transformation, where plants treated with a larger fraction of FR light had larger elongation compared to trees treated with higher fractions of R light. Both factors were able to explain 82% of the variability (Figure 4. Available as 3D figure, Figure S1). A total of 54% of the variability was explained by the light treatments and the origin of the ecotypes could explain 38% when the different variables were tested independently.

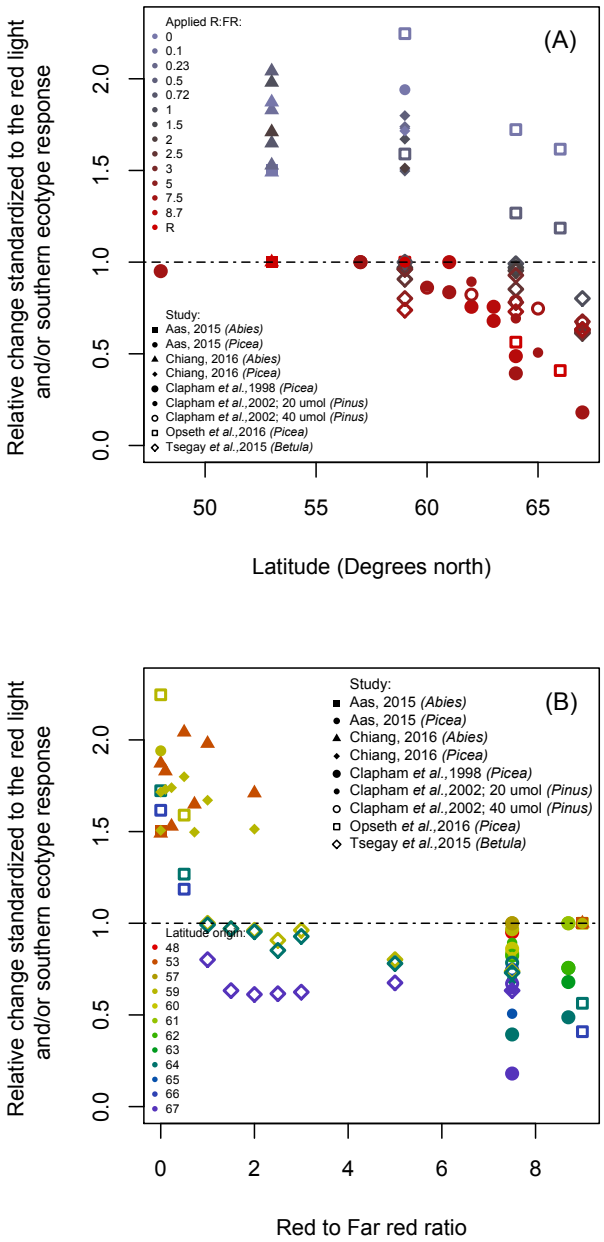

Figure 4. Effect of day light extension with different light qualities on seedling growth in selected temperate and boreal tree species: Relative changes of growth plotted (**A**) against the latitudinal origins under different red to far red ratios (R:FR ratios) and (**B**) against different R:FR ratios applied in trees from different latitudinal origins. The data were collected from work performed with seedlings of selected tree species (three evergreen conifers: *Picea abies*, *Pinus sylvestris*, *Abies lasiocarpa* (Clapham *et al.,* 2002, Mølmann *et al.,* 2006, Aas 2015, Chiang, 2016, Opseth *et al.,* 2016, Chiang *et al.,* 2018) and one deciduous broadleaved species (*Betula pendula*) (Tsegay, 2005), that were exposed to the different light quality treatments for 35–50 days. The additional legends give the first author, publication year, and tree genus. Data from Clapham *et al.* (2002) were derived from two experiments with 20 and 40 µmol photons m^{-2} s^{-1} day extension light, respectively. The light treatments correspond to day extensions with different R:FR ratios and main day light periods of 9–12 h with similar light quantities in W m^{-2}. In the different experiments needle or plant height was measured as the growth response variable. Each study was standardized to the effect of red (R) light in the southern ecotype (when more than ecotype was included; see methods for details).

4. Discussion

Atmospheric constitution, e.g., the presence of clouds, can alter the composition of the light spectra. In our measurements, clouds increased the blue (B, 400–500 nm) light fraction by up to 10% in solar elevation angles above 20° through a reduction of direct light, which also led to a corresponding reduction of the red (R, 600–700 nm) light fraction in a similar magnitude. The green (G, 500–600 nm) light fraction was less affected by weather conditions, mainly due to a potentially 50% lower scattering compared to that of the B fraction (Strut, 1871). R:FR ratios between weather conditions were not significantly different at high solar elevation angles but changed sharply at low solar angles.

It is well known that from solar elevation angles of –12° at the last two stages of twilight, i.e., the nautical and civil twilight (0° to −6° and −6° to −12°, respectively), the most substantial fraction of the spectra corresponds to the B light wavelength (Smith, 1982, Goldberg and Klein, 1977, Spitschan *et al,* 2016). This is mainly due to a lack of direct radiation and a longer path length of the scattered sunlight through the atmosphere, which increases the probability of Rayleigh scattering of light by small atmospheric molecules and aerosols. With increasing solar elevation angles, there is an initial increase in the B light fraction together with a reduction of the fraction of R light (Figure 2). This initial increase in B light has been reported previously in a city environment at lower solar elevation angles than in our study (Spitschan *et al,* 2016).This shift that was not present in a rural scenario, was explained by the presence of high-pressure sodium lamps as the city's main illumination source, were the timing for such a shift may accordingly depend on the city's illumination regime. The absence of this increase in rural scenarios and the low magnitude of this effect may indicate that this change should not play an important role as a biological signal in natural ecosystems. Once that the relative amount of direct light increases, a quick reduction of B light occurs, together with an increase of R light, mainly due to a shorter sunlight path length through the atmosphere that reduces the amount of B light refraction and therefore the B fraction in the sun spectra (Garner and Allard, 1923). In contrast, G light tends to keep an asymptotic slower increase from lower to higher solar elevation angles with light quality reaching a steady state in solar angles higher than 10°.

The higher proportion of R light at twilight in cloud-covered conditions derives from the strong reflectance of R light from clouds into the lower atmosphere and the higher absorbance of B light by clouds. The intensity of this effect depends on the elevation of the clouds, its density, and its position on the horizon (Zagury, 2012). Many studies on radiation light quality with a more physical focus reported higher percentages of R light during sunrise and sunset

than in the current study, mainly due to the direction of the used sensor and the aperture's angle. Zagury (2012), for example, used a 25° aperture facing in the direction of the light source. This technical difference allows the sensor to detect exponentially more direct light and ignores the mostly diffuse light coming from other directions. The reduced amount of measured scattered light, which consists mostly of shorter wavelengths, therefore, increases the relative amount of R compared to FR light. Although the angle at which plants sense the light depends on the leaf angle, the measured values from the horizontal measurements and the inclusion of light coming from different angles as reported in our study here, are, on average, likely more similar to the light quality detected by plant leaves under natural conditions.

A very strong effect of the weather conditions at low solar elevation angles was found for the R:FR ratio, possibly partly explaining the larger differences between natural R:FR ratio measurements reported by previous authors (*e.g.* Smith, 1982, Ragni *et al.,* 2004, Yamada *et al.,* 2009). Although changes of the R:FR ratio in the presented magnitudes (Figure 2) have shown biological effects in short-term experiments with herbaceous plants (Hughes *et al.,* 1984), this effect has not been found in the few tree species investigated so far (Opseth *et al.,* 2016, Chiang *et al.,* 2018). In contrast, many annual plants show high plasticity to lower fluctuation on the R:FR ratio conditions (Morgan and Smith, 1979). This may indicate that the previously investigated tree seedlings species may require several generations to adapt to the different R:FR ratios.

At higher latitudes, the period of daytime under modified sun light spectrum at twilight is exponentially longer compared with lower latitudes, especially during the spring and autumn. These prolonged periods of low solar elevation angle and the respective change of spectral light quality at higher latitudes might be used as pace-setting signals for plant biological processes in perennial plants, like bud break, growth, or bud set. In woody plant species that have broad latitudinal distributions, such as the trees used in our literature review, ecotypes from southern latitudes have been shown to require less radiation to keep growing than conspecific ecotypes from the northern distribution edge. For example, Mølmann *et al.* (2006) tested different radiation intensities and showed that the effect of FR and R light treatments also depended on light intensity and plant origin, e.g., 1.7 W m^{-2} of FR light completely prevented bud set in more southern ecotypes (from 59 and 64° N latitude) of Norway spruce seedlings, while in a more northern ecotype (from 66.5° N latitude) only 43% bud set was reached. In all three ecotypes, lower light intensities, independent of the light quality, were not able to prevent bud set.
Several studies have suggested differential sensitivities of plant growth to light quantity and quality depending on their latitudinal origin (Clapham *et al.,* 2002, Tsegay, 2005, Mølmann *et*

al., 2006, , Aas 2015, Chiang, 2016, Opseth *et al.,* 2016, Chiang *et al.,* 2018) showing an interaction between the effect of light qualities and latitude (Mølmann *et al.,* 2006, Opseth *et al.,* 2016). The analysis of such data in the current study (see Figure 4A, B; Figure S1), also showed a distinct growth response to changed R:FR ratios depending on the latitudinal origin of the plants studied. The re-analysis of the combined data, clearly confirms the findings of the individual studies that an increased amount of FR light strongly promotes growth, compared to R light alone (Figure 4B). However, the previously observed interaction between the light treatments and the population origin was not found to be significant anymore (P_{value} = 0.4). Therefore, we propose that northern ecotypes tend to be as sensitive to light quality changes as southern ecotypes, but with higher light quantity requirements. The interaction between light quality and latitudinal origin on the growth of seedlings of temperate and boreal tree species proposed by previous authors, may actually be the result of two underlying, complementary responses: Firstly, an interaction between the light quantity and light quality (requiring higher amounts of FR light than R to reach similar results) and secondly, an interaction between the population origin and the light quantity (with northern ecotypes requiring more light than southern ecotypes). Remarkably, this is not contradictory to the results previously described by other authors, but a broader analysis may be needed to unequivocally reveal the relationship of the light quality and quantity requirements in tree seedlings from different latitudinal origins. Our analysis, together with our continuous light analysis suggests, that northern (or high latitude) ecotypes have adapted to longer photoperiods of modified light quality (mainly with respect to the R:FR ratio), which could have a more important role for biological processes like growth or bud set than in more southern (or low latitude) ecotypes of the same species. Although northern ecotypes may grow less under specific R:FR light conditions compared to southern ecotypes, at similar, non-saturating amounts of applied energy (in W m^{-2}), the amplitude of the effect of the light treatments between just R and FR light remain similar across the different latitudes (Figure 4A). This indicates that the accumulated amount of energy applied may play a more important role than the used light quality. Of course, effects of light quality and quantitiy on trees are occurring on top of other, fundamental environmental drivers, especially temperature were the effect of light quality have shown to be temperature dependent (Clapham *et al.,* 1998). Thus, phenology and growth of trees species from boreal and temperate climates are regulated by temperature, light quantity, quality, and photoperiod, where the relative importance of each of these is likely dependent on the species origin latitude (Vince-Prue *et al.*, 2001, Tanino *et al.,* 2010, Olsen, 2010, Olsen *et al.,* 2012).

5. Conclusions

Here, we supply easily transferable continuous data of changes in light quality through the day and year in dependency of different weather conditions. These results are highly relevant from a plant biological perspective since the recorded wavelength areas are among the important determinants of plant growth and development. Such data is required to design LED systems simulating natural variation in light quality and quantity, which is becoming increasingly relevant today because an increasing number of plant growth facilities are using LED systems as the main source of radiation, and more natural light spectra are desirable.

Here, we also corroborate our hypothesis that the extended periods with modified light spectra at high latitudes correlates with the light requirements of seedlings of boreal and temperate tree species. This suggest that in addition to other ecologically highly important factors such as temperature and photoperiod, changes in light quantity and quality play an important adaptive role on seedlings of woody plants at higher latitudes.

Supplementary Materials: The following material is available online at https://www.mdpi.com/1999-4907/10/8/610/s1, Figure S1: Effect of day extension with different red to far red ratios (R:FR ratios) on trees from different latitudinal origins, Table S1: Summary of the previous experiments used for the analysis, Table S2: Average light proportions of blue, green, and red and red to far red ratio under different solar elevation angles and two contrasting weather conditions.

Author Contributions: C.C., J.E.O., D. Bånkestad, and G.H. designed the experiment; C.C. performed the experiment; C.C. and D. Basler analyzed the data and prepared the figures; D. Basler and J.E.O. validated the results; C.C. and G.H. wrote the initial draft of the paper. All authors provided inputs and suggestions and approved the manuscript for submission

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Chapter 2

Reaching natural growth: Light quality effects on plant performance in indoor growth facilities.

Abstract

To transfer experimental findings in plant research to natural ecosystems it is imperative to reach near to natural-like plant performance. Previous studies propose differences in temperature and light quantity as main sources of deviations between indoor and outdoor plant growth. With increasing implementation of light emitting diodes (LED) in plant growth facilities, light quality is yet another factor that can be optimized to prevent unnatural plant performance. We investigated the effects of different wavelength combinations in phytotrons (i.e. indoor growth chambers) on plant growth and physiology in seven different plant species from different plant functional types (herbs, grasses and trees). The results from these experiments were compared against a previous field trial with the same set of species. While different proportions of blue (B) and red (R) light were applied in the phytotrons, the mean environmental conditions (photoperiod, total radiation, red to far red ratio and day/night temperature and air humidity) from the field trial were used in the phytotrons in order to assess, which wavelength combinations result in the most natural-like plant performance. Different plant traits and physiological parameters, including biomass productivity, SLA, leaf pigmentation, photosynthesis under a standardized light and the respective growing light and chlorophyll fluorescence, were measured at the end of each treatment. The exposure to different B percentages induced species-specific dose response reactions for most of the analysed parameters. Compared with intermediate B light treatments (25 and/or 35% B light), extreme R or B light enriched treatments (6% and 62% of B respectively) significantly affected the height, biomass, biomass allocation, chlorophyll content and photosynthesis parameters, differently among species. Principal component analyses (PCA) confirmed that 6% and 62% B light quality combinations induce more extreme plant performance in most cases, indicating that light quality needs to be adjusted to mitigate unnatural plant responses under indoor conditions.

Keywords: Light quality, blue light, red light, LED, phytotrons, photosynthesis, photomorphology

Introduction

Temperature and light are principal determinants of plant growth, as plants react to environmental conditions in their development. With improvements in controlled environment capabilities, the use of indoor cultivation systems has increased worldwide, both for research and production applications. It enables plant production when outside conditions are harsh, improving flexibility, control and predictability compared with outside growth. One of the problems, that especially plant researchers are confronted with, is a clear difference between plants grown under indoor versus outdoor conditions. These differences are naturally limiting the transferability of results from indoor experiments to natural systems. Several experiments have tried to replicate outdoor growth in indoor facilities, but low correlations have been found (Junker *et al.,* 2015; Hohmann *et al.,* 2016). Poorter *et al.*, (2016) suggested that this difference comes mainly from the different photothermal ratio (PTR), the ratio between the daily light integral and the daily mean temperature, which is generally much lower in growth chambers experiments. The low PTR in indoor experiments derives mainly from the low and constant used irradiances, compared with the higher and variable natural sunlight conditions. In general, the conditions in indoor facilities lead to higher specific leaf area (SLA), leaf nitrogen content and relative growth rate, as well as lower maximum photosynthesis (A_{max}), plant height and shoot dry weight (SDW), compared with outdoor experiments (Poorter *et al.,* 2016).

Due to high photosynthetic efficiency of blue (B) and red (R) light, high electrical efficiency of B and R LEDs, as well as high technical requirements to create sun-like LED spectra (Thiel *et al.,* 1995; Fujiwara and Sawada, 2006), most existing indoor plant growth facilities with LED lighting systems use mixtures of mainly B and R light. However, different LED lamp use different proportions of B and R LEDs, or B and R in combination with some other LED types, such as white and far-red, resulting in very different lighting environments among indoor growth facilities. In addition, the lack of a common protocol for reporting and measure LED light irradiance further limits the comparability between experiments (Cocetta *et al.,* 2017). Many studies have investigated the plants responses to different B to R ratios. These studies revealed that independent of the light intensity a required minimum percentage of B light is necessary to maintain the activities of photosystem II and I (Miao *et al.,* 2016). Hogewoning *et al. (*2010 a) suggested that at least 7% of B light is necessary to reproduce near to natural plant growth. Under monochromatic light, drastic effects after long exposures have been observed, including non-natural morphologies with parameters such as shoot elongation, specific leaf area (SLA), chlorophyll concentration and photosynthetic performance being

affected (Furuyama *et al.,* 2014; Hernandez *et al.,* 2016; Piovene *et al.,* 2015; Shengxin *et al.,* 2016).

The vast majority of studies related to light quality effects on plants have been conducted under low light levels (*e.g*. 20 µmol m^2 s^{-1} Macebo *et al.,* 2011; 100 µmol m^2 s^{-1} Hogewoning *et al.,* 2010 a and Hernandez and Kubota.,2016; 150 µmol m^2 s^{-1} Kim *et al.,* 2004;180 µmol m^2 s^{-1} Herrera *et al.,* 2018; 215 µmol m^2 s^{-1} Pennisi *et al.,* 2019; 330 µmol m^2 s^{-1} Schuerger *et al.,* 1997) with a few exceptions (*e.g*. 550 µmol m^2 s^{-1} Shengxin *et al.,* 2016), even though interactions between light quantity and quality have been reported previously (Furuyama *et al.,* 2014). Finally, it is also important to consider other light quality related parameters, *e.g.* the effect of red to far red ratio (R:FR). The applied light conditions in indoor cultivation typically have a much higher R:FR ratio (or a complete absence of FR) compared with sunlight conditions, which affects plant photosynthesis, morphology and development (*e.g.* Bae and Cho, 2008; Hogewoning *et al.,* 2010 a; Hernandez and Kubota, 2016; Hernandez *et al.,* 2016; Kim *et al.,* 2004; Shengxin 2016; Zhen and van Iersel, 2017). Once the R:FR ratio is corrected to more natural values, a more natural-like growth may be achieved despite big deviations from sunlight in other parts of the plant biologically active radiation (280-800 nm; *e.g.* Hogewoning *et al.,* 2012)

The aim of this study, as a first step on a series of experiments to reach natural-like growth under indoor conditions, was to investigate the effects of varying proportions of B and R light within walk-in growth chambers (phytotrons) on growth and physiological traits of plants from different functional plant groups. We also compared these experiments with a previous field-trial with the same set of species and expected more natural-like growth in closer to natural light spectra. The inclusion of seven different species form different functional plant types further enabled us to identify, if light quality effects plant performance differently among species and plant types. In contrast to many previous studies, we explicitly applied more natural-like R:FR ratios and light intensities, and the plants were exposed to temperatures and air humidities based on the pre-measured field trial to approximate the results to similar, but simpler, outside conditions.

Material and Methods

Plant materials and pre-growing conditions

For this study, we investigated young plants of 7 species from different functional plant types to include the species as source of variation: trees represented by black alder (*Alnus glutinosa*

L., provenance HG4, Zurich, Switzerland) and Scotch elm (*Ulmus glabra* HUDS., provenance Merenschwand, Aargau, Switzerland), herbs represented by basil (*Ocimum basilicum* L. var Adriana), lettuce (*Lactuca sativa* L.), melissa (*Melissa officinalis* L.) and radish (*Raphanus raphanistrum* L. subsp. sativus), and grasses represented by winter wheat (*Triticum aestivum* L.). For the experiments, all plants were raised from seeds. The seeds of both tree species were purchased from the Swiss federal institute for forest, snow and landscape research, WSL, Birmensdorf, Switzerland. All herb seeds were provided form Wyss Samen und Pflanzen AG, Zuchwil, Switzerland, and *Triticum* seeds were supplied form Sativa AG, Rheinau, Switzerland. In the subsequent, the species will continuously be referred to by their scientific genus name for clearness. Due to the different germination speeds the timing of sowing was different for the species as follows: seeds of *Alnus* and *Ulmus* were sown in 20 cm x 40 cm x 2 cm trays with commercial substrate (pH 5.8, 250 mg L^{-1} N, 180 P_2O_5 mg L^{-1}, K_2O 480 mg L^{-1}, Ökohum, Herrenhof, Switzerland) 43 days before the start of the experiments and were let to germinate under 190 µmols m^{-2} s^{-1} of photosynthetic photon flux density (PPFD: 400-700 nm) with 25% Blue (B: 400-500 nm), 32% Green (G: 500-600 nm) and 41% Red (R: 600-700 nm) light and a R to far red ratio (R:FR .655-665 nm and 725-735 nm; according to Sager *et al.,* 1988) of 5.1 for 23 days. Twenty days before the start of the experiment the light was increased to 240 µmols m^{-2} s^{-1} PPFD, with a R: FR of 5.1, to acclimate the plants to higher intensity levels. Thirteen days before the start of the experiment *Melissa* seeds were sown in the same type of trays and under the same conditions. 6 days before the start of the experiments the remaining species were sown in the same type of trays and under the same environmental conditions, with exception of *Triticum* which was sown immediately in round 2 L pots with a density of 15 seeds per pot (13.5 cm diameter, Poppelmann, Lohne, Germany). During the germination and pre-treatment period, the different seedlings were at 25 ºC / 50 % relative humidity during daytime and 15 ºC / 83 % relative humidity during night, with 10 hours day and one-hour light/temperature/humidity ramping pre and post day.

At the start of the experiment all species, excluding *Triticum*, were transplanted to the same type of 2 L pot previously used for *Triticum*, with a single individual in each pot. Moreover, *Triticum* was thinned to 10 plants per pot. The pots were filled with the same substrate as used in the germination trays, and 4 g of Osmocote slow release fertilizer (Osmocote exact standard 3-4, Scotts, Marysville, Ohio, USA), containing 16% total N, 9% P_2O_5, 12% K_2O and 2.5% MgO, was added to each plot. All plants were watered daily in the morning throughout the experiment.

The pre-growing procedure was repeated 3 times for this study: First, for the field-trial that was used as reference for the phytoron experiments, and then twice for the different light treatments of the phytotron experiment. (see *control and light quality treatments* below). No significant difference in initial height or biomass was found at the start of the experiments within species for the different replications (data not shown).

Control and light quality treatments

To establish a control treatment as a reference point for natural growth, all seven target species were grown in a field trial for 35 days (4 August 2017 - 7 September 2017) at the botanical garden of the University of Basel, Switzerland. Throughout the field trial, the *in situ* climate and the natural sunlight spectrum was recorded (see below). Following the field trial, we exposed plants from the seven different species to four mixtures of B and R light, that can be expressed as B/R ratio or as percentage of B light in four walk-in Phytotrons (1.5 m x 2.5 m) with full control of temperature, air humidity and light quality and quantity (prototypes, Enersign GmbH, Basel, Switzerland). To unify nomenclature with previous studies, the four different light treatments will be referred to by their respective B light proportion (Table 1 and supplementary Fig. 1). The light treatments were designated based on previous literature (Hogewoning *et al.,* 2010 a), measurements of natural light done *in situ* (Chiang et *al.,* 2019) and technical capacities of the phytotrons at the average light intensity of the outdoor treatment. For each treatment, the replication per species was 9 pots (with either one or more individuals per pot depending on species; see above). In all light treatments, the average PPFD from the field trial (575 µmol m^{-2} s^{-1}) was provided at the average height of the different species using 18 LED panels for each chamber consisting of a mixture of B, G, R and FR LEDs per panel (prototypes, DHL-Licht, Hanover, Germany). The LED lighting system of each chamber was mounted on movable ceilings, which height can be adjusted by the environmental control software of the chambers. To preserve similar light level at average plant height, the height of the lamps was adjusted twice along the experiment. Based on the field trial conditions, the photoperiod was set to 13 hours and 5 minutes, giving a constant daily light integral (DLI) of 27.1 mol $m^{-2}day^{-1}$ in all light treatments. Similar to the light conditions, temperature and humidity during day and night were set to average field trial conditions: 22ºC/ 66%RH and 18ºC/ 79%RH, for day and night, respectively, with a period of one-hour ramping between day and night. A uniform temperature and humidity distribution within each chamber was ensured by a constant vertical air stream from below. To avoid border and space effects, all plants were randomly distributed within each phytotrons on two tables. The tables were rotated by 90°every

day. Each light treatment was replicated twice (two separate runs of all four light combinations), where the distribution of the chambers was random between the two runs.

At the end of the 35 days experimental period a suite of measurements was conducted in the field trial and the phytotron experiments. A description of the measured parameters is given in the following paragraphs. Due to limitations imposed by the lamp characteristics a higher R:FR ratio compared with outdoor (1.8 vs. 1.1) was applied in order to reach the targeted light intensities. No UV light was applied.

Table 1: Spectral characteristics of sunlight and of the indoor light treatments, based on the measured spectra shown in Fig. 1.

Treatment \ Characteristic	**Field trial**	**6 % B**	**25 % B**	**35 % B**	**62 % B**
Blue (%)	28	6	25	35	62
Green (%)	36	16	16	16	16
Red (%)	36	78	59	49	22
R:FR ratio	1.1	1.8	1.8	1.8	1.8

Climatic growth conditions

In order to apply most natural conditions within the phytotrons, the climate from the field trial at the botanical garden of the University of Basel, Switzerland was recorded throughout the 35 days growth period. Relative humidity, temperature, and PPFD were measured every 5 minutes with a weather station (Vantage pro2, Davis, Haywards, California, USA). In addition, sunlight spectra in the waveband 350 - 800 nm were recorded every minute using a spectrometer (STS, OceanOptics, Florida, United States) that was equipped with an optical fiber and a cosine corrector (180º field-of-view ; CC-3-UV-S, OceanOptics) placed by the weather station's PAR sensor facing upwards. The spectrometer was associated to a Raspberry Pi 2 computer for automatic sampling, integration time adjustments and data storage. *A posteriori*, the spectra were used to calculate photon flux densities within specific wavebands: PAR, B light, G light, R light and R:FR ratio. The light measurements were verified by comparing the data from the weather station with the data from the spectrometer readings. The data from the field trial were used to calculate average diurnal and nocturnal temperature, air humidity and PAR conditions for the phytotron treatments.

Morphological parameters.

By the end of the 35 days growth period, the plant height was measured as total height from the substrate to the apical tip. In the case of long inflorescences (*Raphanus*) or plants without a clear stem (*Triticum*), extended leaf length was recorded as height, and in case of *Lactuca*, no height was recorded. Two full-grown leaves from the top three mature leaves were collected from each plant to measure leaf area (LI-3100, Licor, Licoln, Nebraska, USA) and calculate the specific leaf area (SLA) in $cm^2\ g^{-1}$on a dry leaf weight basis. Dry weight (DW) was measured separately for leaves, stems and roots after 10 days drying at 80ºC in a drying oven (UF 260, Memmert, Schwabach, Germany). Because of the lack of a clear stem, only total aboveground and root biomass was measured for *Lactuca*, *Melissa* and *Triticum*. All reported organ weights and the below to above ground biomass ratio (root:shoot-ratio) refer to plant dry mass.

Chlorophyll fluorescence and chlorophyll content

One night before the end of the experiment, fast chlorophyll fluorescence induction was measured on one of the top three leaves in four randomly chosen plants of each species and treatment by using a continuous excitation fluorometer, (Pocket PEA, Hansatech instruments Ltd, Nordfolk, UK). The plants were dark adapted for at least 20 minutes before recording photosynthetic maximum quantum yield (Fv/Fm) and the absolute performance index (PI) of the leaves.

At harvest, two discs of 1.13 cm^2 area from the top four leaves were punched and stored in a 1.5 mL Eppendorf tube together with four to six glass beads of 0.1 mm diameter for later chlorophyll analysis. The tubes were quickly frozen in liquid nitrogen and then kept at -80ºC until analysis. At the day of chlorophyll measurement, the tubes were agitated two times for 10 seconds to triturate the tissue using a mixing device (Silamat S6, Ivoclar Vivadent, Schaan, Liechtenstein). After adding 0.7 mL. of acetone to each tube, they were agitated again for 10 seconds and then centrifuged at 13000 rpm at 4ºC for 2 minutes. 0.25 mL of the supernatant were dissolved in 0.75 mL of acetone, and the samples absorption spectra were measured using a spectrometer (Ultrospec 2100 pro, Biochrom, Holliston, USA). Chlorophyll a and b concentrations, chlorophyll a to b ratio (Chl a, Chl b and a:b ratio, respectively) and total carotenoid concentrations as $mg\ g^{-1}$, were calculated from the spectra using the values at 470, 646 and 663 nm as described in Wellburn (1994).

Leaf gas exchange

Six days before the end of the experiment, a light response curves of net CO_2 leaf-exchange was measured in one of the top three leaves in three randomly chosen plants per species and treatment using a LI-6800 photosynthesis system (LI-COR, Lincoln, Nebraska, USA). The light response curves were measured under two different light spectra: i) a standardized artificial light spectrum, composed by 70% R and 30% B (in the following referred to as 'standardized light') provided by the chamber head light source to study photosynthesis of the different species under a uniform light spectrum, and, ii) the respective growing light spectrum (in the following referred to as '*in situ* spectrum') provided by using a transparent, clear-top chamber head (Clear-top leaf chamber 6800-12A, LI-COR) to study photosynthesis of the different species under their respective growing spectra and avoid any bias on photosynthesis from a non-adapted spectrum. Twelve different light intensities: 2000, 1500, 1000, 800, 600, 400, 200, 100, 50, 25, 10 and 0 μmol m^{-2} s^{-1} of PPFD were used for light response curves with the artificial light spectrum. Due to lower maximum irradiance in the phytotrons limited by the light quality being applied (see above), the light response curves for the *'in situ'* growing light were measured only up to a maximum radiation of 700 μmol m^{-2} $s^{-1}$of PPFD (700, 480, 380, 200, 100, 60, 30, 20, 17, 15 and 0 μmol m^{-2} $s^{-1}$of PPFD). All leaf CO_2-exchange measurements were conducted at 400 ppm CO_2, 60% relative air humidity and 20ºC leaf temperature, with 60 to 120 seconds as threshold for stability after each light change intensity. Stability of readings was assumed when the difference of the slopes between IRGA's were smaller than 0.5 μmol mol^{-1} sec^{-1} and 1 mmol mol^{-1} sec^{-1} for CO_2 and H_2O, respectively.

For each light curve, 12 different light models were fitted (Lobo *et al.,* 2013), including a model for photo-inhibition (Eilers and Peeters 1988). For each species and treatment, the model with the best fit (lowest sum of squares) was selected (details in Lobo *et al.,* 2013). The selected model was then used to calculate the following four values from the light response curve: maximum photosynthesis within the range of measured light (A_{max}), quantum yield of the CO_2 fixation (α) as the slope of the linear curve between 0 and 100 μmol m^{-2} s^{-1} of PPFD, dark respiration (DR) and the light compensation point (CP) of photosynthesis.

Statistical analysis.

To evaluate the effect of the light treatments, a two-way analysis of variance (ANOVA) was performed for all measured parameters, considering the species and different treatments as fixed factors and the two replicates of each treatment as random effect. The significance of the

random factor was evaluated using a restricted likelihood ratio test. The data was checked for normal distribution, independence and homogeneity of the variance.

To enable the direct visible and statistical comparison of the treatment effects across species, each measured trait was normalized relative to its mean value on the field trial for each species. (Raw trait average values per species and treatments are available in Table S1). The normalized data was used to perform a one-way ANOVA, considering the treatments as fix factor and species as random factors (Table S2). A Tukey pairwise multiple comparison test was used as post hoc analysis. In several cases when all indoor light treatments differ from the field trial, an additional one-way ANOVA was performed without the field trial to highlight the individual response differences to the different light treatments (Supplementary table 3).

Finally, to identify the specific traits that have the maximum variation between treatments and to quantify which treatment gave the less extreme response compared to the outdoor trial, a principal component analysis (PCA) was performed separately for each species, using the different measured traits as input values. To perform a PCA analysis, the same number of observations is required for each variable but due to fewer light measurement, chlorophyll measurements and fluorescence measurements than the number of plants used for biomass measurements, in each species and treatment, the missing values of chlorophyll content and light parameters were imputed using normal distribution with the same average and standard deviation of the available data. All analyses were done using R (version 3.6.1, R Core team) using the package plyr for data processing and lm4, car, RLRsim, emmeans for data analysis and multicomp and vegan for statistically significant representations.

Results

Plant growth and biomass allocation

There was a significant interaction between the light treatments and the different species on the total plant height at the end of the experiments (Table 2), where the relationship with the field trial was species dependent. Some species, e.g. *Alnus* and *Melissa*, were significantly smaller independent of the light treatment, while other, e.g. *Ocimum*, were taller than the same species in the field trial. Compared only among the phytotron treatments, all species had shorter plants at higher percentages of blue (B) light (62%), which was most pronounced in *Alnus* and *Melissa* (58 and 52% lower height respectively compared with the 6%B treatment; Fig. 1 A). Other species like *Ocimum* and *Triticum* were less affected by changes of B light but follow the same trend (20 and 15% lower height respectively compared with the 6%B treatment; Fig. 1 A). In several of the tested species, there was a significant difference in plant height between the two

intermediate B treatments (25 and 35 %B). Averaged across species, 6% B light produced 22 % taller plants that were statistically significantly different from the two intermediate treatments,

Table 2: P-values for the different measured traits in both experiments.

	Fix factors			**Random factors**
	Light quality	**Species**	**Light quality x Specie**	**Replicate**
Variable				
Biomass and Morphology				
Height*	< 2.2x10^{-16}	< 2.2x10^{-16}	< 2.2x10-16	5x10-4
Dry weight leaves	1.16E-05	< 2.2x10^{-16}	< 2.2x10-16	1.5x10-3
Dry weight shoot**	1.03E-08	< 2.2x10^{-16}	2.37E-14	-
Dry weight roots	1.26E-05	< 2.2x10^{-16}	< 2.2x10-16	< 2.2x10-16
Total dry weight	8.74E-05	< 2.2x10^{-16}	< 2.2x10-16	< 2.2x10-16
Root to Shoot ratio	1.39E-11	< 2.2x10^{-16}	< 2.2x10-16	< 2.2x10-16
SLA	0.1024	< 2.2x10^{-16}	< 2.2x10-16	7.9x10-3
Chlorophyll				
Chlorophyll a (mg g^{-1})	4.90E-07	< 2.2x10-16	3.47E-14	< 2.2x10-16
Chlorophyll b (mg g^{-1})	< 2.2e-16	< 2.2e-16	< 2.2e-16	5.62E-14
Chl a: b ratio**	1.85E-05	< 2.2e-16	5.98E-06	-
Carotenoids (mg g^{-1})	1.49E-13	< 2.2e-16	2.78E-13	< 2.2e-16
Fv/Fm**	2.53E-08	< 2.2e-16	0.003297	-
Standardized light				
Max photosynthesis**	0.03074	4.42E-05	3.09E-08	-
Quantum yield**	2.44E-06	1.94E-12	-	-
Dark respiration**	0.4026571	9.16E-12	6.89E-05	-
Compensation point	0.008619	< 2.2e-16	5.48E-11	< 2.2e-16
In-situ' light				
Max photosynthesis**	6.52E-06	1.25E-12	-	-
Quantum yield**	6.45E-06	1.93E-07	-	-
Dark respiration**	-	4.06E-06	-	-
Compensation point	0.3041	4.19E-16	1.74E-05	< 2.2e-16

*Lettuce was removed from these analyses

**Interactions or factors were removed from the analysis due non-significance.

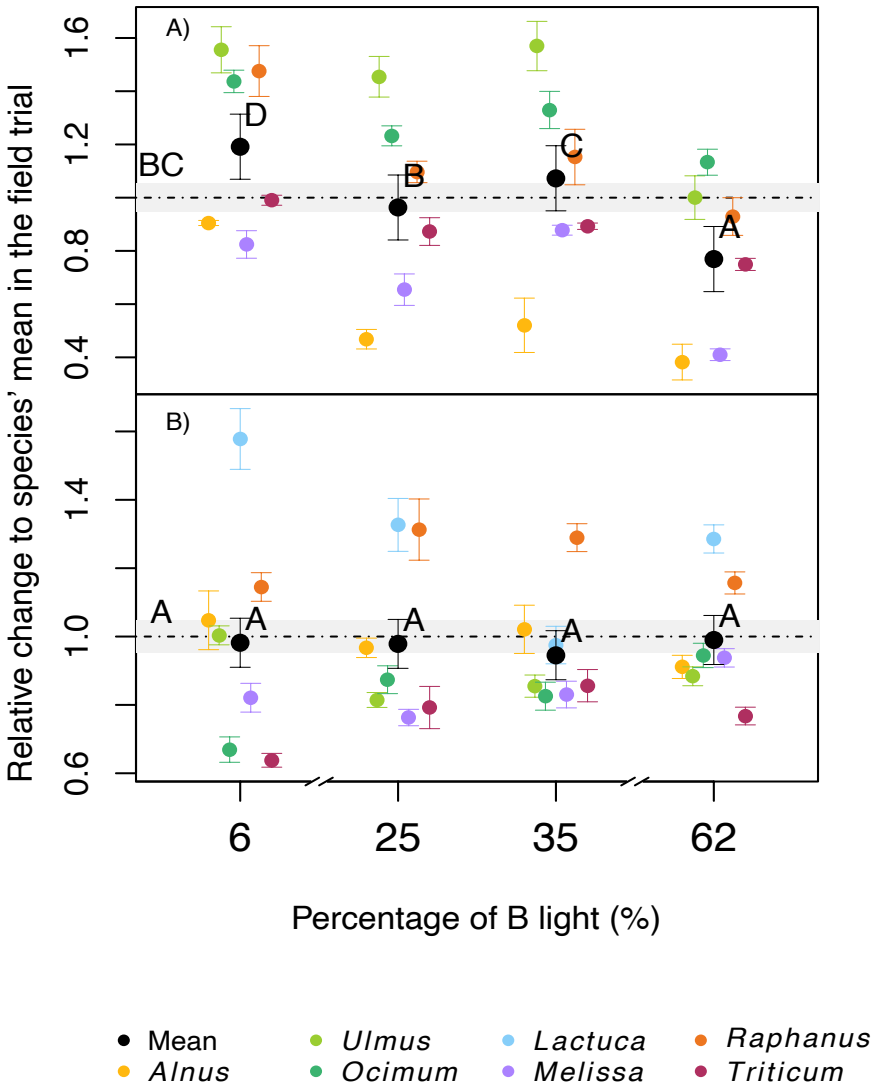

Figure 1: Fold change on: plant height (A) and SLA (B), relative to the average field trial (dotted line). Coloured dots are the average of each species in both experiments runs (n=18), the black dots are the average values across all 7 species (n=126). Error bars indicate the standard errors. The grey area corresponds to the standard error of the field trial. Different letters indicate statistically difference between groups with experiment replicate and species as a random effect.

while in the other extreme, 62% B light yielded a statistically significant shortening of plants by about 20 % compared with the average across treatments (Fig. 1 A). A dose respond was obtained for specific leaf area in several species (SLA, Fig. 1 B). Unlike the height results and due to the species-specific reactions to the light treatments, the average response did not significantly differ neither within the light treatments nor between the light treatments and the outdoor control. *Lactuca* and *Alnus*, *e.g.*, had significant higher SLA at 6% B compared with other light treatments, while other species, *e.g. Raphanus* and *Triticum* had higher values at 25 or 35% B light compared with 6 or 62 % B light.

There were significant interactions between the light treatments and species for the dry biomass of leaves, shoots and root as well as for the total dry biomass (Table 2). Similarly to plant height and SLA, the relationship of plant biomass at the different light treatments with the field control was species dependent. Independent of the species, there was generally a lower leaf biomass at 62% B light compared with 6% B light. This was especially the case for the two tree species tested, where *Alnus* and *Ulmus* were most sensitive to low percentages of B light (Fig. 3A). On average across all species, in none of the light treatments leaf biomass differed significantly from the outdoor control. Nevertheless, plants exposed to 6% B had 35% higher leaf biomass than plants exposed to 62%B (Fig. 3A) Similar results were obtained for shoot biomass where, across all species, plants grown at 62% B had a significantly lower shoot biomass compared with all the other light treatments, but similar values as in the field trial (Fig. 3B). In contrast to the aboveground biomass, the effects of light quality on root biomass was different among all species (Fig. 3 C). In comparison to the field trial, four species (*Ulmus*, *Lactuca*, *Ocimum*, *Triticum*) had significantly higher root biomass in the phytotron treatments, while in three species (*Raphanus*, Alnus, *Melissa*) it was very similar compared to the field trial (Fig. 3C). Across all species, there was not a strong effect of light quality on root biomass among the light treatments, but a trend to higher root biomass at 6% B (Fig. 3C). Total biomass production followed the same trend as found for the individual plant organs, with a significant interaction between light treatment and species (Table 2); higher values under indoor conditions independent of the light treatment compared with the field trial and increasing biomass with decreasing percentage of blue light (data not shown).

With respect to the effect of light quality on the allocation of biomass, there was a significant interaction between light treatment and species for the root to shoot (r:s) mass ratio (Table 2). Almost all species had significant higher r:s values in the phytotrons compared to the field trial independent of the light treatment, with *Triticum* showing even an 4 to 8 times higher investment to roots compared to the field control (Fig. 2 D). In some species (*e.g. Alnus* and *Ocimum*), 6% and 62% B light induced higher r:s ratios than 25 and 35% B light, while other species (e.g. *Melissa* and *Ulmus*) were almost indifferent with respect to light quality (Fig. 2 D).

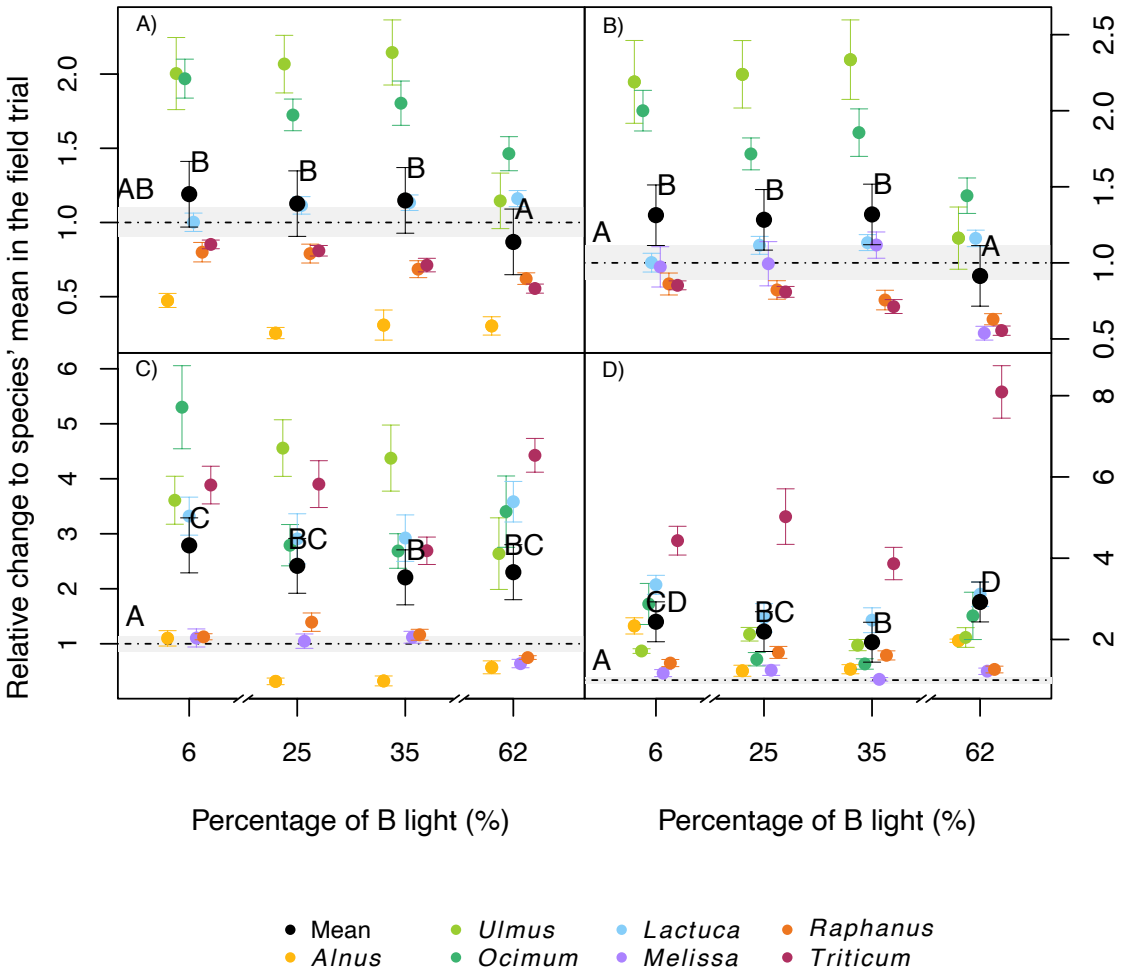

Figure 2: Fold change on: leaves (A), shoot (B), roots (C) and root to shoot ratio (D), as dry weight relative to the average value of the field trial (dotted line). Coloured dots are the average of each species in both experiments runs (n=18), the black dots are the average values across all 7 species (n=126). Error bars indicate the standard errors. The grey area corresponds to the standard error of the field trial. Different letters indicate statistically difference between groups with experiment replicate and species as a random effect.

Leaf pigmentation

There were significant interactions between the different treatments and species on the pigment concentrations in leaves (Table 2). Also, the relationship of the different light treatments to the field trial was species dependent, but all investigated species exhibited higher Chl a concentration in leaves at 62 % B light compared to the other light treatments (strongest effect in *Lactuca*) and the lowest Chl a concentrations at 6 % B light (Fig. 3 A). On average across all species, 6%B was the only treatment significantly different from the field trial with 24% lower concentration of Chl a. The effect on Chl b was similar to that of Chl a, with smaller effects of the light quality on the total amount of Chl b (data not shown). As a result, the average a:b ratio across all species was not significantly different across light treatments, but significantly higher

than in the field trial (Table 2, Fig. 3 B). The concentrations of carotenoids in leaves, showed overall very similar reactions to light quality as chlorophyll, with increasing concentrations at higher proportions of blue, and an interaction between the light treatment and species (Fig. 3 C, Table 2). Like chlorophyll and carotenoids, Fv/Fm values, show significant interaction between the species and the light treatments (Table 2). Almost all species in the phytotron treatments with 25, 35 and 62 % B had Fv/Fm values close to the field trial values (Fig. 3 D), except Ocimum were this one revealed higher Fv/Fm values indoors than in the field trial. Averaged across all species, Fv/Fm was significantly lower than in the field at 6% B (Fig. 3 D). Performance index (Pi) absolute values followed the same trend as Fv/Fm (data not shown, Table S1).

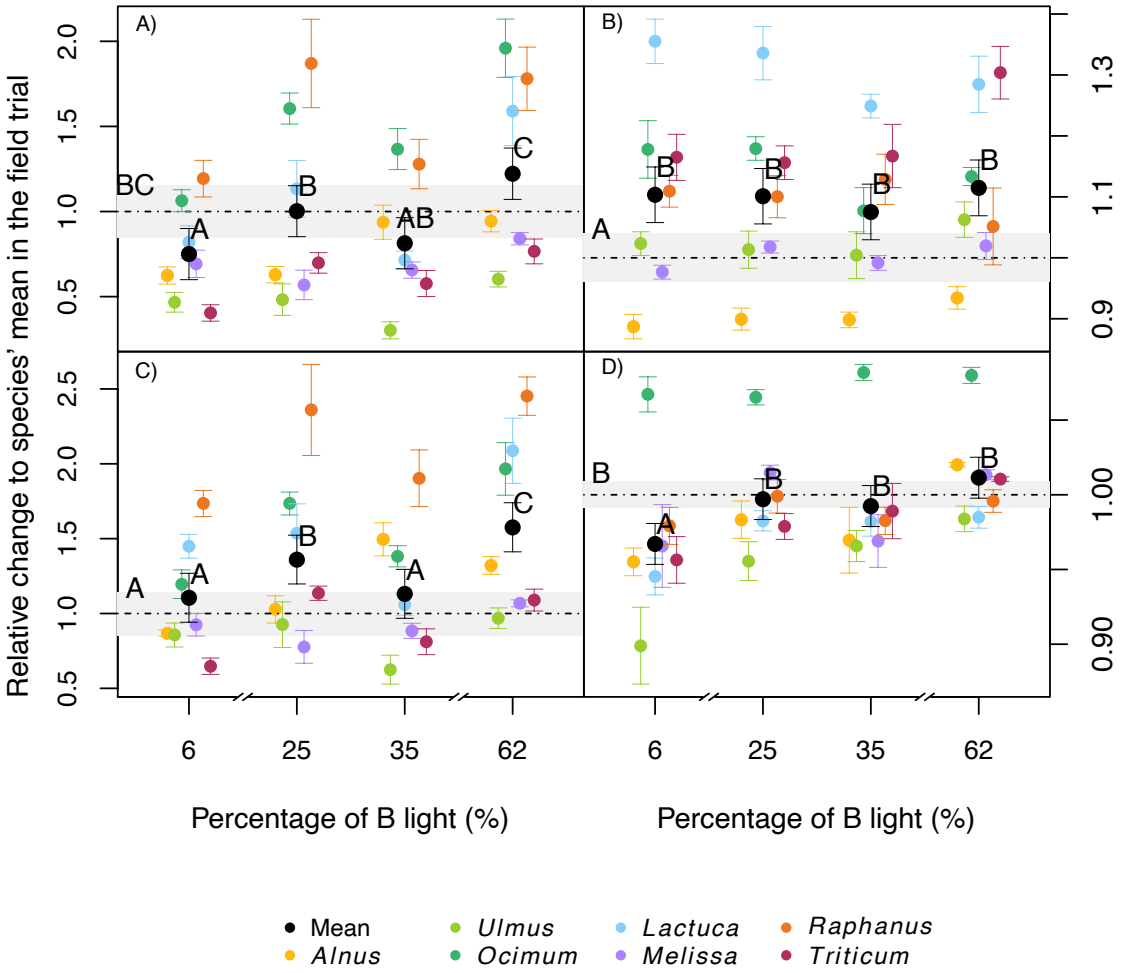

Figure 3: Fold change on Chlorophyll a (A), Chlorophyll a:b ratio (B), carotenoids content (C) and Fv/Fm values (D) relative to the average value of the field trial (dotted line). Coloured dots are the average of each species in both experiments runs (n=18), the black dots are the average values across all 7 species (n=126). Error bars indicate the standard errors. The grey area corresponds to the standard error of the field trial. Different letters indicate statistically difference between groups with experiment replicate and species as a random effect.

Photosynthesis and leaf respiration

In contrast to the other plant traits tested, all species reacted very uniformly to the light treatments in all measured photosynthesis and leaf gas exchange parameters, and no significant interaction between treatment and species effect was found (Table 2). When measured with the standardized light, maximum photosynthesis (A_{max}) was, on average across all species, significantly higher at 62%B compared with the field trial (Fig. 4 A). Meanwhile, when the same parameter was measured under the *in situ* light, higher values were reached at either 25% or 35%B light compared with the field trial (Fig.4 B). The quantum yield of the CO_2 fixation (α) had similar trends to A_{max}, where on average no light treatment was significantly higher than the field trial when the standardized light was used. 62% B light was the only treatment to induce higher α values than the other light treatments (Fig. 4 C). When α was measured using the *in situ* light, higher values were reached at either 6%, 25% or 35%B compared to the field trial (Fig. 4 D).

The photosynthetic light compensation point (CP) and the dark respiration of leaves (DR) were significantly different among species (Table 2). Averaged across all species, there were no significant effects of the treatments on CP when the standardized light was used, but with the *in situ* light significantly lower values were reached under 6 and 25% B conditions compared with 35 and 62% B and the field trial (data not shown). DR was on average significantly lower in plants exposed to 62%B light compared with other light treatments and the field trial when the standardized light was used (Fig. 4 E). This was not the case for the *in situ* light, were although several species had higher DR values than the field trial, no significant difference was found between the treatments for the average across species (Fig. 5 F).

Principal component analysis (PCA)

A principal component analysis (PCA) for each species revealed a clustering of each treatment with various degrees of overlap (Fig. 5); from easily differentiable groups between light treatments in some species, *e.g. Alnus*, *Lactuca* and *Triticum*, to a more continuous gradient among treatments, *e.g. Melissa* and *Raphanus*. *Alnus, Ocimum, Lactuca, and Triticum* showed a large variability between treatments from outdoor (field trial) to indoor conditions, while the different light treatments tended to cluster close to each other. This was not the case for *Melissa,* Raphanus *and Ulmus* where the field trial was not clearly separated from the phytotron treatments (Fig. 5). The two intermediate treatments (25% and 35% B) yielded responses closer to the average (*i.e.* the centre of the figure) in most species. The loadings for

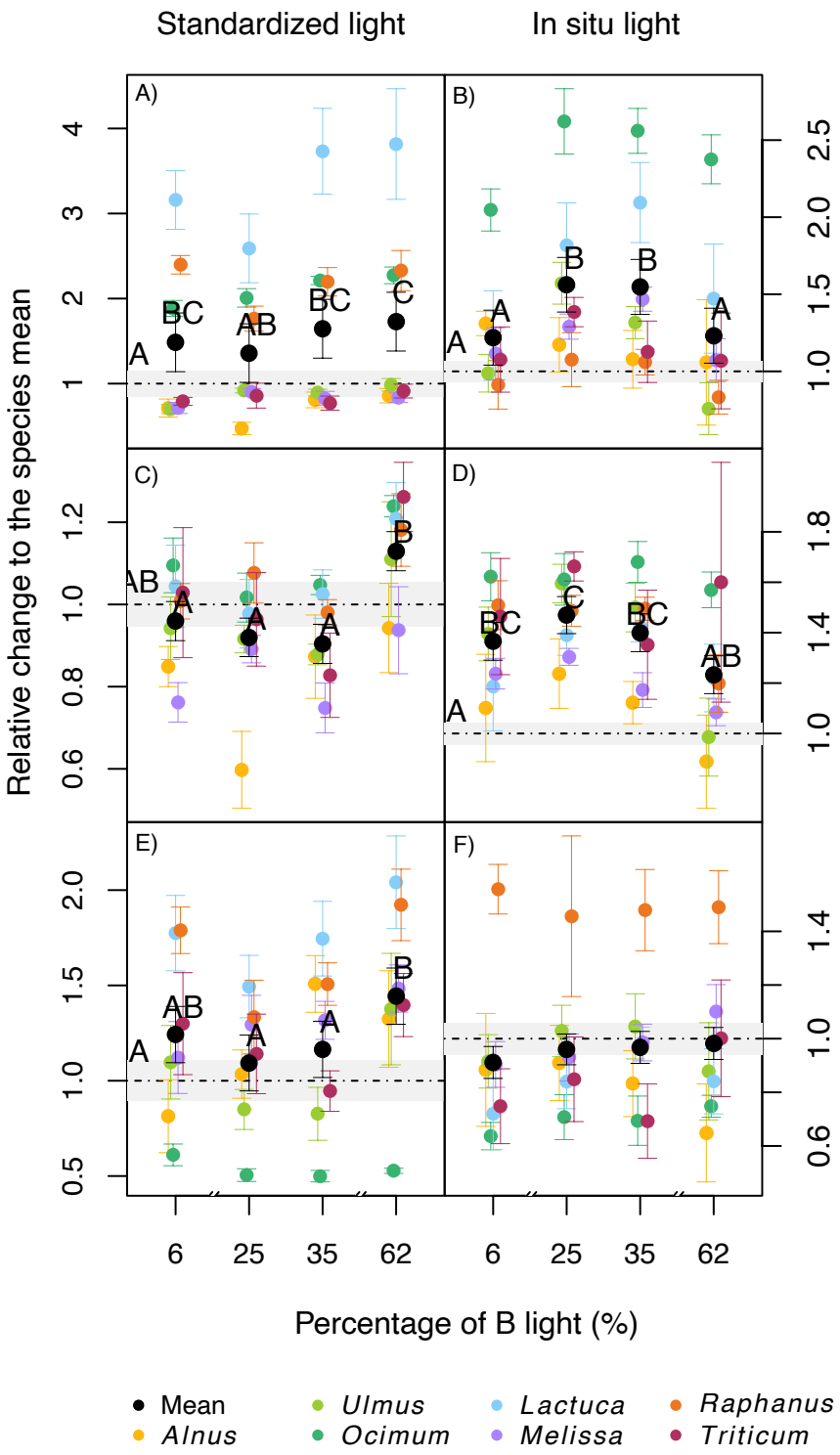

Figure 4: Fold change on maximum photosynthesis (A_{max}, A and B), quantum yield of the CO_2 fixation curve (α, C and D) and dark respiration (DR, E and F) relative to the average value of the field trial (dotted line). Values were measured with either a standard light with 70% B light and 30% R light ('standardized light') or the actual '*in situ*' light (see methods for details). Coloured dots are the average of each species in both experiments runs (n=18), the black dots are the average values across all 7 species (n=126). Error bars indicate the standard errors. The grey area corresponds to the standard error of the field trial. Different letters indicate statistically difference between groups with experiment replicate and species as a random effect.

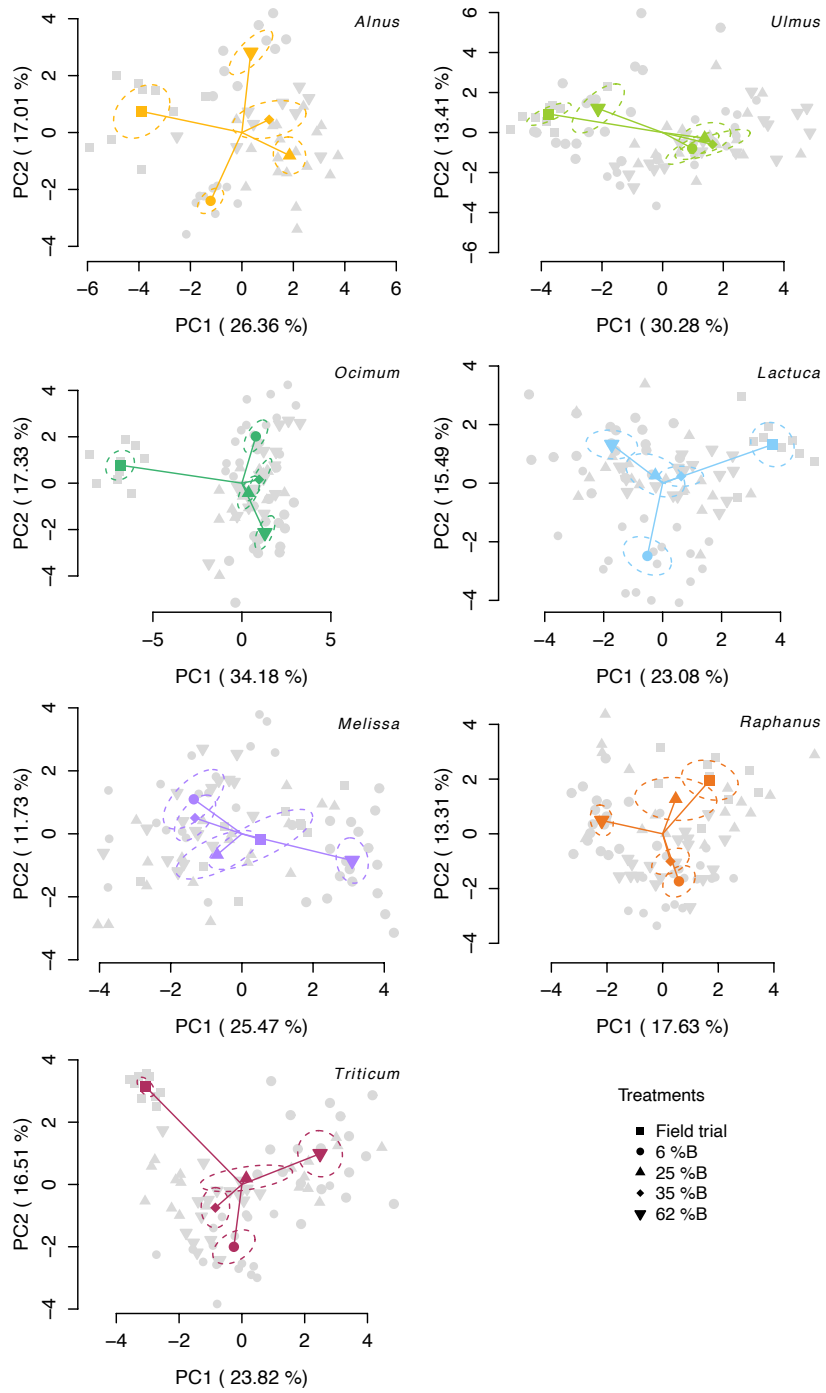

Figure 5: Principal component analysis (PCA) of the measured traits of each specie grown under 6% B, 25% B, 35% B and 62% B light. Each lighter point (n=18) corresponds to a plant and solid ones to the average weighted centroids of each light treatment, where the name of each specie is mentioned in the respectively upper right corner. Ellipses correspond to the standard error of the weighted centroids with a confidence of 95%.

score calculations were also plotted to determine the importance of each factor. No single parameter was specifically responsible for the variation across treatments and between species, except for CP in *Ocimum* growing in the field trial (Supplementary Fig. 2). Independent of the species the first two components explained between 31% and 43% of the total variability.

Discussion

Previous studies investigating the effect of the spectral light quality on plant performance were mainly focused on single species, and they generally did not directly compare findings with natural conditions. In the present study, we deliberately investigated a suite of species from different functional plant types to determine how different they react to the treatments. By applying the same mean climatic conditions indoors as in the initial field trial, we could determine which LED light conditions are generating the most natural-like plant performance. Our results showed clear differences within the light treatments and between the light treatments and the field trial on most measured plant traits, whereby the effect sizes were highly species-specific, while effect directions were similar among species, with the clear exception of SLA and root biomass production. As expected, light treatments with a very extreme blue: red (B:R) ratios (6 and 62% B) induced more extreme ('unnatural') values in most plant traits than treatments with a more balanced B:R ratio (25 and 35% B).

Light quality effects on morphology

Previous studies that compared indoor with outdoor plant growth were often biased by a higher plant density in the indoor condition (Poorter et al., 2016). In our study, we therefore deliberately kept exactly the same plant densities between the field and the phytotron trials to avoid any stand density bias on plant morphology.

The effects of B light percentages on plant morphology have been previously reported in numerous studies (Dougher and Bugbee, 2001; Hogewoning *et al.,* 2010 a and 2010 b; Hogewoning *et al.,* 2012; Terfa *et al.,* 2012; Gautam *et al.,* 2015; Piovene *et al.,* 2015; Shengxin *et al.,* 2016). In general, B light is sensed by the cryptochrome system, where under high irradiances or high levels of B light, plants exhibit shorter and stunned growth (*e.g.* Lin *et al.,* 1998, Hogewoning *et al.,* 2010 a; Hernandez and Kubota, 2016). It is also known that a total lack of B or R light negatively affects plant performance, including growth rate, height and several other parameters. *E.g.,* Hernandez *et al.,* (2016) found that tomato plants grew shorter under either B or R light mixtures compared with only B or R light. The higher hypocotyl extension when B or R light was not present was then associated to lower anthocyanin concentrations, indicating that a mixture of B and R light is required for an optimal growth

Previous studies have shown that under high levels of B light, there is an increase of the palisade cell area, which can lead to an increase of leaf thickness (*e.g.* Hogewoning *et al.,* 2010a, Shengxin *et al.,* 2016, Hernandez *et al.,* 2016). However, this B light-induced increase in leaf thickness does not necessarily have to translate into a lower SLA (Zheng and Van Labeke,

2017). Dougher and Budgee (2001) identified that the direction of the effect of B % light on SLA is very species dependent. Independent of the applied light quality, Poorter *et al.,* (2016) found that on average, indoor experiments tend to produce plants with higher SLA compared to field grown plants, mainly due to higher temperatures and lower light quantity in indoor facilities. In our study, which applied the average temperature and light quantity as in the field trial, the SLA of most species was similar between plants growing in the phytotrons and in the field.

Stem, leaf, root and total dry biomass under the different treatments followed largely the trend in plant height. The lower biomass at high B % can thus be explained by a stronger inhibition of stem elongation by B light due to an increased cryptochrome activity (Hernandez and Kubota, 2016). In addition, the stunted growth of plants at high B % leads to an increased self-shading of leaves and decrease of light interception, which has been proposed to result in negative consequences for the whole plant productivity (Hogewoning *et al.,* 2012). Although the individual species reacted differently between phytotrons and field trial, on average, a significantly higher plant biomass within our phytotron treatments compared with the field was found (except the 62% B treatment). In contrast, Poorter *et al.,* (2016) reported lower biomass under indoor conditions compared with field grown plants up to 10% depending on species and functional group. This might be explained by the fact that, in contrast to other indoor experiments, we deliberately applied the same average temperatures and light strength in the phytotrons as were measured in the field trial.

While the effect of light quality on the aboveground organs was quite similar among species in the current study, the direction of the effect on roots was clearly species dependent, with species like *Alnus* and *Ocimum* exhibiting higher root growth at very low and high B %, and species like *Raphanus* and *Ulmus* showing increased root production at intermediate B percentages (25 and 35 % B). Up to date, only scarce information is available on light quality effects on belowground plant productivity. A previous study by Yorio *et al.,* (2001) reported that under 10% B mixed with 90% R light, there was a higher root production in *Lactuca*, *Raphanus* and *Spinacia*, compared with plants grown under pure R light. Nhut *et al.,* (2003) found that mixtures of B and R light stimulate the production of roots compared with pure R light in strawberry plantlets. Independent of light quality, we found significantly enhanced root production in the phytotron treatments in all species except *Alnus*. As Poorter *et al.,* (2016) indicated, indoor climatization might induce root zone conditions that differ markedly from field conditions, leading to altered root production and consequently, profoundly changed plant growth. Because all plants in our experiment were regularly watered in the field and phytotron

treatments, we can exclude that the observed higher root productivity in the phytotrons result from different water availability between indoor and field trials. However, pot soil temperature was not monitored, and it is likely that it differed significantly between indoor and field conditions, partly due to the lack of infrared radiation in the LED lamps.

Light quality effect on leaf pigmentation

The leaf pigmentation (*i.e.* the concentration of chlorophyll and carotenoids) changed strongly with light quality in our study. Under natural sunlight, cryptochrome activity is reduced at high radiation, thereby signalling strong light conditions to the plant. The same effect can be achieved under experimental conditions by exposing plants to high percentages of B light (Lin and Heins, 1997). The high proportion of B light in our 62 % B treatment thus triggered the enhanced production of photosynthesis pigments, despite the fact that the other treatments with lower B % had the same PPFD. In fact, the low concentrations of Chl a and b in plants that have been treated with low levels of B light or monochromatic R light in previous studies, have even led to photo-oxidative stress in plants due to an increase of O_2^- and H_2O_2 radicals that induce cellular damage (Hogewoning *et al.,* 2010 a ; Shengxin *et al.,* 2016). Barnes and Bugbee (1992) proposed that a minimum of 20-30 μmol $m^{-2}s^{-1}$ of B light is necessary to reach natural-like growth and morphologies, even if such a minimum requirement for B light appears to be highly species-specific (Dougher and Bugbee, 1998). Likely because all of our light treatments included at least 6 % of B light, we did not observe light quality related stress effects in our experiment, but we identify that even with over 30 μmol $m^{-2}s^{-1}$ of B light (at 6% B), higher percentage of B can increase the photosynthetic capacity, indicating that it is not just the quantity of B light, but also its relationship with other wavebands in the spectrum. Interestingly, most species showed higher Chl a:b ratios in the phytotrons compared to the field trial. This effect has been observed previously in indoor-grown plants by Vialet-Chabrand *et al.,* (2017), who attributed it to the lack of fluctuating light conditions in indoor facilities.

Like chlorophyll, the production of carotenoids was also significantly increased at 62% of B light compared with 6% B (and 35% B), but only the 62% B treatment induced higher carotenoids concentration than in the field trial. Hogewoning *et al.* (2010a) reported an increase of carotenoids in cucumber plants when B was increased to 50% in the light spectra. An increase of carotenoids has been shown to work as an accumulative protection mechanism, correlating with high intensity light or light spectra rich in B. For example Shengxin *et al.* (2016) found that Fv/Fm of rapeseed leaves got reduced under monochromatic B or R light treatments,

compared with mixtures of B and R. They attributed this to a higher PS II damage and linked the higher concentrations of carotenoids to a protection mechanism against oxygen radical formation. This is in line with our Fv/Fm results, where lower percentages of B in the applied spectra induce, on average, small but significant differences of the Fv/Fm values in almost all investigated species.

Light quality effects on photosynthesis

When A_{max} was measured under the same standardized light conditions (30% B and 70% R) in the current study, plants under 63 % B showed on average significantly higher A_{max} compared to plants under 25 % B and the field trial. This could be partially explained by the increased chlorophyll concentrations in 63% B treated plants (see above). Previously higher A_{max} have been linked to higher levels of stomatal conductance and nitrogen concentration, were this last one is correlated to Rubisco, cytochrome, proteins and chlorophyll content (Matsuda *et al.,* 2004). A higher A_{max} has also been suggested to partially derive from an instantaneous stimulation of photosynthesis (*i.e.*, during the exposure to the light within the gas-exchange chamber) due to the lack of adaptation to the standardized light condition (Hogewoning *et al.,* 2010a). In our case using 70% R in plants adapted to 62% B may promote a higher A_{max}, meanwhile this may not be the case in plants adapted to lower percentages of B light, and therefore higher percentages of R light. Kim *et al.* (1993) have shown that in *Pisum sativum* about 4 days were necessary to reach full photosynthetic acclimation after a transition from a PSI to a PSII stimulating light environment and *vice versa*. Similarly, Hogewoning *et al.,* (2007) showed in duckweed, that 6 days were needed to fully acclimate to different light conditions, using the Chl a:b ratio as control parameter.

In contrast to the measurements with standardized light, when the leaf CO_2 exchange of the plants used in the current study was measured under the respective *in situ* light conditions, A_{max} was significantly lower at very low (6%) or very high (62%) B light conditions, despite the higher concentration of chlorophyll at 62% B or small differences in SLA (Fig. 1 B). In a similar but more extreme experiment, several long-term studies reported lower net photosynthesis or A_{max} in plants raised under monochromatic B or R light (Hogewoning *et al.,* 2010 a; Piovene *et al.,* 2015; Shengxin *et al.,* 2016). Hogewoning *et al.* (2010a) also reported dysfunctional photosynthesis in cucumber plants, grown under pure R light and a dose response curve in A_{max} when the B % was increased up to 50% B, with no further increase of A_{max} beyond 50 % B. The increase of A_{max} with B percentages was associated with a reduction of the SLA, an increase of N and chlorophyll per leaf area, and higher stomatal conductance under

mixtures of B and R light compared with only B or R (Hogewoning *et al.* 2010a). Matsuda *et al.,* (2007) reported an increase of A_{max} in spinach plants exposed to a 1:1 B: R radiation at 300 μmol m^2 s^{-1}, compared with just B light, associated with increased leaf N concentration. Shengxin *et al. (*2016) showed that dark adapted Fv/Fm values were higher (as an indicator for less photo-stress) under mixtures of B and R light, compared with monochromatic B or R light, further supporting the above findings.

The effect of the treatments on photosynthesis was also visible in the quantum yield of the CO_2 fixation curve (α) of the investigated species. Similar to A_{max}, a more natural level of B light may explain a higher efficiency when an '*in situ*' light was used for our gas-exchange measurements, with significantly higher values indoor than in the field trial. Similar results were reported by Hogewoning *et al.,* (2010) at 15-30% B compared with 50% B. This effect may indicate the evolutionary adaption of species to the sunlight spectrum, with higher quantum yield under a more natural B:R ratio (*circa* 33% of B in the sunlight spectrum, Chiang *et al.,* 2019). Other conditions with extreme levels of B or R light may require the adaptation to each light condition, where CO_2 fixation may have a wavelength dependence related to absorption properties of the different pigments involved. Terashima *et al.,* (2009) described three major causes for the wavelength dependency of the quantum yield: absorption by photosynthetic carotenoids, absorption by non-photosynthetic pigments and an imbalanced excitation of the two photosystems, where an imbalance in excitation will result in quantum yield losses (Pfannschmidt, 2005; Zhen and van Iersel, 2017). It has been shown that the right light stimulus, with light qualities that match properly the species-specific ratio of PSII and PSI, is the key to high quantum efficiency of photosynthesis under diverse light qualities (Chow *et al.,* 1990). The light compensation point of photosynthesis (CP) was generally not affected by light quality. Similar results have been observed in previous cases (*e.g.* Furuyama *et al.,* 2014; Shengxin *et al.,* 2016).

In the current study, the average dark respiration (DR) using the standardized light, independent of the species, was relatively lower at 62% B compared with the other light treatments or the field trial. Atkin *et al.,* (1998) described in Tobacco, that observed changes in DR were dependent on the previously applied irradiance (tested between 0 to 300 μmol photons $m^{-2}s^{-1}$). An instantaneous stimulation of the photosystems in low light adapted plants due the stimulus of an intensity radiation burst was hypothesized. Although the total photon flux was the same between treatments in our study, similar short time effects on DR might have occurred

when plants were exposed to a high intensities and light spectrum that they were not adapted to.

Principal component analysis

The PCA analyses performed in this study affirmed that the effects of light quality on plant performance are highly species dependent and that changes in the B % of the light spectra may help to promote more natural like growth. Applying light with a spectrum with similar B and R light proportions to sunlight is proposed to avoid physiological plant responses to a lack or excess of B light (which might also differ among species). Although 7% B has been recommended to avoid dysfunctional photosynthesis (Hogewoning *et al.,* 2010 a), this study indicates that levels of 25 to 35% B light in the spectrum are needed in indoor conditions to avoid undesired (*i.e.* unnatural) effects of the light spectrum on plant growth. No specific trait was identified across the different species to have a higher importance than others (Supplementary Fig. 2), where the ranking of importance of each measured parameter was species dependent. Independent of this, the PCA clearly indicated that other environmental variables should be controlled (*e.g.* air flux, soil temperature) or more precisely mimicked in indoor growth facilities if natural-like growth is required. A similar approach was used by Annunziata *et al.,* (2017) to understand the difference between indoor and outdoor experiments, with a focus on Arabidopsis's metabolism where a clearer clustering of the indoor and outdoor conditions was obtained, with similar values of the first and second component to the ones presented here (first and second component explaining 28 and 15% of the variance, respectively compared with 24 and 15 % average across species in our study).

Conclusion

The applied light spectra in this study significantly influenced plant morphology, pigment concentration and photosynthesis. Less deviating responses compared with the field trial were reached with either 25% or 35% of B light in almost all species. Hence, if natural like plant growth is desired in indoor plant cultivation, the application of a balanced light spectrum is generally recommended. However, spectral quality of the light source is only one of many factors that can potentially bias plant performance. In this study, we thus aimed to apply similar climatic conditions within the growth chambers as were measured in the field trial that was used to compare outdoor with indoor growth. Nevertheless, we still found significant differences between phytotron and field grown plants in most of the investigated plant traits. This highlights the difficulties to exactly reproduce natural plant performance in indoor growth

facilities, as well as the necessity to include the simulation of additional environmental factors (e.g. replication of natural minimum and maximum temperature, humidity and irradiance changes, wind speed and direction, etc.) in indoor experiments with plants.

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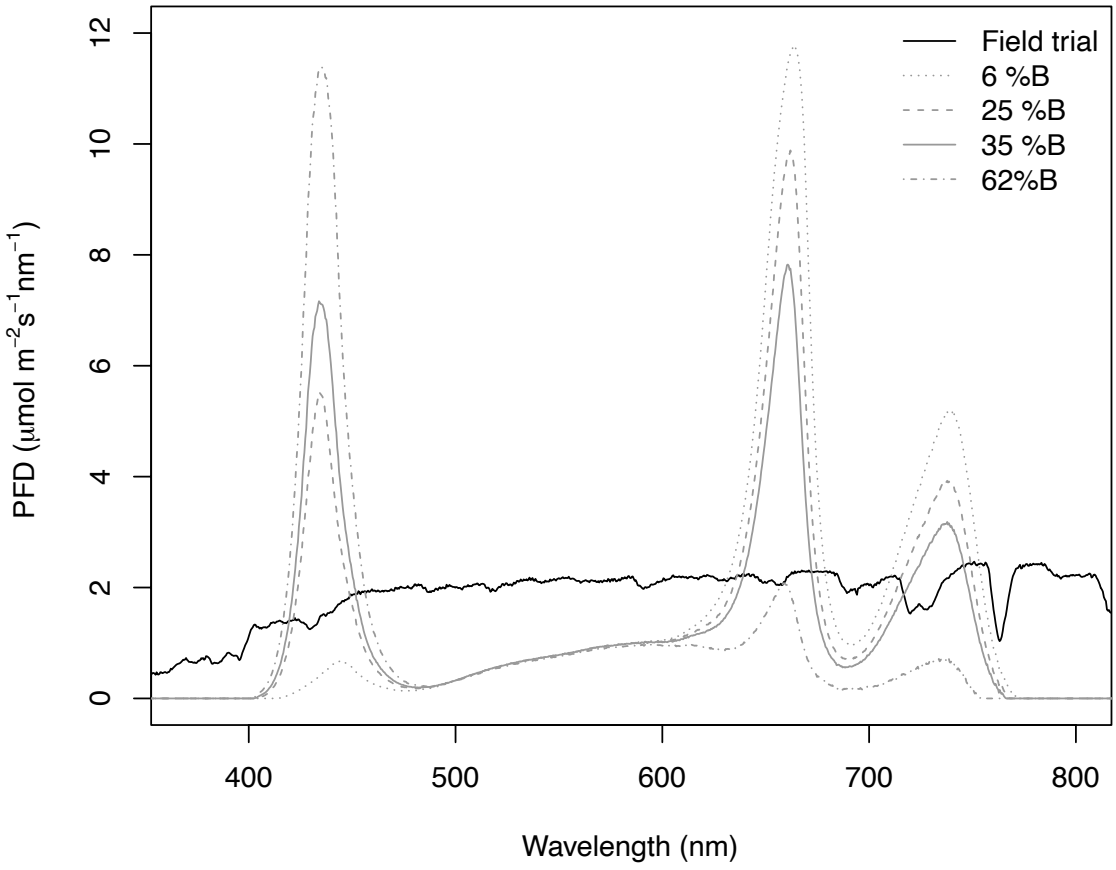
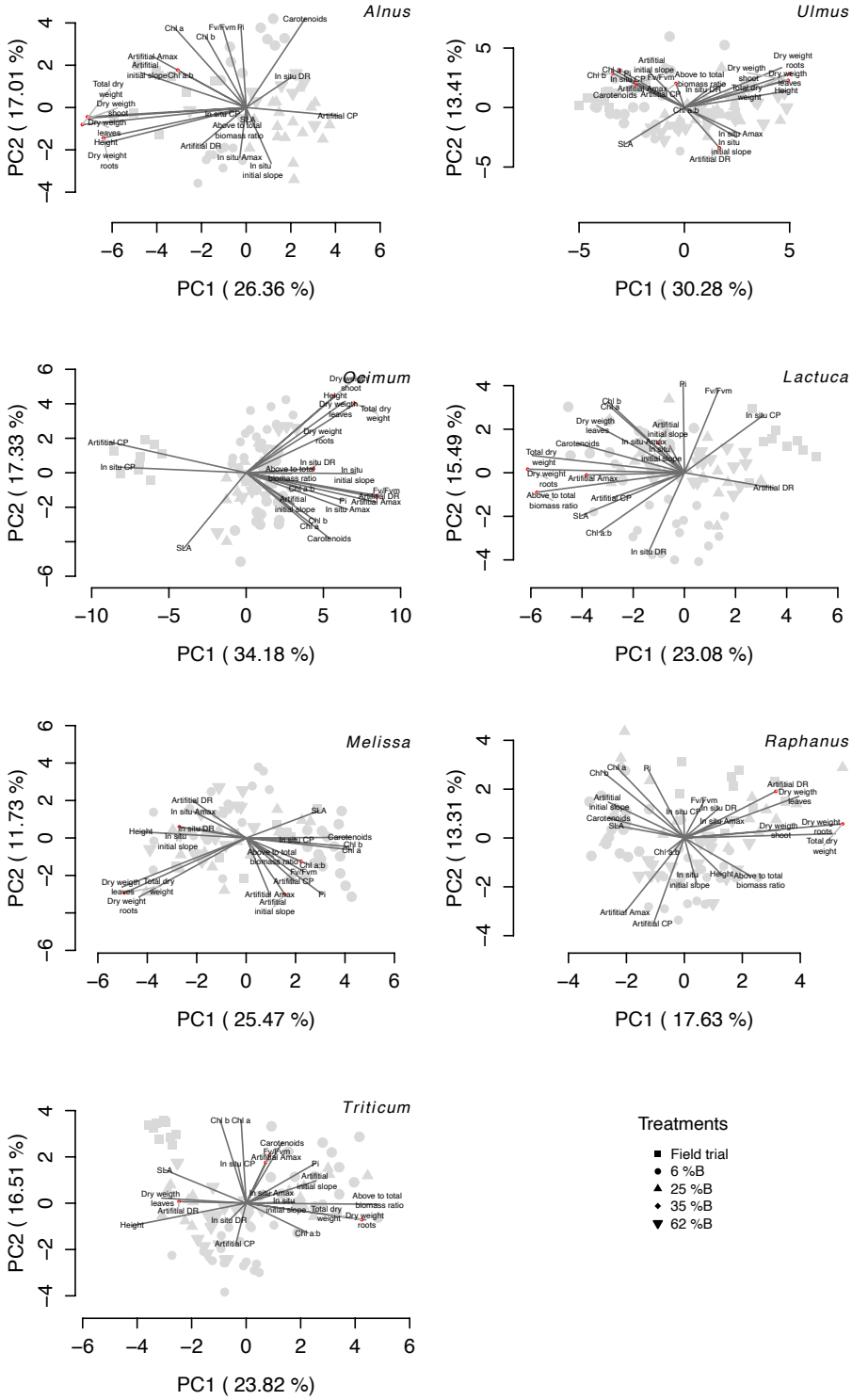
Supplementary material



Supplementary Fig. 1: Applied spectra for the field trial and each of the different light treatment where 6%, 25%, 35% and 62% refers to the percentage of blue light as percentage of the PPFD (*i.e.* including far-red). The integrated area between 400 and 700 nm corresponds to an approximately 575 µmol $m^{-2}s^{-1}$ of photosynthetic photon flux density in each case.



Supplementary Fig. 2: Principal component analysis (PCA) of the measured traits of each specie grown under 6% B, 25% B, 35% B and 62% B light together with the importance of the different measured traits in each specie. Lighter point (n=18) corresponds to a plant and solid ones each measured trail.

Species	**Alnus**					**Ulmus**				
Trial\Treatment	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**
Biomass and Morphology										
Height*	22.32±1.2	20.2±0.21	10.45±0.82	11.62±2.28	8.53±1.5	31.18±3.44	48.51±2.7	45.35±2.38	48.95±2.89	31.19±2.55
Dry weight leaves	1.06±0.1	0.5±0.05	0.27±0.04	0.33±0.11	0.32±0.07	1.33±0.28	2.66±0.32	2.75±0.26	2.85±0.29	1.52±0.25
Dry weight shoot	0.51±0.05	0.22±0.03	0.13±0.02	0.15±0.05	0.12±0.02	0.66±0.16	1.69±0.23	1.71±0.19	1.79±0.23	0.79±0.16
Dry weight roots	0.52±0.05	0.58±0.07	0.17±0.03	0.17±0.05	0.3±0.06	0.55±0.13	1.98±0.24	2.5±0.28	2.4±0.33	1.45±0.36
Total dry weight	2.09±0.19	1.3±0.13	0.57±0.09	0.64±0.2	0.74±0.15	2.54±0.56	6.34±0.77	6.96±0.66	7.05±0.82	3.76±0.75
Root to Shoot ratio	0.34±0.03	0.8±0.07	0.42±0.05	0.44±0.04	0.68±0.01	0.27±0.02	0.46±0.02	0.57±0.05	0.5±0.04	0.55±0.07
SLA	34.76±1.24	36.41±2.99	33.61±0.99	35.49±2.45	31.67±1.18	28.35±0.85	28.45±0.79	23.08±0.62	24.23±0.92	25.05±0.78
Chlorophyll										
Chlorophyll a (mg g^{-1})	7.35±0.97	4.58±0.37	4.62±0.35	6.88±0.74	6.93±0.46	6.8±0.43	3.18±0.39	3.28±0.63	2.06±0.34	4.1±0.31
Chlorophyll b (mg g^{-1})	1.45±0.25	1.02±0.11	1±0.07	1.49±0.15	1.45±0.11	1.54±0.09	0.71±0.09	0.74±0.15	0.45±0.07	0.88±0.09
Chl a: b ratio	1.94±0.18	1.68±0.04	1.99±0.18	2.9±0.21	2.56±0.11	1.15±0.09	0.98±0.09	1.06±0.17	0.72±0.11	1.11±0.08
Carotenoids (mg g^{-1})	5.12±0.23	4.55±0.1	4.61±0.09	4.6±0.07	4.79±0.09	4.42±0.05	4.53±0.09	4.48±0.14	4.44±0.17	4.7±0.13
Fv/Fm	0.78±0.01	0.74±0.01	0.77±0.01	0.76±0.02	0.8±0	0.81±0	0.73±0.02	0.77±0.01	0.78±0.01	0.79±0.01
Standardized light										
Max photosynthesis**	14.35±1.48	10.22±1.52	6.78±1.05	11.6±1.33	12.31±1.21	12.87±1.9	9.01±0.68	11.82±0.36	11.51±0.5	12.68±0.95
Initial slope	0.044±0.001	0.037±0.002	0.026±0.004	0.038±0.004	0.041±0.005	0.045±0.002	0.043±0.003	0.042±0.002	0.04±0.001	0.05±0.006
Dark respiration	-1.05±0.15	-0.86±0.2	-1.09±0.13	-1.59±0.16	-1.39±0.27	-1.33±0.22	-1.46±0.26	-1.13±0.14	-1.1±0.19	-1.83±0.39
Compensation point	22.67±4.33	21.67±3.76	41.33±4.29	38.67±2.33	29±2.65	24.33±2.85	25.83±3.76	25.5±2.64	25±3.46	28.17±1.38
In-situ' light										
Max photosynthesis	8.37±0.29	10.97±0.66	9.81±1.48	9.03±1.57	8.86±3.41	7.3±0.39	7.21±0.89	11.47±0.99	9.6±0.76	5.53±1.23
Initial slope	0.044±0.002	0.048±0.009	0.054±0.006	0.049±0.004	0.039±0.008	0.034±0.002	0.048±0.004	0.055±0.003	0.052±0.003	0.034±0.005
Dark respiration	-1.74±0.07	-1.54±0.37	-1.58±0.24	-1.45±0.21	-1.13±0.32	-1.27±0.15	-1.16±0.13	-1.3±0.12	-1.33±0.15	-1.11±0.23
Compensation point	30.33±2.67	37.67±19.19	26.67±4.17	26.67±4.67	26.33±3.38	30.33±0.88	18.83±1.19	20.83±1.92	22.83±2.98	24.67±3.21

Supplementary table 1: Raw average values by measured trial for each treatment and specie. Plus minus values standard errors of these.

Species	**Ocimum**					**Lactuca**				
Trial\Treatment	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**
Biomass and Morphology										
Height*	21.17±1.38	30.42±0.89	26.09±0.79	28.15±1.48	23.99±1.03	-	-	-	-	-
Dry weight leaves	1.18±0.15	2.32±0.15	2.03±0.13	2.12±0.18	1.72±0.14	8.97±0.49	8.98±0.55	10.01±0.53	10.17±0.47	10.42±0.49
Dry weight shoot	0.37±0.04	0.78±0.06	0.62±0.04	0.75±0.07	0.51±0.05	-	-	-	-	-
Dry weight roots	0.36±0.06	1.93±0.28	1.02±0.14	0.98±0.11	1.24±0.24	3.21±0.3	10.65±1.11	9.33±1.46	9.37±1.36	11.5±1.18
Total dry weight	1.91±0.23	5.03±0.31	3.67±0.27	3.85±0.33	3.47±0.3	12.18±0.77	19.64±1.53	19.34±1.83	19.54±1.72	21.91±1.43
Root to Shoot ratio	0.24±0.02	0.7±0.12	0.37±0.04	0.34±0.03	0.63±0.14	0.35±0.02	1.18±0.08	0.9±0.12	0.88±0.11	1.1±0.11
SLA	20.7±1.32	13.86±0.77	18.09±0.84	17.09±0.85	19.56±0.74	25.07±1.5	39.57±2.23	33.26±1.94	24.45±1.38	32.23±1.03
Chlorophyll										
Chlorophyll a (mg g^{-1})	2.19±0.29	2.33±0.14	3.51±0.2	2.99±0.26	4.29±0.37	2.51±0.53	2.06±0.24	2.84±0.42	1.8±0.13	3.99±0.51
Chlorophyll b (mg g^{-1})	0.54±0.08	0.49±0.03	0.74±0.05	0.7±0.08	0.93±0.09	0.57±0.12	0.35±0.04	0.5±0.08	0.33±0.02	0.71±0.09
Chl a: b ratio	0.55±0.08	0.66±0.05	0.96±0.04	0.77±0.04	1.09±0.1	0.68±0.14	0.99±0.05	1.05±0.13	0.73±0.05	1.43±0.15
Carotenoids (mg g^{-1})	4.08±0.05	4.8±0.19	4.81±0.08	4.39±0.15	4.62±0.06	4.38±0.33	5.93±0.16	5.84±0.19	5.46±0.09	5.62±0.2
Fv/Fm	0.77±0.02	0.83±0.01	0.82±0	0.84±0	0.84±0	0.85±0.01	0.8±0.01	0.84±0.01	0.84±0.01	0.84±0.01
Standardized light										
Max photosynthesis**	10.18±2.77	19.17±0.95	20.43±1.1	22.52±0.49	23.13±1	4.63±0.99	14.62±1.6	11.99±1.87	17.28±2.34	17.67±3.01
Initial slope	0.043±0.002	0.047±0.003	0.044±0.003	0.045±0.001	0.053±0.001	0.043±0.003	0.045±0.004	0.042±0.004	0.044±0.003	0.052±0.004
Dark respiration	-3.99±0.29	-2.44±0.23	-2.02±0.13	-1.99±0.12	-2.11±0.06	-1.12±0.15	-1.99±0.22	-1.68±0.18	-1.96±0.22	-2.29±0.27
Compensation point	91.33±3.84	49±5.22	45.5±4.43	42.5±1.65	36.17±2.14	16±2.08	41±4.15	40.33±7.82	41.5±4.93	38.67±3.03
In-situ' light										
Max photosynthesis	6.73±0.3	13.78±0.92	17.65±1.42	17.24±0.98	16±1.07	5.77±0.65	7.11±1.67	10.48±1.59	12.08±1.5	8.49±2.04
Initial slope	0.037±0.001	0.059±0.003	0.059±0.004	0.061±0.003	0.057±0.003	0.038±0.002	0.045±0.007	0.053±0.003	0.055±0.002	0.047±0.005
Dark respiration	-2.8±0.21	-1.78±0.14	-1.98±0.23	-1.95±0.26	-2.1±0.11	-1.85±0.17	-1.33±0.25	-1.56±0.19	-1.81±0.12	-1.56±0.22
Compensation point	76.33±3.38	28.17±2.61	33±4.38	31.67±5.63	34.67±3.33	38.33±6.12	23.67±3.29	27.83±4.61	30.83±2.43	24.5±3.27

Supplementary table 1 (continuation): Raw average values by measured trial for each treatment and specie. Plus minus values standard errors of these.

Species	**Melissa**					**Raphanus**				
Trial\Treatment	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**
Biomass and Morphology										
Height*	26.75±0.76	22.05±1.38	17.51±1.58	23.48±0.5	10.97±0.59	5.84±0.19	8.62±0.56	6.41±0.24	6.73±0.61	5.43±0.41
Dry weight leaves	2.93±0.48	2.85±0.39	2.91±0.43	3.27±0.25	1.58±0.13	2.35±0.18	1.88±0.15	1.86±0.15	1.61±0.13	1.46±0.09
Dry weight shoot	-	-	-	-	-	0.53±0.06	0.6±0.07	0.51±0.04	0.57±0.08	0.35±0.03
Dry weight roots	1.73±0.33	1.92±0.28	1.82±0.23	1.94±0.19	1.11±0.13	4.41±0.51	4.97±0.24	6.14±0.74	5.13±0.44	3.3±0.15
Total dry weight	4.67±0.81	4.77±0.65	4.73±0.63	5.21±0.43	2.69±0.25	7.3±0.67	7.46±0.41	8.52±0.87	7.31±0.56	5.12±0.18
Root to Shoot ratio	0.58±0.03	0.68±0.05	0.72±0.07	0.59±0.03	0.7±0.05	1.52±0.13	2.16±0.13	2.56±0.22	2.44±0.17	1.92±0.13
SLA	38.1±2.97	31.28±1.6	29.08±0.91	31.64±1.49	35.73±1.02	23.71±0.98	27.15±0.99	31.13±2.12	30.57±0.97	27.43±0.77
Chlorophyll										
Chlorophyll a (mg g^{-1})	8.04±1.54	5.57±0.65	4.58±0.7	5.27±0.39	6.76±0.29	2.72±0.8	3.24±0.29	5.08±0.71	3.48±0.39	4.84±0.51
Chlorophyll b (mg g^{-1})	1.82±0.3	1.31±0.15	1.03±0.15	1.22±0.09	1.54±0.1	0.67±0.16	0.74±0.06	1.17±0.15	0.79±0.08	1.16±0.07
Chl a: b ratio	1.94±0.39	1.79±0.15	1.5±0.21	1.71±0.1	2.07±0.04	0.63±0.18	1.09±0.06	1.49±0.19	1.2±0.12	1.55±0.08
Carotenoids (mg g^{-1})	4.35±0.18	4.25±0.05	4.43±0.04	4.31±0.05	4.43±0.1	3.92±0.24	4.35±0.1	4.32±0.14	4.43±0.16	4.13±0.25
Fv/Fm	0.81±0.01	0.78±0.02	0.82±0	0.78±0.01	0.82±0	0.83±0.01	0.82±0.01	0.83±0.01	0.82±0.01	0.83±0.01
Standardized light										
Max photosynthesis**	19.11±2.88	13.64±1.24	17.2±0.81	15.78±1.59	15.96±1.07	10.31±1.25	24.72±1.12	18.19±1.52	22.66±1.72	24.02±2.44
Initial slope	0.054±0.004	0.041±0.003	0.048±0.002	0.04±0.003	0.051±0.006	0.053±0.002	0.053±0.002	0.057±0.004	0.052±0.002	0.062±0.005
Dark respiration	-1.08±0.08	-1.21±0.2	-1.4±0.17	-1.42±0.11	-2.11±0.56	-1.23±0.11	-2.19±0.15	-1.63±0.24	-1.85±0.14	-2.36±0.23
Compensation point	18.33±0.88	26±3.81	27.67±3.7	33.67±2.03	35±4.23	20.33±2.85	40.67±3.22	26.67±5.3	34.83±2.68	35.17±3.05
In-situ' light										
Max photosynthesis	11.08±0.33	12.34±1.87	14.29±0.9	16.26±0.86	11.88±1.54	13.77±1.97	12.57±2.16	14.81±2.41	14.59±1.14	11.46±1.51
Initial slope	0.051±0.004	0.064±0.003	0.067±0.002	0.06±0.004	0.056±0.003	0.046±0.003	0.069±0.004	0.068±0.003	0.069±0.002	0.055±0.005
Dark respiration	-1.63±0.09	-1.47±0.14	-1.51±0.14	-1.6±0.11	-1.79±0.16	-1.05±0.01	-1.64±0.1	-1.53±0.32	-1.56±0.16	-1.57±0.14
Compensation point	24.33±1.2	20.5±2.19	21±2.8	26±3.49	26.5±3.27	22.33±1.33	20.67±1.38	21±4.77	21.5±2.38	25.17±6.28

Supplementary table 1 (continuation): Raw average values by measured trial for each treatment and specie. Plus minus values standard errors of these.

Species	**Triticum**				
Trial\Treatment	**Outdoor trial**	**6%B**	**25% B**	**35%B**	**62%B**
Biomass and Morphology					
Height*	52.41±1.37	51.9±0.98	45.73±2.73	46.78±0.63	39.27±1.19
Dry weight leaves	12.26±0.46	10.45±0.36	9.92±0.44	8.74±0.56	6.8±0.37
Dry weight shoot	-	-	-	-	-
Dry weight roots	21.04±2.43	81.74±7.21	82.1±8.96	56.6±5.24	93.12±6.46
Total dry weight	33.3±2.34	92.19±7.36	92.02±8.77	65.34±5.38	99.91±6.53
Root to Shoot ratio	1.76±0.25	7.78±0.62	8.82±1.2	6.79±0.7	14.21±1.13
SLA	31.45±0.67	20.07±0.63	24.92±1.94	26.93±1.49	24.14±0.81
Chlorophyll					
Chlorophyll a (mg g^{-1})	7.12±0.44	2.88±0.34	4.97±0.43	4.11±0.54	5.45±0.52
Chlorophyll b (mg g^{-1})	1.89±0.07	0.67±0.09	1.15±0.11	0.98±0.17	1.14±0.14
Chl a: b ratio	1.45±0.05	0.94±0.08	1.65±0.07	1.18±0.13	1.58±0.11
Carotenoids (mg g^{-1})	3.75±0.12	4.37±0.14	4.34±0.1	4.38±0.19	4.89±0.16
Fv/Fm	0.83±0	0.79±0.01	0.81±0.01	0.82±0.02	0.83±0
Standardized light					
Max photosynthesis**	16.92±1.13	13.39±0.78	14.52±2.48	13.04±1.4	15.44±1.37
Initial slope	0.056±0.004	0.058±0.009	0.054±0.006	0.046±0.006	0.071±0.005
Dark respiration	-1.5±0.11	-1.95±0.4	-1.72±0.31	-1.42±0.16	-2.1±0.25
Compensation point	23±2	28.83±6.52	26.17±3.49	30±4.97	22.83±2.97
In-situ' light					
Max photosynthesis	9.12±0.47	9.82±1.93	12.62±0.87	10.27±1.82	9.73±2.83
Initial slope	0.044±0	0.064±0.01	0.073±0.003	0.059±0.009	0.07±0.021
Dark respiration	-1.9±0.05	-1.42±0.26	-1.61±0.3	-1.31±0.26	-1.9±0.41
Compensation point	35±0	21.67±2.53	19.17±3.57	19±2.24	21.17±3.39

Supplementary table 1 (continuation): Raw average values by measured trial for each treatment and specie. Plus minus values standard errors of these.

Supplementary table 2: P-values for the different measured traits in both experiments using normalized data.

	Fix factors	**Random factors**
	Light quality	**Specie**
Variable		
Biomass and Morphology		
Height*	$< 2.2x10^{-16}$	$< 2.2x10^{-16}$
Dry weight leaves	3.54E-05	$< 2.2x10^{-16}$
Dry weight shoot	1.67E-09	$< 2.2x10^{-16}$
Dry weight roots	7.91E-08	$< 2.2x10^{-16}$
Total dry weight	4.95E-09	$< 2.2x10^{-16}$
Root to Shoot ratio	5.88E-07	$< 2.2x10^{-16}$
SLA	0.4753	$< 2.2x10^{-16}$
Chlorophyll		
Chlorophyll a (mg g^{-1})	5.73E-11	$< 2.2x10^{-16}$
Chlorophyll b (mg g^{-1})	8.51E-11	$< 2.2x10^{-16}$
Chl a: b ratio	2.37E-05	< 2.2e-16
Carotenoids (mg g^{-1})	7.59E-13	$< 2.2x10^{-16}$
Fv/Fm	4.02E-08	$< 2.2x10^{-16}$
Standardized light		
Max photosynthesis**	3.73E-05	$< 2.2x10^{-16}$
Initial slope	2.54E-07	< 2.2e-16
Dark respiration	< 2.2e-16	< 2.2e-16
Compensation point	0.003218	< 2.2e-16
In-situ' light		
Max photosynthesis	5.66E-07	$< 2.2x10^{-16}$
Initial slope	8.14E-07	1.00E-04
Dark respiration	-	$< 2.2x10^{-16}$
Compensation point	0.003684	< 2.2e-16

Chapter 3
Reaching natural growth: The significance of light and temperature fluctuations on plant performance in indoor growth facilities

Abstract:
Recommendations for near-natural plant growth in indoor conditions have been described without considering environmental fluctuations, which might have important consequences for researchers and plant producers when comparing results from indoor facilities with natural ecosystems or production. Poorter *et al.* (2016) proposed that differences in temperature, light quantity and the lack of their variation are sources of deviations between indoor and outdoor experiments. Here, we investigated the effect of fluctuating light, temperature and humidity in an indoor environment on plant performance. 7 plant species from different functional plant types were grown outdoors during summer and spring. The same species were then grown in indoor growth chambers under different scenarios of climate complexity in terms of fluctuations: 1) fixed night and day conditions, 2) daily sinusoidal changes and 3) variable conditions tracking the climate records from the field trials. In each scenario, the average of the environmental variables were the same as in the respective field trial. Productivity-, gas exchange- and leaf pigment-traits were measured in all plants at the end of the experiments. The plant trait responses were highly dependent on species and treatment, but some general trends were observed. The variable condition yielded lower biomass compared to the fixed and sinusoidal conditions, together with a higher specific leaf area and chlorophyll concentrations. A principal component analysis (PCA) across all plant traits in response to climatic conditions, suggested that at least a sinusoidal fluctuation is recommended for a more natural-like plant performance in indoor growth facilities. However, prevailing significant differences for several traits between field- and indoor-grown plants even under variable climates indicate that additional factors than those controllable in standard phytotrons (*e.g.*, wind speed and direction, leaf and soil temperature) can bias plant performance in indoor facilities.

Keywords: Dynamic light, dynamic temperature, natural growth, controlled environment.

Introduction

From a scientific and commercial point of view, natural-like growth in plants is desired in indoor facilities (Matsubara, 2018). Although it is well known that several environmental interactions may affect plant phenology, and therefore the output of experiments, it is common practice to apply static environmental conditions in indoor experiments. Fixed day and night time conditions may be oversimplified and may lead to results significantly deviating from natural outdoor conditions (Poorter *et al.,* 2016). Two of the most important environmental factors that affect plant growth are light and temperature. It is well-known that instantaneous and daily fluctuations of temperature and light, can affect plant performance in both positive and negative ways (*e.g.* Myster and Moe,1995; Kaiser *et al.,* 2015). Myster and Moe (1995) reviewed the effect of the difference between day and night temperatures, where a positive difference between day and night enhances plant height, chlorophyll content and leaf orientation (more upright position), mainly due to an increase in cellular elongation. Since cell metabolism is not linearly related to temperature, an increase in temperature may induce a stronger effect than a decrease in temperature of the same magnitude. Rapid changes in temperature can adapt the plants for less favourable conditions (*i.e.* "hardening"; Matsubara, 2018). Daily and instantaneous changes in light have also been studied in detail previously. From these studies it is known that changes in light along the day may induce lower biomass but also higher maximal photosynthesis (A_{max}), especially per unit of leaf mass (Vialet-Chabrand *et al.,* 2017). Fast fluctuations in light intensity have been shown to reduce photosynthesis and biomass in the long term (Kaiser *et al.,* 2018), partly related to increases in radical oxygen species (ROS) and interactions with other environmental factors. Under increasing light, higher leaf temperatures could close stomata and limit photosynthesis (Yamori, 2016) meanwhile under reductions of light, light use efficiency will be slowed down due to relaxation of energy dissipation (Kromdijk *et al.,* 2016). Interestingly, fluctuating light has in some cases been shown to promote several photosynthesis related parameters, especially in partially shaded leaves (Kaiser *et al.,* 2017). Several studies have measured the effect of light or temperature variations on plant performance under semi-controlled and controlled conditions but simultaneous comparisons with outdoor growth are scarce in the literature.

Having static climatic conditions in indoor plant production and research is often practical and logical, but the generated knowledge and results may not extrapolate well to other conditions as the important factor of environmental fluctuations is missing (Poorter *et al,* 2016; Matsubara, 2018). Hence, contradictory results have been found when similar treatments have been applied in indoor and outdoor experiments. *E.g.,* Strømme *et al.,* (2015) found that for

Populus tremula in outside conditions an increase in temperature promotes bud break, meanwhile other authors in indoor conditions claimed a delayed bud break at increasing temperature (Søgaard *et al.,* 2008; Kalcits *et al.,* 2009). These results showcase the difficulties to translate indoor results to real-world conditions, and when trials have been conducted to replicate outdoor growth in indoor facilities, low correlations have been found (Junker *et al.,* 2015, Hohmann *et al.,* 2016). Poorter *et al.,* (2016) suggested multiple reasons why this may occur, where the main differences may come from lower light quantities, higher plant density and shorter durations of indoor compared to outdoor experiments. Other sources of variation have been pointed out, including age of the plants, leaf temperature, soil temperature, soil microorganism, lack of UV light and the light quality in indoor experiments (Poorter *et al.,* 2016). When the effect of light quality was studied under constant day and night conditions of temperature, light quality has been shown to affect plant morphology (Hogewoning *et al.,* 2010; Hernandez and Kubota, 2016).

The aim of this study was to compare and quantify the effects of fluctuating environmental conditions on several important plant traits (productivity, gas-exchange and leaf pigmentation), aiming to reach a close to natural plant performance under indoor growth conditions. A range of species from different functional groups were included in two 35 days field trials where the in-situ climate was recorded and plant traits were measured at the end of the trials. The same plant species were then grown in indoor experiments in phytotrons under different levels of complexity of light, temperature and air humidity fluctuations, simulating the outdoor conditions. We hypothesized that, applying steady, average climatic conditions will lead to plant growth that deviates most from natural growth, while the application of real fluctuations of temperature, humidity and light will produce plants that show similar performance to field grown plants.

Materials and methods.

Plant material and pre-growing conditions

In total, we investigated 7 species from different functional plant types: trees represented by black alder (*Alnus glutinosa* L., provenance HG4, Zurich, Switzerland, Swiss federal institute for forest, snow and landscape research (WSL), Switzerland) and scotch elm (*Ulmus glabra* HUDS., provenance Merenschwand, Aargau, Switzerland, WSL, Switzerland), herbs represented by basil (*Ocimum basilicum* L. var Adriana, Wys Samen Pflanzen, Switzerland), lettuce (*Latuca sativa* L., Wys samen pflanzen, Switzerland), melissa (*Melissa officinalis* L., Wys Samen Pflanzen, Switzerland) and radish (*Raphanus raphanistrum* L. subsp. sativus var.

Marabelle, Wys Samen Pflanzen, Switzerland) and finally grasses represented by winter wheat (*Triticum aestivum* L., Sativa, Switzerland). In the following, the species will always be referred to by their scientific genus name for clarity. Due to the different germination speeds, the timing of sowing was different for the species as follows: Seeds of black alder and scotch elm were sown in 20 cm x 40 cm x 2 cm trays with commercial substrate (pH 5.8, 250 mg L^{-1} N, 180 P_2O_5 mg L^{-1}, K_2O 480 mg L^{-1}, Ökohum, Herrenhof, Switzerland) 43 days before the start of the experiments and let germinate under 190 µmols m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) with a red to far red ratio (R:FR) of 5.1 for 23 days. 20 days before the start of the experiment the light was increased to 240 µmols m^{-2} s^{-1} PPFD, with a R: FR of 5.1, to acclimate the plants to higher light levels. 13 days before the start of the experiment *Melissa*, and 6 Days before the start of the experiments the rest of the species were sown in the same type of trays and under the same environmental conditions with exception of *Triticum*, which was sown directly in round 2 L pots with a density of 15 seeds per pot. During the pre-growing period the seedlings were exposed to 25/15 ºC and 50 / 83 % relative humidity (RH) for day and night, respectively, with a daylength of 10 hours and one-hour light/temperature/humidity ramping pre and post daytime.

At the start of the different treatments, all species except *Triticum* were transplant to 2 L cylindrical pots of 13.5 cm diameter (Pöppelmann, Lohne, Germany), with a single individuum per pot. The pots were filled with the same substrate as used in the germination trays. During the experiments, all plants were watered daily at the beginning of the day. At the beginning of the experiments, each pot was fertilized with 4 g of a slow releasing fertilizer (Osmocote exact standard 3-4 months, Scotts, Marysville, Ohio, USA) containing 16% total N, 9% P_2O_5, 12% K_2O and 2.5% MgO.

Outdoor trial and environmental conditions

9 plants of each species, pre-grown in the conditions given above, were grown under outdoor conditions for a period of 35 days during summer (4. August 2017 – 7. Spetember 2017) and spring (15. May 2018 – 18. June 2018), respectively in an open site at the botanical garden of the University of Basel, Basel, Switzerland. Both trials were used as control treatments for two separate rounds of phytotron experiments. All pots were placed on a metal grid (the same grid was also used in the indoor runs) with a density of 30 pots per m^2, and all plants were watered daily, to avoid any influence of soil water limitation. Temperature, relative humidity, precipitation, wind speed/direction and PPFD (400-700 nm) was recorded every 5 minutes with a weather station (Vantage pro2, Davis, Haywards, California, USA). In addition, sunlight

spectra in the waveband 350 - 800 nm was recorded every minute using a spectrometer (STS, Ocean Insight, Florida, United States) that was equipped with an optical fibre and a cosine corrector (180° field-of-view; CC-3-UV-S, Ocean Insight) placed next to the weather station's PPFD sensor facing upwards. The spectrometer was connected to a Raspberry Pi 2 computer for automatic sampling, integration time adjustments and data storage. *Posteriori*, the spectra were used to calculate photon flux densities within specific wavebands: PPFD (400-700 nm), blue light (400-500 nm), green light (500-600 nm), red light (600-700 nm) and R:FR ratio (655-665 nm and 725-735 nm; according to Sager *et al.* 1988). The measurements of light were corroborated through the correlation between the data from the weather station and the PPFD calculated from the spectrum.

Experimental phytotron runs

In two different runs corresponding to summer and spring conditions, three different environmental treatments were applied for 35 days in closed walk-in chambers (phytotrons). The plant species, replication and pot density were the same as in the respective field trials (see above). Each phytotron (195 x 130 x 200 cm, L x W x H) was equipped with 18, 120 cm long LED panels consisting of a mixture of individually dimmable B, G, R and FR LEDs per panel with a maximum PPFD of 1200 µmol m^{-2} s^{-1} (DHL-Licht - Prototype, Hangover, Germany) measured at 1 meter from the light source. The LED lighting system of each chamber was mounted on movable ceilings, which height can be adjusted by the phytotron control software to alter the distance to the plants, thereby allowing for precise adjustments of the effective radiation strength at canopy height. The three climatic treatments were as follow: (1) Fixed: constant day and night conditions resembling the average day and night time climate from the 35 days field trial, (2) Sinusoidal: a sinusoidal, average diurnal climate based on the average of every five minute recordings from the 35 day field trial, and (3) Variable: an exact replication (setpoints every 5 minutes) of the recorded temperature, humidity and PFD from the 35 day field trial (Fig. 1., Fig S1). Due to low germination of *Alnus*, this species was not included in the phytotron experiments under sinusoidal spring conditions. In each treatment, the environmental conditions resulted in the same average values as in the respective field trials across the 35 days (Table 1). The used light spectra in the phytotrons (Fig. S2) corresponded to a spectral composition that give more natural plant growth as derived from a previous experiment (Chiang *et al., unpublished*). The light intensity was regulated through changes in electric intensity and roof height keeping similar spectra. For moments where this was not possible in the variable treatment, higher amounts of B and R light were applied, keeping the

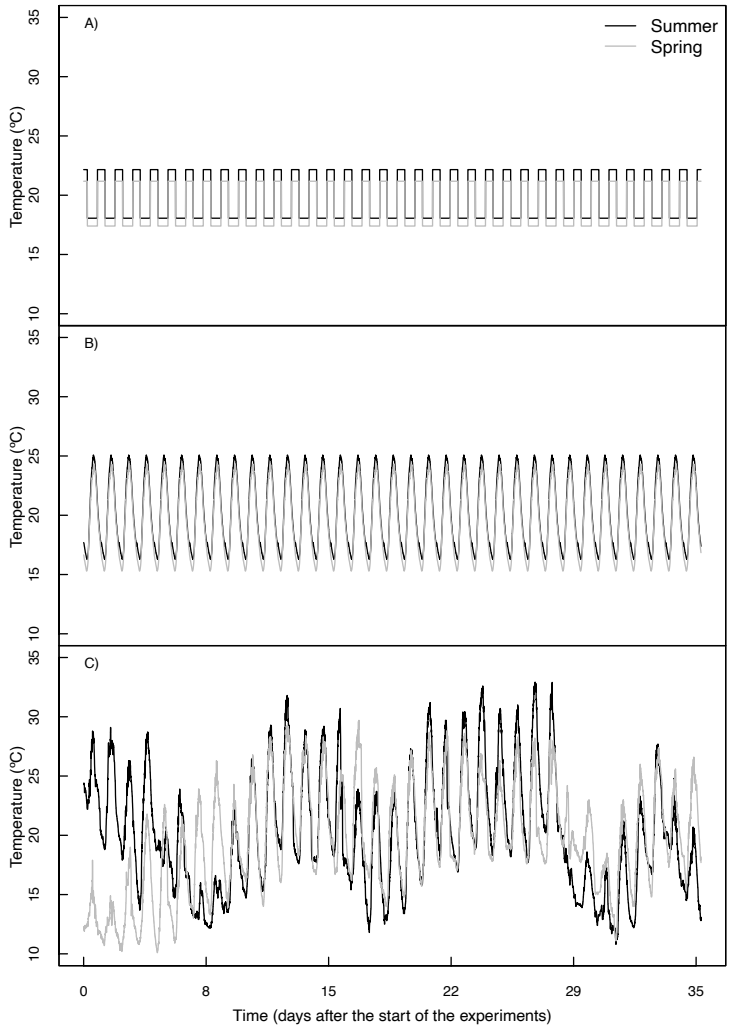


Figure 1: Applied temperatures for each treatment in the summer or spring runs. Upper panel (a): fixed day and night conditions; middle panel (b): sinusoidal diurnal changes(c); lower panel (c): variable changes (real climate tracking)., The corresponding relative humidity (%RH) and light quantity (PPFD as µmol m^{-2} s^{-1}) conditions are available in the supplementary materials (Fig. S1).

Table 1: Average environmental conditions used for the summer and the spring run in the phytotron experiments. The values are means across the respective 35-days grow-periods of the field trials.

	Summer run		Spring run	
	Night	Day	Night	Day
Air temperature(ºC)	18.06	22.16	17.4	21.18
Relative humidity (%)	79.24	64.92	81.73	67.4
PPFD (µmol m^{-2} s^{-1})	0	575.5	0	609
Duration per day (h)	12.95	11.05	14.08	9.92

same previously used B:R ratio. The R:FR ratio was kept at 1.8 for all treatments in the phytotron runs. No UV light was applied in the phytotrons, and the airflow (average value of 0.295 m s^{-1}) in the chamber came from below, ensuring a uniform temperature and humidity distribution within the chambers.

Plant growth and morphology

The height of the plants was measured after 35 days of exposure to the different treatments, as total height from the substrate to the apical meristem. In case of flowering or plants without a clear steam, the extended leaves length was recorded as height, with an exception of *Lactuca* where height was not recorded. Two full-grown leaves from the top three leaves, were taken for each plant to measure its surface area (LI-3100, Licor, Licoln, Nebraska, USA) and dry weight, and calculate specific leaf area (SLA). Dry weight (DW) was measured separately for roots, stem and leaves after 10 days drying at 80ºC in a drying oven (UF 260, Memmert, Schwabach, Germany). Due to the lack of a clearly identifiable stem, only total aboveground and root biomass was determined for *Lactuca*, *Melissa* and *Triticum*. All reported organ masses and the bellow to above biomass ratio (root to shoot ratio; r:s) refer to dry biomasses.

Chlorophyll fluorescence and leaf pigment content

The night before the end of the experiment, fast chlorophyll fluorescence was measured on one of the top four leaves on 4 randomly chosen plants of each species and treatment by using a continuous excitation fluorometer (Pocket PEA, Hansatech instruments Ltd, Nordfolk, UK). The plants were dark adapted for at least 20 minutes (night measurements) and F_v/F_m and Pi absolute (Kalaji *et al.,* 2014) were recorded.

At the end of the experiment, two discs of 1.13 cm^2 from two of the top four fully developed leaves were punched and collected in a 1.5 mL Eppendorf tube together with 4-6 glass beads of 0.1 mm diameter for later chlorophyll and carotenoids analyses. The tubes were rapidly frozen in liquid nitrogen and then stored at -80ºC until analysis. At the day of pigment measurement, the tubes were agitated using a mixing device (Silamat S6, Ivoclar Vivadent, Schaan, Liechtenstein) during two rounds of 10 seconds to triturate the tissue. Then, 0.7 mL of acetone were added to each tube, agitated again for 10 seconds, and then centrifuged at 13000 rpm at 4ºC for 2 minutes. 0.25 mL of the supernatant was taken and dissolved in 0.75 mL of acetone. The spectra of the resultants were measured using a spectrometer (Ultrospec 2100 pro, Biochrom, Holliston, USA). Chlorophyll a, b, chlorophyll a to b ratio and total carotenoid concentrations were calculated from the spectrum using the absorption values at 470, 646 and

663 nm as described in Wellburn (1994) and expressed as mg per g of dry biomass using the average SLA of each species and treatment.

Photosynthesis

6 days before the end of the experiment, 3 light-response curves of net CO_2 leaf-exchange were measured in one of the top three leaves in three randomly chosen plants per species using a portable photosynthesis system (LI-COR 6800, Licor, Lincoln, Nebraska, USA). The light reaction-curves were measured under the applied light spectra in the phytotrons (Fig. S2) using a clear top leaf chamber. Due to the lower maximum irradiance in the phytotron at the same spectra, the light curves with growing light were measured only up to a maximum radiation of 700 μmol m^{-2} $s^{-1}$of PPFD (700, 480, 380, 200, 100, 60, 30, 20, 17, 15 and 0 μmol m^{-2} $s^{-1}$of PPFD) to maintain the spectral quality. All leaf CO_2-exchange measurements were conducted at 400 μmol CO_2, 60% relative air humidity and 20ºC leaf temperature, with 60 to 120 seconds as threshold for stability after each light change. Stability of readings was assumed when the difference of the slopes between IRGAs were smaller than 0.5 and 1 μmol m^{-2} s^{-1} for CO_2 and H_2O.

For each curve, 12 different light models where fitted (Lobo *et al.,* 2013), including a model for photo inhibition (Eilers and Peeters 1988), and the model with the lowest sum of squares was selected in each case. From the selected model, four different parameters were calculated: maximum photosynthesis within the range of measured light (A_{700}), quantum yield for CO_2 fixation (α) as the slope of the curve between 0 and 100 μmol m^{-2} s^{-1} of PPFD, dark respiration (DR) and light compensation point (CP).

Statistical analysis.

To evaluate the effect of the different treatments a two-way analysis of variance (ANOVA) was performed for all the studied variables for each season, considering the species and different treatments as fixed factors (Table 2). The data was checked for normal distribution, independence and homogeneity of the variance.

To facilitate the interpretation of the study across species, we enable the direct visible and statistical comparison of the treatment effects through the normalization of each measured trait relative to its mean value on the outdoor treatment for each species. (Raw trait average values per species, treatments and seasons are available in Table S1 and S2). This data was used to perform a one-way ANOVA, considering the treatments as fixed factor and species as

random factors. As post hoc analysis, a Tuckey pairwise multiple comparation test was used to identify significant differences among treatments.

Finally, to understand the variability of all the measured variables between treatments a principal component analysis (PCA) was performed separately for each species, using all measured traits as inputs. To complete the data set required for the PCA analysis due to fewer gas exchange, pigment and fluorescence measurements than the number of plants, in each species and treatment (n = 9), the missing values of chlorophyll content and light parameters were imputed using normal distribution and keeping the same average and standard deviation of the performed measurements of the respective variables. All analyses were done using R (R Core team, 2019).

Results

Plant growth and morphology

In both runs (summer and spring) there was an interactive effect between treatment and species on plant height (Table 2). On average across all species, the sinusoidal climate change was the only treatment that did not result in significantly different plant heights compared to the field trial (Fig. 2 A, B). Fixed and variable conditions, induced, on average, lower and higher heights than the outdoor treatment in the summer and spring runs, respectively (Fig. 2 A, B). Although there was a considerable spread of the species around the average values, it is interesting to note that under summer conditions, most plants showed lower height growth compared with the field trial, while it was the opposite for the spring conditions (Fig. 2 A, B). Among the 7 species tested, *Triticum* and *Melissa* were the most sensitive species with significantly increased heights under both sinusoidal and variable environments compared with fixed conditions.

An interaction between treatment and species was also found for each trial on total biomass (Table 2), where some species (especially *Alnus*, *Melissa* and *Raphanus*) showed large deviations from the outdoor results in one or both phytotron runs (Fig. 2 C, D). However, when averaged across species, lower total biomass was reached under the variable conditions compared with the fixed condition independent of the run (Fig. 2 C, D). In addition, the species mean total biomass did not differ significantly from the outdoor trials under the variable climate, while it was significantly increased in the fixed climate in both runs, and in the sinusoidal treatment for the summer run. The biomass of individual organs largely followed the total biomass trends in all treatments and in both runs (Table S1).

Table 2: P-values of the two-way ANOVA for all measured plant traits, separated for the summer and spring run. Non-significant P-values (α = 0.05) are given in italic.

		Summer			Spring		
	Variable	Treatment	Species	Treatment x Species	Treatment	Species	Treatment x Species
Biomass and Morphology	Height**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Dry weight shoot	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Dry weight roots	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Total dry weight	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Root to shoot ratio	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	SLA	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Chlorophyll	Chlorophyll a (mg g^{-1})	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Chlorophyll b (mg g^{-1})	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Chl α:β ratio	< 0.001	< 0.001	< 0.001	*0.095*	< 0.001	-
	Carotenoids (mg g^{-1})	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	FvFm	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Pi	< 0.001	< 0.001	< 0.001	*0.445*	< 0.001	-
Photosynthesis	Max. photosynthesis	< 0.001	< 0.001	-	*0.0567*	< 0.001	-
	Light compensation point	*0.060*	< 0.001	-	< 0.001	0.005	-
	Quantum yield for CO2 fixation	< 0.001	< 0.001	0.006	0.003	< 0.001	-
	Dark respiration	*0.5223*	< 0.001	-	< 0.001	< 0.001	< 0.001

** Lettuce was not included in this analysis

-: The variable was removed from the analysis due non statistically significant.

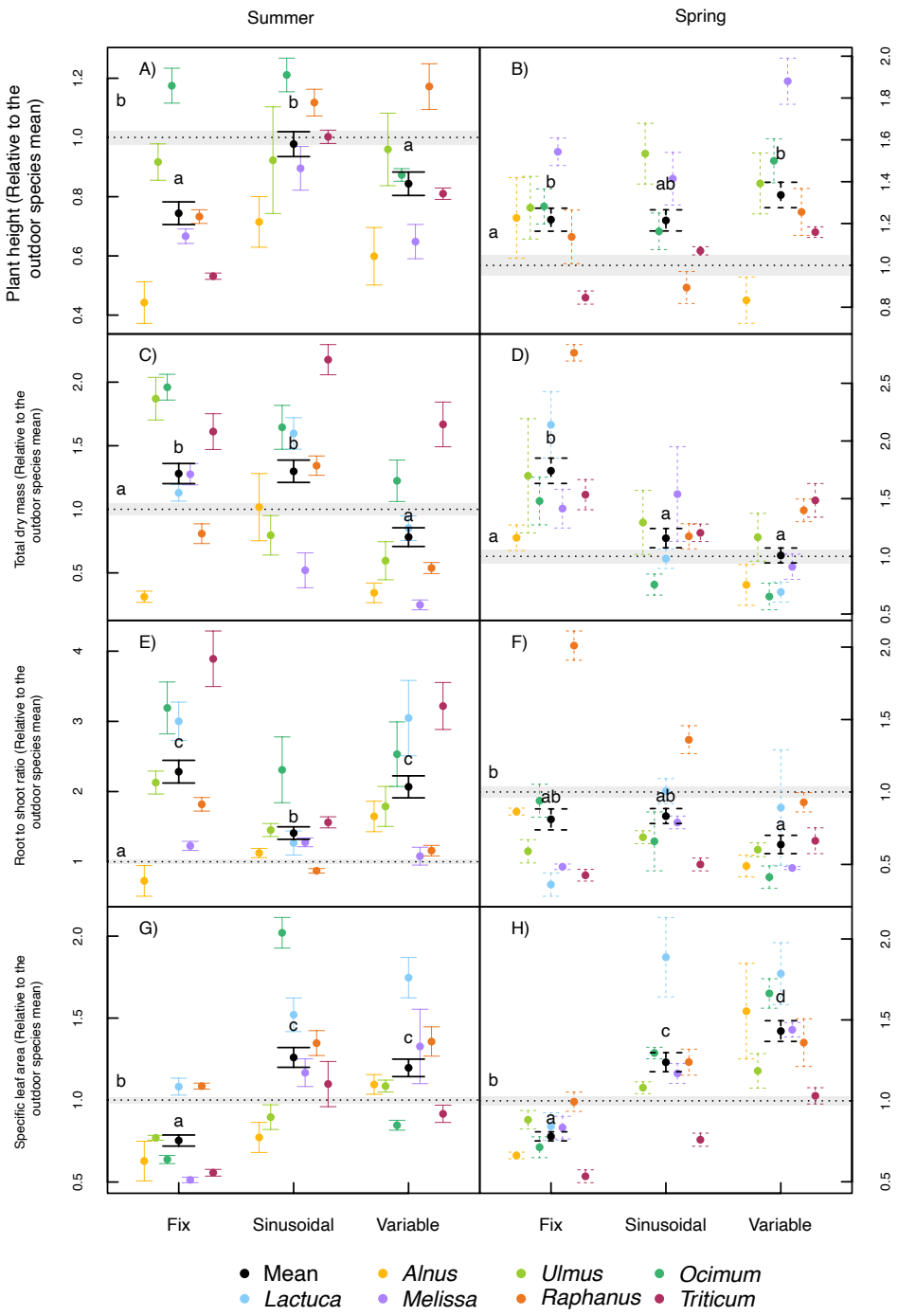


Figure 2: Plant height (A, B), total plant dry mass (C, D), root to shoot ratio (E, F) and specific leaf area (G, G) of the difference species normalized relative to the outdoor species mean under the two different runs (summer and spring). Error bars correspond to the standard error. N=9 for each species. Letters indicate significant differences ($P<0.05$, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect, separately for each run. *Alnus* was not included in the spring trial under the sinusoidal treatment.

Like for all other growth traits, there was also a significant interaction between treatment and species on the root to shoot ratio (r:s; Table 2). In the summer run, higher ratios were obtained in the three indoor treatments compared with the outdoor treatment in almost all the species (Fig.2 E, F). This was not the case in the spring run, where lower values were obtained with the exception of *Raphanus*. *Raphanus* had higher r:s ratios under fixed and sinusoidal conditions compared with outdoor or variable conditions. On average across species, only the variable treatment yielded significantly lower values (*i.e.* higher allocation to shoot biomass) than the outdoor treatment in the spring run.

In both runs and for most species, the sinusoidal and variable conditions induced a higher specific leaf area (SLA) compared to the field trial, while SLA tended to be lower under the fixed conditions (Fig. 2 G, H). However, although the trend was similar among species, an interaction was found between treatments and species on the SLA as well (Table 2). The effect of the different treatments within trials was biggest for *Lactuca* and *Basil*, where the difference on SLA was almost two-fold among treatments.

Leaf pigmentation and leaf gas-exchange

Compared with the fixed treatment, higher concentrations of chlorophyll *a* were reached under variable environmental conditions in all species and both runs, but this effect was stronger in the spring run (Fig 3 A, B). An interaction between species and treatment was found in both runs (Table 2). The sinusoidal treatment, on average across species, did not differ from the outdoor treatment, meanwhile fixed conditions induced lower concentrations compared with the outdoor treatment (Fig 3 A, B) in both runs. Chlorophyll *b* followed the reactions of chlorophyll *a* in most of the species (Table S1 and S2). Hence, the chlorophyll *a:b* ratio was similar among treatments (Fig. 3 C, D). Nevertheless, there was a significant interaction between species and treatments for the summer, but not for the spring run (Table 2). On average, higher chlorophyll *a:b* ratios were recorded only under fixed conditions compared with all the other treatments in the summer run (Fig 3 C, D).

F_v/F_m values were around 0.8 in the field trial and all phytotron treatments (Table S1, S2), indicating the absence of significant stress in all treatments. However, F_v/F_m values were higher under sinusoidal conditions compared with the fixed treatments in both runs, and also under the variable treatment in the spring run (Fig. 3 E, F). A significant interaction between species and treatment was found for F_v/F_m (Table 2), as well as for the fluorescence Pi value (data not shown).

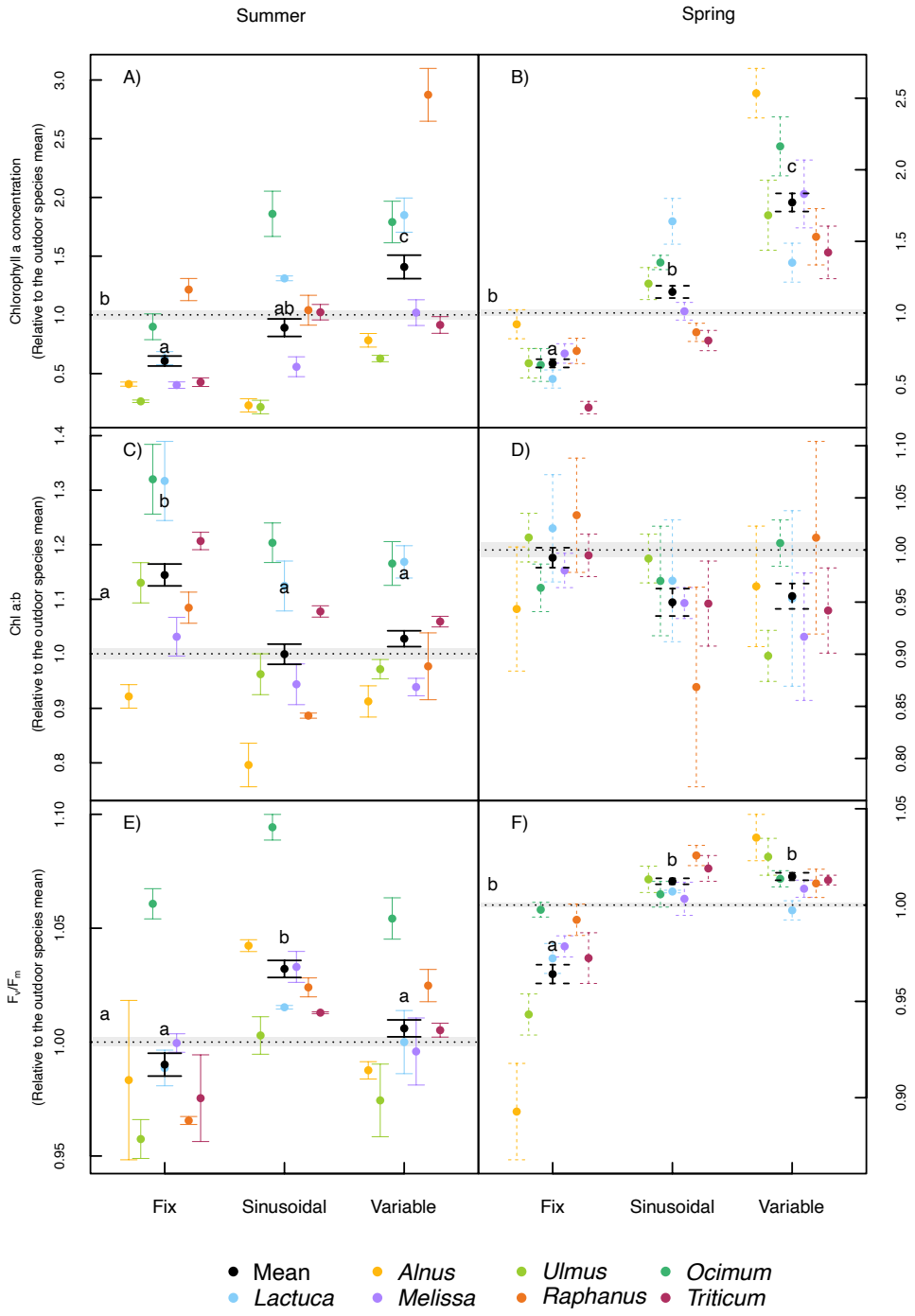


Figure 3: Chlorophyll a concentration (A, B), chlorophyll a to b ratio (C, C) and F_v/F_m values (D, E) of the difference species normalized relative to the outdoor species mean under the two different runs (summer and spring). Error bars correspond to the standard error. N=4 for each species. Letters indicate significant differences ($P<0.05$, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect, separately for each run. *Alnus*, was not included in the spring trial under the sinusoidal treatment

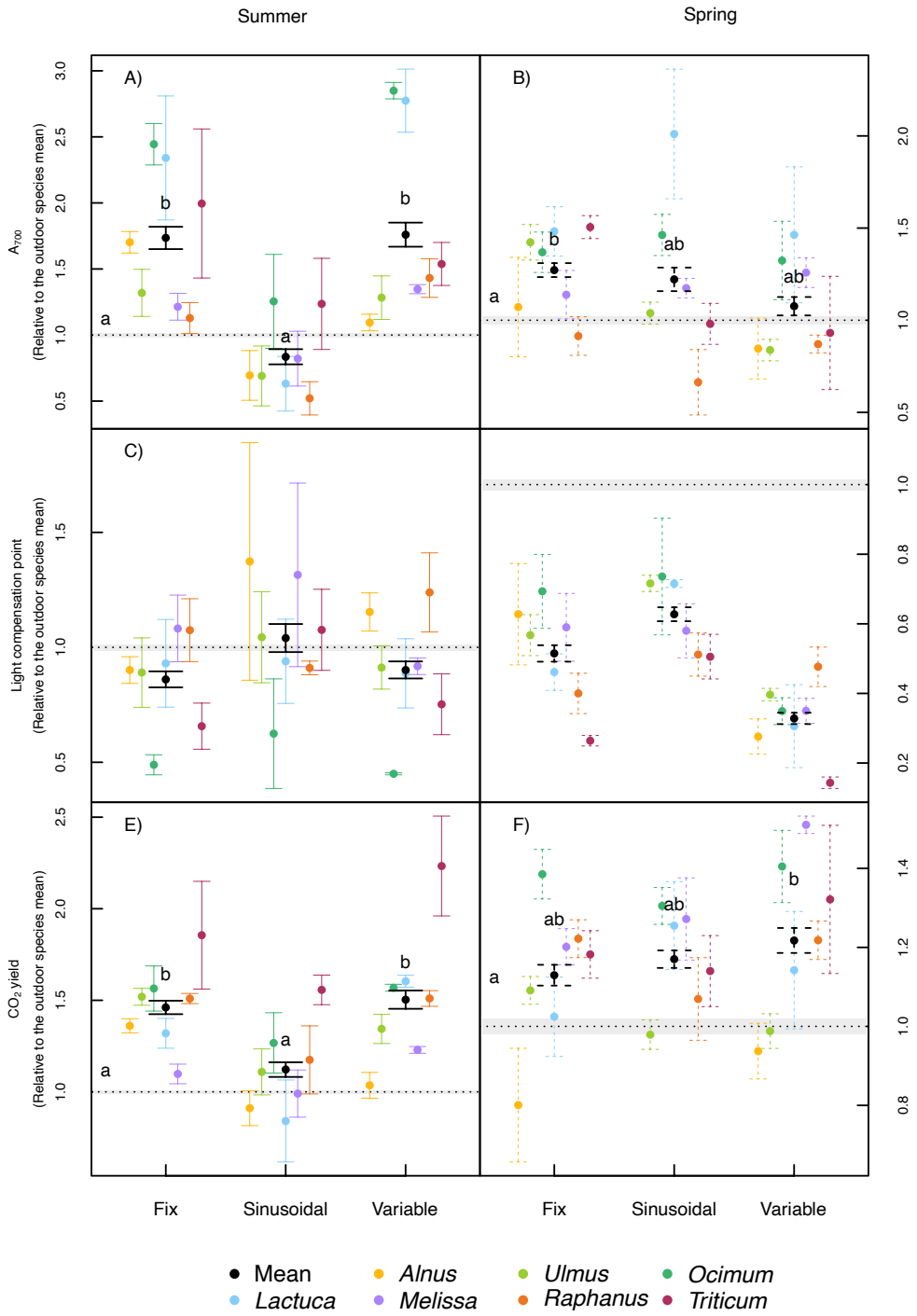


Figure 4: Maximum photosynthesis (A, B), Light compensation point (C, D) and CO_2 yield of photosynthesis (E, F) of the difference species normalized relative to the outdoor species mean under the two different runs (summer and spring). Error bars correspond to the standard error. N=3 for each species. Letters indicate significant differences ($P<0.05$, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect, separately for each run.

In contrast to most growth traits, no interaction was found between the treatment and species on A_{700} in both runs, and additionally, there was no statistically significant effect of treatment in the spring run when species and treatment were considered fixed variables (Table 2). On average across all species, plants had higher A_{700} values compared to the outdoor treatment under the fixed climatic treatments in both runs, and under the variable treatment in the summer run (Fig. 4 A, B), largely driven by the strong reactions of *Lactuca* and *Ocimum*. The light compensation point of net photosynthesis was not significantly affected by the different treatments in the summer trial (Table 2). The light compensation values from the spring trial showed no interactive effect between treatment and species, but much lower compensation points were reached for all the indoor treatments compared with the outdoor treatment (Fig. 4 C, D). This trend was strongest for the variable conditions, which induced lower compensation points on average across species than the other two indoor treatments. Only in the summer run, an interactive effect was found between treatment and species for the quantum yield of CO_2 fixation (α) (Table 2). On average across species, α was higher during the summer trial under fixed and variable conditions, compared with the sinusoidal and outdoor treatments, especially influenced by *Triticum*. In the spring run, the average α across species was significantly higher under the variable conditions compared with the outdoor treatment, while no significant difference to the outdoor treatment was found for the sinusoidal and the fixed treatment (Fig. 4 E, F). There was no treatment effect on leaf respiration in the dark in the summer run, but a significant treatment and treatment × species interaction in the spring run, with higher values, therefore lower values of respirations, in the fixed and variable treatments (Table 2, Table S1, S2).

Principal component analysis (PCA)

A PCA performed separately for each species and the two runs showed clear separations among the treatments in most cases (Fig. 5). The sinusoidal and variable treatments were often more clustered whilst the fixed and outdoor treatments were furthest from the mean, although this was not always the case. Interestingly, within a species, the treatment grouping was very different between the summer and the spring run (Fig. 5). Within the PCAs, traits of the same type (biomass, pigments, photosynthesis) tended to point in similar directions (Fig. S2), with the exception of light measurements related parameters, that often pointed along different axes within a species. Independent of the species or run, the effect of the different factors had similar weights, and the first two principal comments explained, on average 56.99% of the total variance (standard deviation = 3.54%).

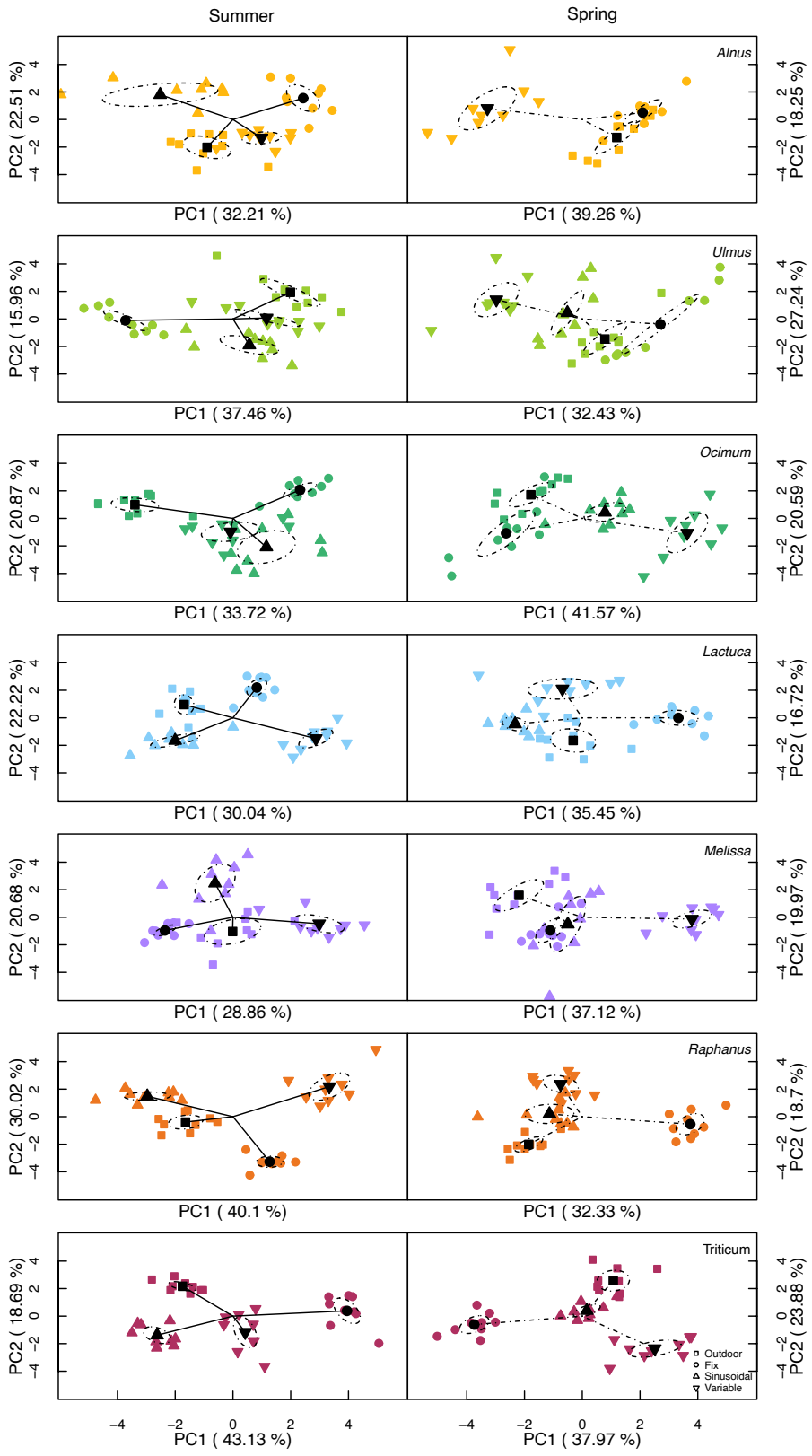


Figure 5: Principal component analysis of each species in the two runs (summer and spring). Ellipsoid calculated using the standard error of the plotted points. *Alnus* was not included in the spring trial under the sinusoidal treatment

Discussion

The incorporation of environmental variability has previously been recommended for more natural-like plant growth (Poorter *et al.,* 2016; Annunziata *et al.,* 2017; Annunziata *et al.,* 2018; Matsubara, 2018), but is rarely applied in phytotron studies due to practical reasons and as well as technical limitations of the growth chambers. Within this study, we found significant differences in almost all investigated plant traits among the different climatic scenarios applied indoors, but also strong differences with the plants from the out-door trials. Although there was an overall trend to more similar traits to the outdoor plants in phytotron runs that simulated the real temperature and humidity variations, this was not the case for all traits. Importantly, we also found a high species-specificity. Overall, we could show that the type of environmental variation does affect plant morphology and photosynthetic capacity, in line with previous studies (e.g. Kaiser *et al,* 2018; Yamori 2016), and with studies that looked at specific metabolic processes (e.g. Annunziata *et al.,* 2017; Annunziata *et al.,* 2018).

Plant growth and morphology

Although the effect size of climatic variability on plant height differed between the summer and spring run, in both runs the sinusoidal treatment did not differ from the control in average across species. It has been suggested that a larger difference between day- and night-time temperature can stimulate the production of abscisic acid, which may enhance stem elongation and help to respond to changes in the environmental conditions (*e.g.* Jensen *et al*., 1996; Thingnaes *et al.,* 2003). On the other hand, it has also been proposed that daily light fluctuations can induce shorter plants (Poorter *et al.,* 2016). The presented results suggest that in our case the effect of the diurnal temperature amplitude had a larger effect on height than the effect of fluctuating light.

The reduction in total plant biomass under fluctuating climates was one of the strongest effects in this study and has also been reported in several previous experiments (*e.g.* Porteer, 2016; Vialet – Chabrand *et al.,* 2017; Annunziata *et al.,* 2018). In our case, the difference between treatments was more marked in the spring run, in which not only the totally variable conditions but also the sinusoidal treatment did not differ from the outdoor control. This may propose that the main effects of a fluctuating environment without extreme conditions, may lie in the amplitude between the daily minimum and maximum temperatures, as previously mentioned by Annunziata *et al.* (2018). Annunziata *et al.,* (2017) could show for *Arabidopsis thaliana* that there is a lower accumulation of starch during the day under fluctuating environments, which also implies that the plants have less starch available during the night for

growth and metabolism (Sitt and Zemma, 2012). Additionally, lower night temperatures will also reduce the consumption of C reserves because of reduced metabolic and growth activity (Pilkingston *et al.,* 2015). Annunziata *et al.,* (2018) showed that under variable light and temperature, 90% more biomass could be reached in *A. thaliana* compared with fluctuating light and fixed temperature, corroborating the relevance of joint environmental fluctuations for plant growth.

The observed differences in root:shoot ratios between phytotron and outdoor experiments might result from deviating pot soil temperatures. Although pot temperature was not monitored or controlled in our experiment, it can be assumed that under natural sunlight, the soil is likely to warm up more than under the LED lights in the phytotrons. Differences between air- and soil-temperature may play an important role in the allocation of reserves and new biomass production (*e.g.* Randeni and Caesar, 1986; Domish *et al.,* 2001) In our experiment, the differences in root:shoot ratios between indoor and outdoor conditions show the reversed pattern to the stem biomass, where the root:shoot ratio was higher in all phytotron treatments in the summer run, but lower in the spring run compared with the control (Fig. 2). Although the 35-days mean temperatures were similar between the summer and the spring run, the spring run had considerably lower temperatures in the first couple of days (Fig. 1). The potentially stronger effect of the above-mentioned outdoor soil warming at the cooler spring days, might have led to an early divergence in the biomass-allocation between outdoor and phytotron plants that affected the whole experiment differently than in the summer run. Although several reports are available about the effects of light and temperature fluctuations on plant growth and physiology (Annunziata *et al.,* 2017; Vialet – Chabrand *et al.*, 2017; Mathew *et al.,* 2018; Annunziata *et al.,* 2018), none of them focused on biomass allocation changes.

Plants that were treated with fluctuating climates produced thinner leaves with significantly higher SLA values compared to plants treated with the fixed conditions. This result is in line with a recent indoor study which found that the SLA in *Arabidopsis thaliana* was up to 25% higher with a thinner spongy mesophyll layer under fluctuation levels of light and constant temperatures, compared with a fixed light treatment lacking light fluctuations (Vialet-Chabrand *et al.,* 2017). Under natural conditions, bigger climatic fluctuations, especially the presence of cold temperatures and high solar radiation are generally associated with the production of hardened leaves and smaller SLA values. Especially, sunlight adapted leaves tend to be thicker compared with shade adapted leaves, as a result of the compromise between the increase in chloroplast surface area for CO_2 dissolution, due to low affinity of rubisco for CO_2, and the production costs of thicker leaves (Tershima *et al.,* 2006). The fact that we found

increasing SLA values with increasing climatic variation suggests that neither the applied minimum and maximum temperatures, nor the applied light peaks were extreme enough to induce thicker or denser leaves. Finally, our study as well as the above-mentioned experiments by Vialet- Chabrand *et al.,* (2017) and Annunziata *et al.,* (2017 and 2018) were performed without UV light, which is known to reduce stem elongation and SLA (*e.g*. Jenkins, 2014).

Leaf pigmentation and photosynthesis

To our knowledge, an increase in chlorophyll under fluctuating environmental conditions has not been reported so far. Higher light intensities have been related with lower Chl a and b concentrations in leaves (on a dry matter basis), but temperature and water availability have been identified as the most important factors for the synthesis of chlorophyll (Li *et al.,* 2018). Erwin and Heins (1995) showed a positive correlation between the diurnal difference between day and night time temperatures difference and total chlorophyll leaf concentrations in *Dendranthema* and *Chrysanthemum*. Similar, chlorophyll concentrations were significantly higher in the variable compared to the fixed climate treatments in the current study. Similar to Vialet -Chabrand *et al.* (2017), who reported higher chlorophyll *a:b* ratios in *Arabidopsis* under fixed vs. variable light conditions (4.27 vs 3.72 in average respectively), we did find significant changes in chlorophyll *a:b*, especially between the fixed and variable condition (Table 2).

It is broadly assumed that fluctuating light can increase the photosynthetic capacity of plants (*e.g.* Abu-Gosh *et al.,* 2016; Vialet- Chabrand *et al.,* 2017; Kaiser *et al.,* 2017; Mathews *et al.,* 2018). In line with this notion, we found increased F_v/F_m values in the sinusoidal and variable compared to the fixed treatments in our experiments. Vialet-Chabrand *et al.,* (2017) also reported higher F_v/F_m values under variable light conditions, which might be related to a higher PSII capacity. In contrast, several authors have shown lower F_v/F_m values under varying environmental conditions. Yamori (2016) explained such reductions mainly by a photoinhibition of PSI under fluctuating light, while other abiotic stressors were linked to a photoinhibition of PSII. Additionally, to this, it has been proposed that fluctuations in temperature over the day and night may have a reparative effect in both photosystems, due to a better circadian clock adjustment (Annunziata *et al.,* 2018), resulting in higher F_v/F_m values under fluctuating environments.

Like for the photosynthetic capacity, previous studies have indicated that A_{max} may be enhanced in plants that grow in fluctuating light environments. Vialet – Chabrand *et al.* (2017) highlighted that this effect becomes especially evident if photosynthesis values are calculated

per leaf mass instead of leaf area, meanwhiles Mathews *et al.,* (2018), demonstrated that the time of the day when the measurements are conducted have an important impact on the effect size of light fluctuation on photosynthesis. Especially in the morning fluctuating light treatments tended to produce higher A_{max} than at midday, compared with both sinusoidal and square light treatments. Poorter *et al.,* (2016) reported generally lower values in indoor vs. outdoor experiments, especially in woody species, probably also driven by the often higher SLA of indoor vs. outdoor plants.

In our study, there were no strong differences in the light compensation point as well as CO_2 yield of photosynthesis among the phytotron runs, but significantly lower compensation points in the phytotrons compared to field grown plants in the spring run, and higher CO_2 yields in indoor plants under simulated summer and spring conditions. The latter might be a consequence of the different light spectral composition of the used LED lamps in the phytotrons (with a higher proportion of blue and red light compared to the sun spectrum). Vialet-Chabrand *et al.,* (2017) did not find any difference in photosynthetic light compensation point either when comparing fixed and fluctuating light treatments on Arabidopsis thaliana under constant temperature, meanwhiles light intensity was shown to play a more important role.

When all the different variables were analysed through the PCA (Fig. 5 and S3), several species tend to have either the fixed or the variable treatment separated furthest from the rest of the treatments. Even though the average environment variables were all the same for the different treatments within runs, the different trials could be easily separated by the PCA in all species. Annunziata *et al.* (2017) used a fixed and a sinusoidal light treatment against natural light to evaluate metabolism effects in *Arabidopsis* under stable temperature, explained similar amount of the variance variability between light treatments and indoor and outdoor plants by the first two principal components (37-34 and 23-16 % for the first and second component, respectively). However, differently to the current study, Annunziata *et al.* (2017) found a much clearer separation between the outdoor treatment and the indoor treatments, but lower difference between the indoor treatments. *Posteriori*, Annunziata *et al.,* (2018) demonstrated that after removing the temperature fluctuation under either fluctuating or fixed light, there was an increase in scattering of the PCA values between treatments, hypothesizing that fluctuating of temperature had a stronger effect than light fluctuation on the plants' metabolism in their experiment scenario.

In conclusion, the current study indicates that implantation of fluctuations of temperature and light quantity are relevant at morphological and photosynthetic level to reach

more natural-like plant performance in indoor growth facilities. However, it became also clear that although we were able to closely reproduce outdoor conditions in terms of temperature, humidity and irradiance in our phytotrons, there were still significant differences prevailing in most investigated traits compared with field grown plants. Other factors that could not be reproduced in our setup (*e.g.* soil temperature offsets, wind speed and directions) thus might have significantly added to the observed differences between indoor and outdoor grown plants. Although our results were generally species-specific, some general trends could be revealed. Especially, we recommend to not use fixed day and night time conditions, but to grow plants at least under sinusoidal climate fluctuations, if trait measurements on indoor plants are used for extrapolations to, and models of, natural systems.

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Supplementary material

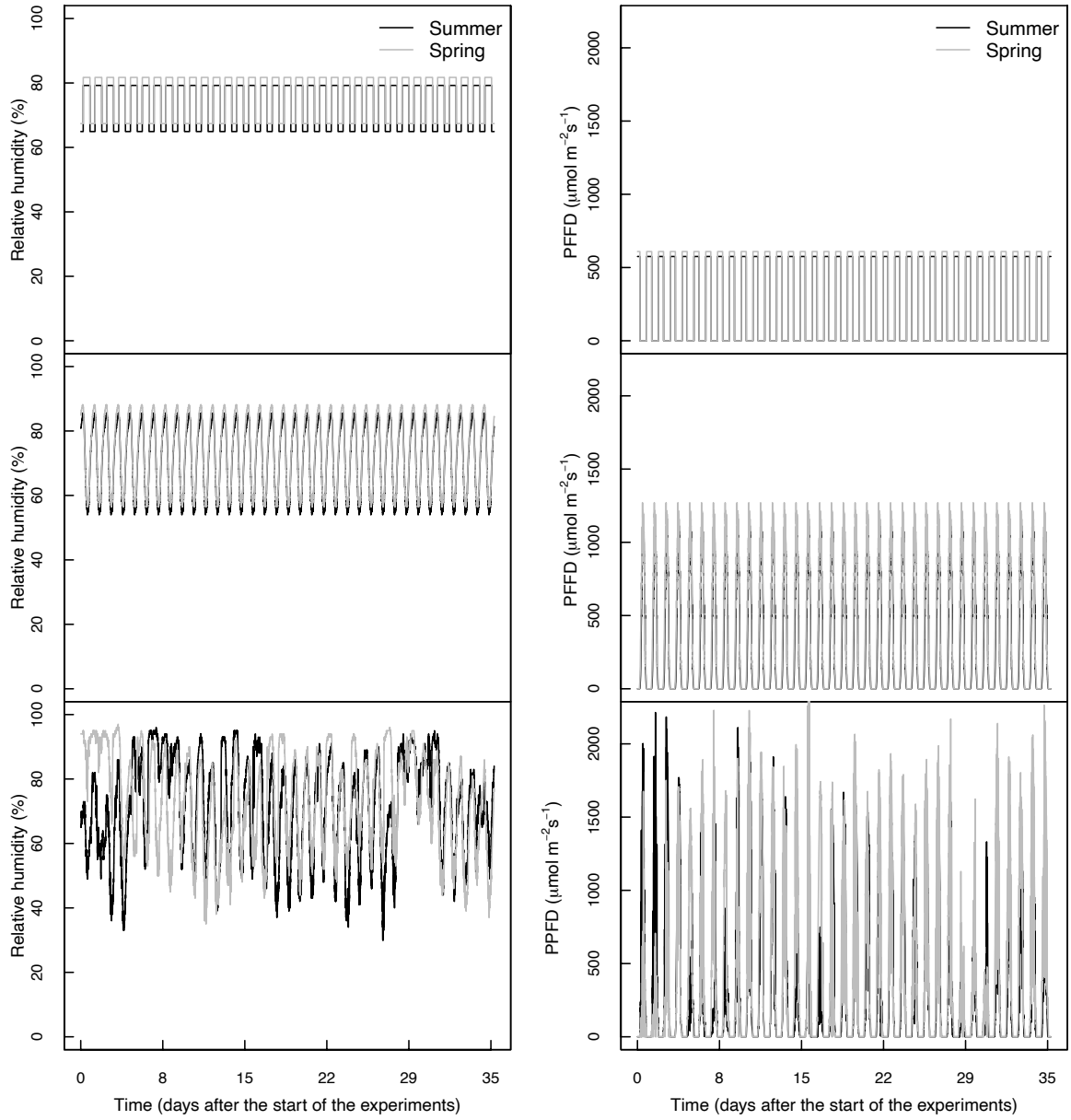

Figure S1: Applied relative humidity (%) and PPFD (µmol m^{-2} s^{-1}) for each treatment in summer or spring conditions

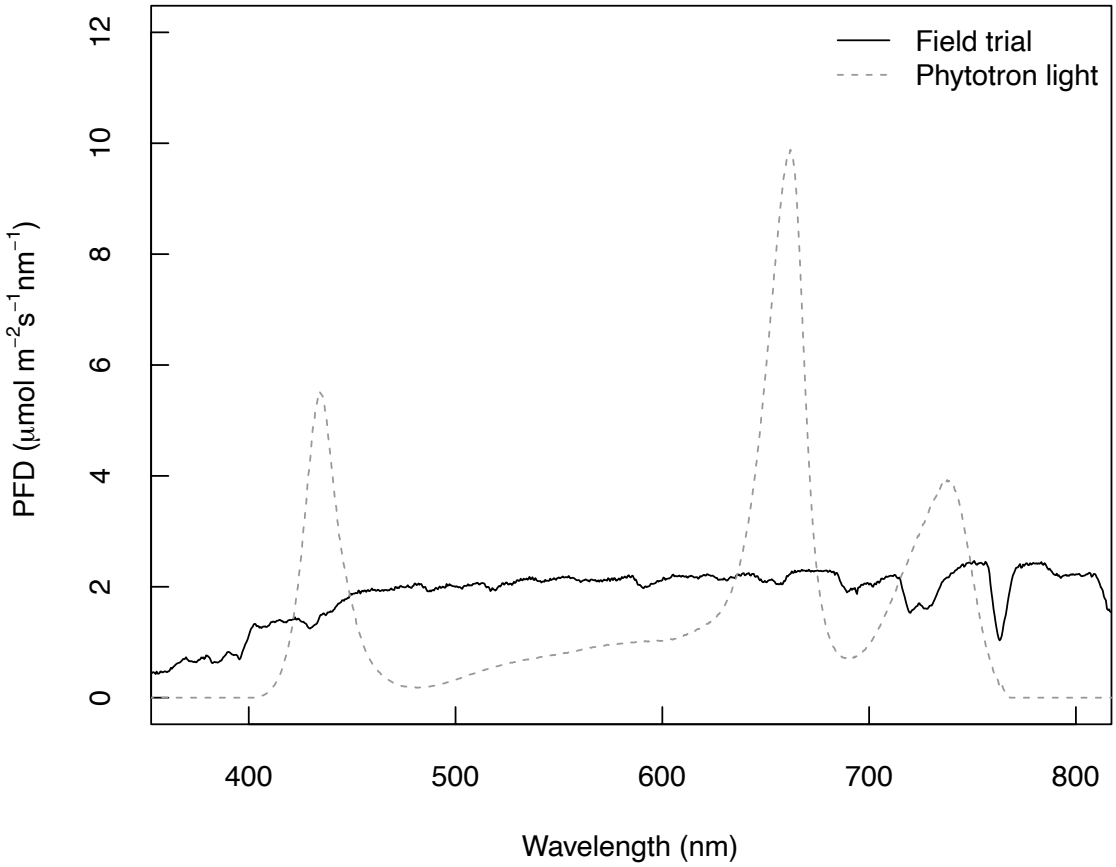

Figure S2: Spectrum examples of the applied light. The field trial example corresponds to a sample of the sun spectra (28% Blue light, 36% Green, 36% Red and R:FR 1.1 in average), meanwhile the phytotron light quality corresponds to the used spectra in the phytotrons (25%B, 16%G, 59%R and R:FR 1.8). The integrated area between 400 and 700 nm corresponds to an approximately 575 µmol $m^{-2}s^{-1}$ of photosynthetic photon flux density in each case

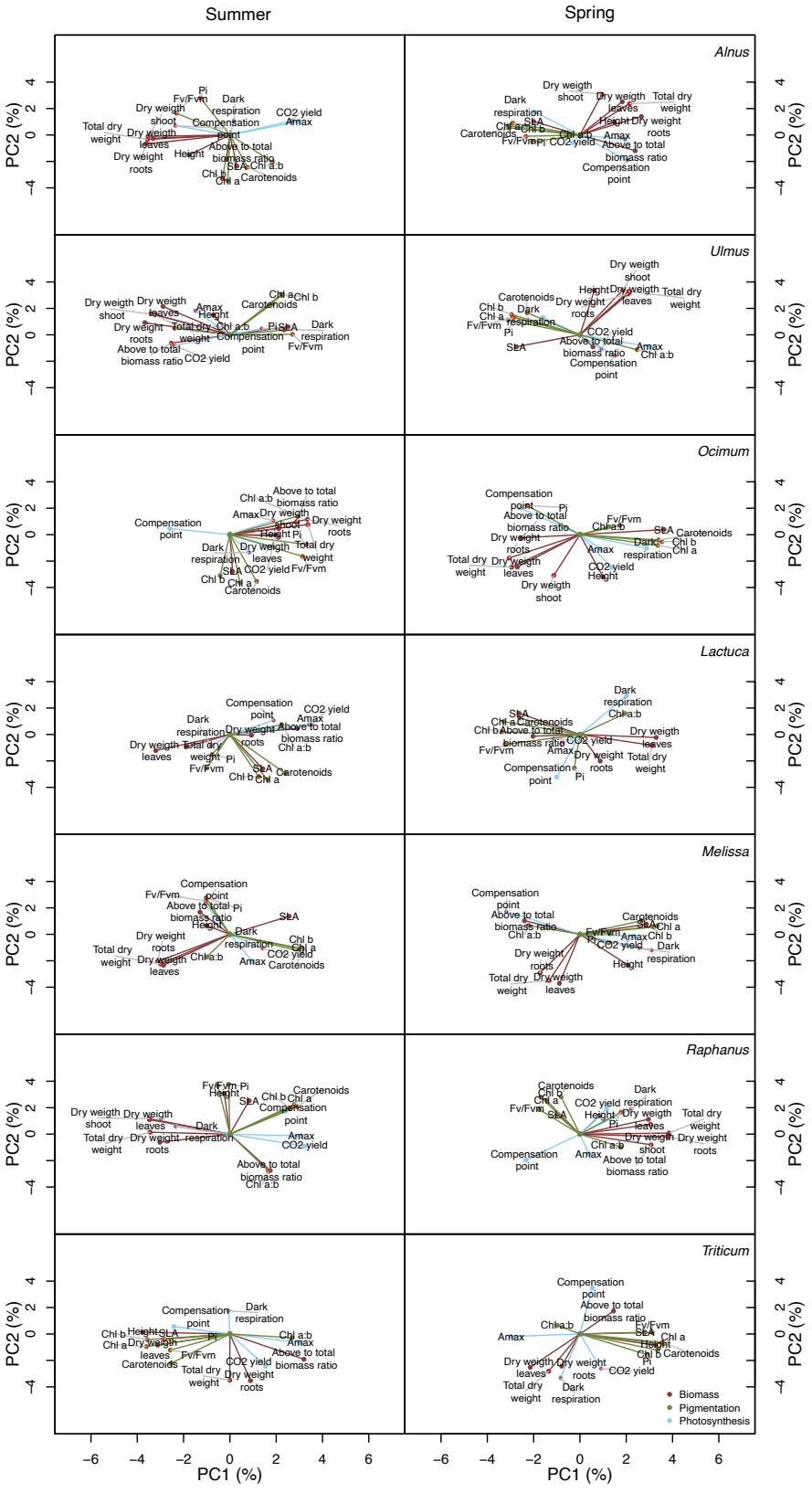

Figure S3: Principal component analysis loadings of each species in two different conditions: Summer and Spring. *Alnus* was not included in the spring trial under the sinusoidal treatment

Table S1: Absolute values of all measured traits for each species and treatment in the 'summer' run. Values are means ± s.e., N=3 to 9 (see methods for details).

Species	Alnus				Ulmus			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	10.88±1.3	13.34±2.09	-	9.07±1.2	26.19±4.48	33.41±3.92	40.18±3.8	36.44±3.82
Dry weight leaves	0.25±0.04	0.27±0.03	-	0.2±0.05	1.42±0.32	3.1±0.91	1.96±0.38	1.98±0.35
Dry weight shoot	0.08±0.01	0.13±0.01	-	0.1±0.03	0.78±0.23	1.31±0.4	1.18±0.28	0.96±0.19
Dry weight roots	0.23±0.04	0.25±0.02	-	0.11±0.03	1.29±0.22	1.52±0.43	1.37±0.33	1.13±0.19
Total dry weight	0.55±0.09	0.64±0.06	-	0.42±0.1	3.49±0.74	5.93±1.73	4.52±0.97	4.07±0.72
Root to Shoot ratio	0.72±0.07	0.62±0.02	-	0.35±0.05	0.61±0.06	0.36±0.05	0.42±0.03	0.37±0.03
SLA	31.76±0.04	21.04±0.65	-	49.35±9.37	25.31±1.65	22.35±1.44	27.35±0.93	29.99±2.69
Chlorophyll								
Chlorophyll a (mg g-1)	2.72±0.16	2.5±0.28	-	6.89±0.47	2.55±0.41	1.65±0.26	3.07±0.28	4.29±0.62
Chlorophyll b (mg g-1)	0.64±0.03	0.64±0.1	-	1.7±0.18	0.61±0.11	0.38±0.05	0.73±0.07	1.13±0.18
Chl a: b ratio	1.34±0.14	1.45±0.14	-	2.59±0.12	0.97±0.13	0.79±0.09	1.1±0.13	1.24±0.17
Carotenoids (mg g-1)	4.25±0.06	4.01±0.25	-	4.1±0.25	4.23±0.09	4.29±0.1	4.2±0.1	3.8±0.1
Fv/Fm	0.79±0.01	0.7±0.02	-	0.82±0.01	0.8±0	0.75±0.01	0.81±0.01	0.82±0.01
Photosynthesis								
Max photosynthesis**	9.73±1.36	10.43±2.62	-	8.23±1.6	6.99±0.6	9.95±0.68	7.26±0.42	5.86±0.41
Initial slope	0.052±0.006	0.041±0.007	-	0.048±0.004	0.04±0.004	0.044±0.001	0.039±0.002	0.04±0.002
Dark respiration	-4.08±0.18	-2.04±0.16	-	-1.24±0.22	-3.1±0.55	-1.92±0.17	-2.21±0.13	-1.39±0.07
Compensation point	79.67±6.77	50±11.59	-	22±4.04	74±4.73	42±4.36	53±1.73	29.33±1.33

Table S1: (continued)

Species	Ocimum				Lactuca			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	17.28±0.33	22.14±1.45	20.09±1.51	25.91±1.82	-	-	-	-
Dry weight leaves	1.48±0.15	2.28±0.3	1.3±0.13	1.06±0.17	6.99±1.07	22.04±3.2	6.93±0.46	6.22±0.9
Dry weight shoot	0.38±0.05	0.55±0.08	0.34±0.04	0.48±0.1	-	-	-	-
Dry weight roots	1.2±0.22	1.68±0.3	0.67±0.21	0.45±0.12	5.79±1.32	5.33±1.04	5.6±0.71	2.6±0.59
Total dry weight	3.05±0.38	4.52±0.63	2.31±0.28	1.99±0.35	12.78±2.27	27.36±3.68	12.52±1.08	8.83±1.11
Root to Shoot ratio	0.63±0.09	0.59±0.07	0.42±0.13	0.26±0.05	0.79±0.1	0.29±0.06	0.8±0.07	0.71±0.32
SLA	19.67±1.16	14.03±1.27	25.47±0.7	32.71±1.79	36.84±2.39	31±3.12	69.54±9.03	65.77±7.05
Chlorophyll								
Chlorophyll a (mg g-1)	2.52±0.15	1.6±0.29	3.41±0.13	5.45±0.52	3.52±0.37	1.9±0.22	5.78±0.56	4.76±0.48
Chlorophyll b (mg g-1)	0.54±0.04	0.36±0.07	0.75±0.06	1.14±0.11	0.66±0.09	0.34±0.03	1.1±0.11	0.93±0.14
Chl a: b ratio	0.86±0.04	0.52±0.08	0.99±0.03	1.41±0.1	1.23±0.09	0.92±0.1	1.97±0.23	1.58±0.17
Carotenoids (mg g-1)	4.75±0.14	4.57±0.11	4.61±0.25	4.78±0.11	5.45±0.23	5.57±0.28	5.29±0.32	5.2±0.46
Fv/Fm	0.83±0.01	0.83±0	0.84±0.01	0.84±0	0.85±0	0.83±0.01	0.86±0	0.85±0
Photosynthesis								
Max photosynthesis**	9.23±1.31	12.64±1.01	13.5±1.03	12.22±1.96	5.17±0.18	7.66±0.69	10.39±1.82	7.56±1.91
Initial slope	0.039±0.004	0.054±0.002	0.051±0.002	0.055±0.004	0.04±0.007	0.041±0.004	0.051±0.004	0.046±0.006
Dark respiration	-2.89±0.28	-2.8±0.28	-2.7±0.56	-1.43±0.18	-3.53±0.61	-1.53±0.07	-2.88±0.32	-1.49±0.6
Compensation point	70.67±5.9	49±7.51	52±11.85	24.67±2.73	77.33±1.86	35.67±4.06	55.33±0.88	23.67±9.21

Table S1: (continued)

Species	Melissa				Raphanus			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	14.4±1.83	22.22±0.95	20.37±1.81	27.08±1.59	7.57±1.29	8.6±0.97	6.77±0.58	9.5±0.85
Dry weight leaves	0.78±0.18	1.56±0.19	1.39±0.37	1.01±0.12	2.83±0.23	4.66±0.16	2.74±0.22	4.06±0.28
Dry weight shoot	-	-	-	-	0.58±0.1	1.28±0.13	0.51±0.05	0.88±0.1
Dry weight roots	0.87±0.23	0.78±0.09	1.16±0.32	0.5±0.06	4.51±0.35	15.94±0.44	6.04±0.64	6.13±0.54
Total dry weight	1.65±0.41	2.34±0.28	2.55±0.68	1.5±0.18	7.92±0.58	21.88±0.56	9.29±0.86	11.07±0.78
Root to Shoot ratio	1.04±0.08	0.5±0.02	0.82±0.05	0.5±0.01	1.35±0.08	2.72±0.14	1.84±0.13	1.26±0.09
SLA	32.02±2.15	26.72±2.24	37.44±1.98	46.08±1.44	25.87±2.93	25.73±1.55	32.05±2.07	35.17±3.81
Chlorophyll								
Chlorophyll a (mg g-1)	4.01±0.41	2.88±0.27	4.06±0.25	7.34±0.95	3.71±0.45	2.73±0.33	3.21±0.24	5.68±0.73
Chlorophyll b (mg g-1)	0.9±0.1	0.65±0.05	0.96±0.05	1.81±0.26	0.91±0.08	0.65±0.07	0.94±0.12	1.43±0.29
Chl a: b ratio	1.55±0.16	1.11±0.09	1.45±0.08	2.48±0.44	1.07±0.08	1.03±0.15	1.22±0.16	1.67±0.26
Carotenoids (mg g-1)	4.47±0.12	4.38±0.07	4.24±0.07	4.1±0.27	4.05±0.16	4.19±0.22	3.52±0.39	4.1±0.37
Fv/Fm	0.82±0	0.81±0	0.83±0.01	0.83±0	0.83±0	0.82±0.01	0.85±0	0.84±0.01
Photosynthesis								
Max photosynthesis**	10.61±0.84	12.08±1.39	12.46±0.56	13.35±0.86	11.86±1.55	10.84±1.24	7.87±2.1	10.33±0.57
Initial slope	0.041±0.003	0.049±0.002	0.052±0.004	0.062±0.001	0.049±0.005	0.06±0.002	0.052±0.005	0.06±0.002
Dark respiration	-2.76±0.19	-1.98±0.25	-2.13±0.08	-1.57±0.1	-3±0.24	-1.54±0.16	-1.75±0.28	-1.81±0.23
Compensation point	66.67±2.96	39.33±6.49	38.67±5.17	23.33±2.4	56.67±6.84	22.67±3.28	29±3.51	27±3.21

Table S1: (continued)

Species	Triticum			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology				
Height*	54.06±1.59	45.74±1.71	57.82±1.06	62.64±1.38
Dry weight leaves	10.35±1.2	29.54±1.89	20.67±1.43	21.67±3.75
Dry weight shoot	-	-	-	-
Dry weight roots	63.53±5.66	83.84±8.76	68.29±4.9	88.07±9.38
Total dry weight	73.87±6.35	113.38±9.66	88.96±5.57	109.73±10.77
Root to Shoot ratio	6.78±0.89	2.89±0.28	3.39±0.3	4.5±0.6
SLA	34.59±3.89	18.48±1.38	26.28±1.4	35.66±1.74
Chlorophyll				
Chlorophyll a (mg g-1)	5.8±0.29	1.96±0.25	4.68±0.41	8.25±1.06
Chlorophyll b (mg g-1)	1.39±0.11	0.47±0.06	1.17±0.07	2.13±0.35
Chl a: b ratio	1.77±0.08	1.04±0.06	1.54±0.13	2.48±0.25
Carotenoids (mg g-1)	4.19±0.13	4.17±0.09	3.97±0.17	3.95±0.17
Fv/Fm	0.82±0.01	0.8±0.01	0.84±0.01	0.83±0
Photosynthesis				
Max photosynthesis**	9.21±1.62	13.86±0.57	9.03±1.02	8.58±2.83
Initial slope	0.049±0.001	0.058±0.003	0.056±0.004	0.065±0.009
Dark respiration	-4.46±0.25	-1.41±0.11	-2.78±0.57	-1.18±0.15
Compensation point	88.33±9.74	23.33±1.33	44.67±5.67	12.67±1.45

Table S2: Absolute values of all measured traits for each species and treatment in the 'spring' run. Values are means ± s.e., N=3 to 9 (see methods for details).

Species	Alnus				Ulmus			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	22.32±1.2	9.88±1.57	15.96±1.91	13.37±2.17	26.67±3.05	24.46±1.64	24.62±4.8	25.59±3.27
Dry weight leaves	1.06±0.1	0.37±0.07	0.93±0.24	0.32±0.07	1.44±0.28	2.02±0.16	0.93±0.17	0.68±0.16
Dry weight shoot	0.51±0.05	0.17±0.04	0.6±0.16	0.16±0.04	0.71±0.15	1.26±0.15	0.63±0.13	0.38±0.09
Dry weight roots	0.52±0.05	0.11±0.04	0.6±0.16	0.24±0.05	0.63±0.14	1.91±0.21	0.65±0.15	0.59±0.18
Total dry weight	2.09±0.19	0.65±0.09	2.13±0.55	0.72±0.16	2.77±0.56	5.19±0.46	2.21±0.43	1.65±0.42
Root to Shoot ratio	0.34±0.03	0.25±0.08	0.39±0.02	0.57±0.07	0.28±0.02	0.59±0.05	0.4±0.03	0.5±0.08
SLA	34.76±1.24	21.83±4.19	26.83±3.18	38.07±2.08	28.71±0.93	22.1±0.43	25.72±2.16	31.16±1.05
Chlorophyll								
Chlorophyll a (mg g-1)	7.35±0.97	3.02±0.13	1.69±0.41	5.76±0.43	6.8±0.43	1.8±0.07	1.47±0.39	4.28±0.18
Chlorophyll b (mg g-1)	1.45±0.25	0.64±0.04	0.43±0.13	1.24±0.13	1.54±0.09	0.36±0.02	0.34±0.08	1±0.03
Chl a: b ratio	1.94±0.18	1.89±0.1	0.94±0.39	2.09±0.15	1.15±0.09	0.64±0.04	0.53±0.13	1.26±0.07
Carotenoids (mg g-1)	5.12±0.23	4.72±0.11	4.08±0.2	4.68±0.15	4.42±0.05	5±0.16	4.26±0.17	4.3±0.08
Fv/Fm	0.78±0.01	0.77±0.03	0.81±0	0.77±0	0.81±0	0.77±0.01	0.81±0.01	0.79±0.01
Photosynthesis								
Max photosynthesis**	8.37±0.29	14.25±0.68	5.81±1.58	9.17±0.52	7.3±0.39	9.62±1.3	5.04±1.65	9.36±1.2
Initial slope	0.044±0.002	0.059±0.002	0.04±0.004	0.045±0.003	0.034±0.002	0.052±0.002	0.038±0.004	0.046±0.003
Dark respiration	-1.74±0.07	-1.77±0.08	-1.8±0.59	-1.72±0.14	-1.27±0.15	-1.59±0.14	-1.52±0.27	-1.48±0.14
Compensation point	30.33±2.67	27.33±1.76	41.67±15.68	35±2.52	30.33±0.88	27±4.58	31.67±6.01	27.67±2.85

Table S2: (continued)

Species	Ocimum				Lactuca			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	21.16±1.38	24.86±1.25	25.62±1.2	18.48±0.45	-	-	-	-
Dry weight leaves	1.25±0.14	1.77±0.15	1.63±0.19	1.27±0.14	8.79±0.54	6.62±0.46	13.08±0.75	4.89±0.29
Dry weight shoot	0.38±0.04	0.54±0.04	0.54±0.06	0.27±0.03	-	-	-	-
Dry weight roots	0.37±0.05	1.62±0.15	1.12±0.26	0.91±0.21	3.15±0.31	6.86±0.56	5.98±0.9	5.28±1.04
Total dry weight	2±0.22	3.92±0.2	3.29±0.35	2.45±0.33	11.94±0.82	13.48±0.78	19.06±1.48	10.17±1.15
Root to Shoot ratio	0.23±0.02	0.73±0.08	0.53±0.11	0.58±0.11	0.35±0.02	1.06±0.1	0.45±0.06	1.08±0.19
SLA	21.37±1.19	13.62±0.54	43.17±2	18.09±0.64	25.14±1.77	27.19±1.29	38.21±2.58	43.91±3.09
Chlorophyll								
Chlorophyll a (mg g-1)	2.19±0.29	1.97±0.24	4.07±0.42	3.92±0.39	2.51±0.53	1.59±0.14	3.29±0.05	4.64±0.37
Chlorophyll b (mg g-1)	0.54±0.08	0.37±0.05	0.84±0.11	0.84±0.12	0.57±0.12	0.28±0.03	0.68±0.03	0.91±0.07
Chl a: b ratio	0.55±0.08	0.64±0.07	1.25±0.15	1.18±0.08	0.68±0.14	0.72±0.06	1.08±0.05	1.57±0.12
Carotenoids (mg g-1)	4.08±0.05	5.38±0.26	4.9±0.15	4.75±0.16	4.38±0.33	5.76±0.32	4.92±0.2	5.11±0.13
Fv/Fm	0.77±0.02	0.82±0.01	0.85±0	0.82±0.01	0.85±0.01	0.84±0.01	0.86±0	0.85±0.01
Photosynthesis								
Max photosynthesis**	6.73±0.3	16.46±1.06	8.45±2.4	19.19±0.42	5.77±0.65	13.5±2.71	3.64±1.19	16±1.38
Initial slope	0.037±0.001	0.057±0.005	0.046±0.006	0.057±0.001	0.038±0.002	0.051±0.003	0.032±0.009	0.061±0.001
Dark respiration	-2.8±0.21	-2.18±0.07	-2.17±0.49	-1.97±0.02	-1.85±0.17	-1.88±0.35	-1.65±0.61	-2.15±0.35
Compensation point	76.33±3.38	37.33±3.33	47.67±18.19	34.33±0.33	38.33±6.12	35.67±7.31	36±7.02	34±5.77

Table S2: (continued)

Species	Melissa				Raphanus			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology								
Height*	25.91±0.81	17.27±0.64	23.21±1.91	16.8±1.5	5.94±0.2	4.36±0.14	6.64±0.27	6.97±0.46
Dry weight leaves	3.62±0.66	4.28±0.3	1.73±0.46	0.87±0.14	2.25±0.18	1.27±0.14	3.42±0.25	1.12±0.06
Dry weight shoot	-	-	-	-	0.53±0.05	0.22±0.02	0.67±0.03	0.24±0.03
Dry weight roots	2.1±0.39	3.01±0.21	1.24±0.33	0.55±0.1	4.7±0.53	4.54±0.44	5.94±0.31	2.66±0.25
Total dry weight	5.72±1.04	7.29±0.48	2.97±0.78	1.42±0.22	7.47±0.68	6.04±0.58	10.03±0.57	4.02±0.32
Root to Shoot ratio	0.58±0.02	0.71±0.04	0.74±0.04	0.63±0.07	1.69±0.13	3.07±0.16	1.47±0.05	1.95±0.13
SLA	40.16±2.58	20.59±0.67	46.87±3.39	53.3±9.11	23±0.92	24.95±0.41	30.99±1.74	31.21±2.05
Chlorophyll								
Chlorophyll a (mg g-1)	8.04±1.54	3.23±0.22	4.49±0.68	8.2±0.88	2.72±0.8	3.3±0.26	2.83±0.35	7.81±0.61
Chlorophyll b (mg g-1)	1.82±0.3	0.72±0.06	1.09±0.16	2.02±0.24	0.67±0.16	0.78±0.07	0.82±0.1	2.09±0.31
Chl a: b ratio	1.94±0.39	1.08±0.08	1.53±0.25	2.56±0.23	0.63±0.18	1.04±0.02	0.97±0.1	2.22±0.19
Carotenoids (mg g-1)	4.35±0.18	4.49±0.15	4.11±0.16	4.08±0.07	3.92±0.24	4.26±0.11	3.48±0.02	3.84±0.24
Fv/Fm	0.81±0.01	0.81±0	0.83±0.01	0.8±0.01	0.83±0.01	0.8±0	0.85±0	0.85±0.01
Photosynthesis								
Max photosynthesis**	11.08±0.33	13.45±1.12	9.1±2.3	14.93±0.38	13.77±1.97	15.53±1.61	7.17±1.73	19.7±2.01
Initial slope	0.051±0.004	0.057±0.003	0.051±0.007	0.063±0.001	0.046±0.003	0.069±0.001	0.054±0.009	0.069±0.002
Dark respiration	-1.63±0.09	-1.67±0.15	-1.68±0.3	-1.51±0.05	-1.05±0.01	-1.76±0.23	-1.35±0.15	-1.95±0.28
Compensation point	24.33±1.2	26.33±3.53	32±9.71	22.33±0.88	22.33±1.33	24±3.06	20.33±0.67	27.67±3.84

Table S2: (continued)

Species	Triticum			
Trial\Treatment	Outdoor	Fix	Sinusoidal	Variable
Biomass and Morphology				
Height*	53.96±1.21	28.68±0.57	54.08±1.21	43.71±1.04
Dry weight leaves	12.29±0.97	6.73±0.44	18.85±0.64	8.11±0.39
Dry weight shoot	-	-	-	-
Dry weight roots	21.6±2.49	47.88±4.56	54.92±3.56	48.41±5.81
Total dry weight	33.89±2.52	54.6±4.77	73.77±4.02	56.52±5.96
Root to Shoot ratio	1.86±0.29	7.24±0.74	2.91±0.15	5.99±0.62
SLA	30.38±0.63	16.91±0.62	33.34±4.18	27.83±1.61
Chlorophyll				
Chlorophyll a (mg g-1)	7.12±0.44	3.04±0.26	7.29±0.47	6.51±0.51
Chlorophyll b (mg g-1)	1.89±0.07	0.67±0.06	1.8±0.12	1.64±0.13
Chl a: b ratio	1.45±0.05	1.23±0.14	2.15±0.22	1.93±0.13
Carotenoids (mg g-1)	3.75±0.12	4.53±0.06	4.04±0.04	3.97±0.04
Fv/Fm	0.83±0	0.81±0.02	0.84±0	0.83±0
Photosynthesis				
Max photosynthesis**	9.12±0.47	18.19±5.14	11.26±3.14	14.02±1.48
Initial slope	0.044±0	0.081±0.013	0.068±0.004	0.098±0.012
Dark respiration	-1.9±0.05	-2.12±0.5	-2.72±0.41	-3.44±1.24
Compensation point	35±0	23±3.51	37.67±6.17	26.33±4.63

Chapter 4
Reaching natural growth: Effect of asynchronous light and temperature fluctuations on plant traits in indoor growth facilities

Abstract: Several studies have recommended the incorporation of environmental fluctuations in indoor experiments if closer-to-natural results in plant experiments are desired (Poorter *et al.,* 2016; Matsubara, 2018). Annunziata *et al.,* 2018, suggest that if these fluctuations are not applied in synchrony, a stress effect could be present since plants have evolved to cope with synchronic environmental fluctuations. Following a series of experiments for plant growth under indoor conditions (Chapter 2 and 3), the present study aims to identify the effect of disparity in fluctuations of two important environmental variables, light quantity and temperature, on the growth of 7 plant species from different functional plant types. A full-factorial combination of light and temperature under fixed or variable conditions was applied in phytotrons and plant performance under these conditions was compared with a previous field trial. In all phytotron treatments, the average light and temperature conditions were the same as in the initial field trial. Productivity-, gas exchange- and leaf pigment-traits were recorded in all species at the end of the experiments. Most plant trait responses were highly dependent on species and treatment, but some general trends were observed. Light fluctuations were mainly responsible for increases in specific leaf area (SLA) and chlorophyll a concentration, as well as for reductions in total dry weigh and chlorophyll a:b ratio. When fixed light conditions were combined with variable temperatures, the plants showed on average lower F_v/F_m values, Amax and CO_2 yield, while under variable light conditions and fix temperatures, F_v/F_m increased compared with fully fixed or variable conditions. Although significant differences of plant traits between the field trial and all phytotron treatments were present (likely due to differences in other parameters that were not controlled in the phytotrons), our results still suggest that a synchronous variation of environmental factors lead to a more natural-like plant growth than if these factors are fixed or vary asynchronous.

Key words: Dynamic light, dynamic temperature, natural growth, controlled environment.

1. Introduction

Due to the sessility of plants, our main source of nutrition, they are constantly exposed to changes in environmental conditions, and in a plant species' evolution, it needs to adapt to the site-specific variation in climate. In agronomic systems, strong deviations from the optimum climate for a crop can negatively affect yields, and since the beginning of farming, humans have selected for crops that can cope with climatic variations and adjusted farming practices to avoid extreme climatic conditions decupling from the environment. By employing glasshouses and indoor growth facilities, plants can be grown under semi-controlled and controlled conditions, thereby increasing crop yield. Besides agriculture, indoor growth facilities are also used in plant sciences to grow target plants independently of the outside climate at constant conditions. However, this absence of climatic variability can induce unnatural plant growth, and in some cases, these simplified scenarios have led to errors in our predictions of plant-climate relations under natural conditions (*e.g.* Junker *et al.,* 2015; Hohmann *et al.,* 2016). Over the last years some authors propose several factors that are mainly responsible for unnatural plant growth in indoor growth facilities, including light quantity, plant density, plant age and the absence of climatic fluctuations (Poorter *et al.,* 2016)

Already Barta *et al.* (1992) suggested that with a high level of control, it would be possible also in indoor growth facilities to mimic natural changes in environmental conditions within minutes, especially when using the new LED lighting technology. However, it took almost 30 years to see these light applications in practice, even though, *i.e.* positives effects of more natural temperature fluctuations have been described before (e.g. Myster *et al.,* 1995). In the last couple of years, several authors suggest that higher levels of light and temperature variation, within a non-stressful range for plants, could have a positive effect on plant development and lead to more natural like plant growth and development (Annunziata *et al.,* 2018; Kaiser *et al.,* 2020). With the advancement of technology, availability and associated cost reduction in equipment (*e.g.* Mitchell and Sheibani, 2020), the possibility to apply more natural-like climatic fluctuations in indoor facilities has become reality and has been recommended by the scientific community (Poorter *et al.,* 2016; Matsubara *et al.,* 2018), but it is still not commonly applied in most plant biological research institutions. Annunziata *et al.* (2017 and 2018) demonstrate that the application of fluctuating temperature and light have an important role in *Arabidopsis*, by revealing plant characteristics that are not expressed under constant conditions, while other factors like light quality may play a secondary role within reasonable limits. In their experiments, Annunziata *et al.,* (2017 and 2018) showed that fluctuations in temperature play a more important role at metabolic level compared with light fluctuations.

Vialet-Chabrand *et al.,* (2017) confirmed that the application of fluctuating light is possible in indoor experiments without adding a higher complexity to their experiments. Kaiser *et al.,* (2020) demonstrate the importance of applying fluctuation climate conditions in genotype selection, where the more sensible accessions of *Arabidopsis* to light fluctuations were the fast-growing accessions under constant light, remarking the importance of applying fluctuating light.

With the higher prevalence of comprehensive climate control in indoor facilities, an increasing amount of strategies that control the environmental variables using algorithms have appeared over the last years. Hemming *et al.* (2019) review an algorithm competition to maximize cucumber yield production in greenhouses, where environmental conditions were managed with different strategies, remarking why it is important, now more than before, to understand and quantify the effect of how plants are affected under different environmental dynamics.

In the previous chapter (Chapter 3), how the environmental dynamics can be controlled to manage plant morphology in several plant species when all climatic parameters are fluctuating in synchrony was investigated. This chapter aim to identify how two different environmental variables (temperature/humidity and light) can affect plant growth, morphology and photosynthesis depending on their dynamics and synchrony in indoor conditions, compared with an outdoor control treatment. The main hypothesise is that under fully fluctuating conditions a lower biomass would be reached due to the stress of changing environmental conditions (*e.g.* Kaiser *et al.,* 2020), together with a more efficient photosynthesis due momentary higher levels of light, where light dynamics play a secondary role compared with temperature dynamics.

2. Material and methods

Plant materials and pre-growing conditions

Similar to chapter 2 and 3 of this thesis, 7 plant species under different climatic treatments in LED-lit walk-in phytotrons were studied. Trees were represented by black alder (*Alnus glutinosa* L., provenance HG4, Zurich, Switzerland) and Scotch elm (*Ulmus glabra* HUDS., provenance Merenschwand, Aargau, Switzerland), herbs were represented by basil (*Ocimum basilicum* L. var Adriana), lettuce (*Lactuca sativa* L.), melissa (*Melissa officinalis* L.) and radish (*Raphanus raphanistrum* L. subsp. sativus), and grasses were represented by winter wheat (*Triticum aestivum* L.). In the following, the species will be referred to by their scientific genus name for clearness. All plants were germinated for 15-42 days at 190 to 240 μmols m^{-2} s^{-1} of photosynthetic photon flux density (PPFD: 400-700 nm) depending on the species and

time of germination. More details on the germination conditions can be found in chapter 2 and 3 of this thesis. During the germination period, the different seedlings were exposed to 25 ºC / 50 % relative humidity during daytime and 15 ºC / 83 % relative humidity during night, with 10 hours day and one-hour light/temperature/humidity ramping pre and post day.

At the beginning of the experiment, all plants were transplanted to round 2 L pots (13.5 cm diameter, Poppelmann, Lohne, Germany) with a single individual in each pot. An exception was *Triticum* that was thinned to 10 plants per pot. The pots were filled with the same substrate as used in the germination trays (pH 5.8, N 250 mg L^{-1}, 180 P_2O_5 mg L^{-1}, K_2O 480 mg L^{-1}, Ökohum, Herrenhof, Switzerland), and 4 g of Osmocote slow release fertilizer (Osmocote exact standard 3-4, Scotts, Marysville, Ohio, USA), containing 16% total N, 9% P_2O_5, 12% K_2O and 2.5% MgO, was added to each plot. All plants were watered daily in the morning throughout the experiment.

Natural field conditions and environmental treatments in the phytotrons

The same reference for natural plant growth as described in chapter 2 and 3 was used, were all seven species were grown in a field trial for 35 days (4 August 2017 - 7 September 2017) at the botanical garden of the University of Basel, Switzerland (Supplementary Fig. S1). Along the field trial, the *in situ* climate and the natural sunlight spectrum was recorded (more details about environmental variables records can be found at Chiang *et al.,* 2019 and chapters 2 and 3). *A Posteriori* the same set of plant species were grown under 4 different air temperature x light treatments as combinations of two levels of complexity: fixed and variable conditions. Air humidity was also modified together with air temperature, giving similar vapour-pressure deficits (VPD) across the applied temperature ranges. The fixed condition treatments correspond to the average values of temperature and light of the 35 days field trial, applied continuously over the average photoperiod, meanwhile the variable conditions follow the recorded values from the field conditions (Supplementary figure 1).

The experiment was performed in four walk-in Phytotrons (1.5 m x 2.5 m) with movable roofs (prototypes, Enersign GmbH, Basel, Switzerland). In each treatment, the environmental conditions resulted in the same average values as in the respective field trial across the 35 days growth period. The airflow in the chambers was applied evenly from below, ensuring a uniform temperature and humidity distribution within the chambers.

The used light spectra (provided by 18 B, G, R, FR-LED panels per chamber, see chapter 2 and 3 for details) in the phytotrons (supplementary figure 2) corresponded to a spectral composition that promotes natural plant growth as derived from chapter 2 of this thesis. The

light intensity was regulated through changes in electric current and roof height keeping similar light spectra across most light intensities. For moments where very high light intensities in the variable light treatments was not possible, higher amounts of B and R light were applied to reach the target intensity, keeping the same B:R ratio. The R:FR ratio was kept at 1.8 for all treatments, and no additional UV light was applied.

Measured parameters.

The same set of plant traits as given in chapter 2 and 3 were measured by the end of the 35 days growth period, corresponding to morphological parameters (plant height; specific leaf area, SLA; Dry weight, DW; and below to above ground biomass ratio, root:shoot-ratio), leaf fluorescence and pigmentation (photosynthetic maximum quantum yield, F_v/F_m; absolute performance index, PI; Chlorophyll a and b concentrations, Chl a and Chl b; chlorophyll a to b ratio, Chl a:b; and total carotenoid concentrations) and photosynthesis parameters measured under the respective growing light source, either sun light in the field treatment or the spectra in the indoor treatments (maximum photosynthesis, A_{max}; quantum yield of the CO_2 fixation as the slope of the linear curve between 0 and 100 μmol m^{-2} s^{-1} of PPFD, α; dark respiration, DR; and the light compensation point of photosynthesis, CP). For more methodological details see chapter 2 and 3.

Statistical analysis.

For each individual species, 9 pots were used as replicates. To avoid border effects all plants were randomly distributed within each phytotrons on two tables, and the tables were rotated by 90°every day.

To evaluate the effect of the treatments, a two-way analysis of variance (ANOVA) was performed for all measured parameters, considering the light and temperature treatments as fixed factors, with two different levels, and the species of each treatment as random effect., without considering the field treatment (Table 1). All data were checked for normal distribution, independence and homogeneity of the variance.

To enable a direct visible and statistical comparison of the treatment effects across species in relation to the field trial, each measured trait was normalized relative to its mean value on the field trial for each species (the raw trait values per specie and treatments are available in supplementary table 1). The normalized data was used to perform a one-way ANOVA, considering each combination of the different treatment levels as fixed factor and species as random factor (supplementary table 2), where the significance of the random factor

was evaluated using a restricted likelihood ratio test. A Tukey pairwise multiple comparison test was used as post hoc analysis.

All analyses were done using R (version 3.6.2, R Core team) and the packages plyr and reshape2 for data processing, lm4, car, RLRsim and emmeans for data analysis, and multicomp and vegan for statistically significant representations.

3. Results

Morphology and biomass distribution

On average across all species, plant height was significantly lower in all indoor environmental treatments compared with the control treatment (Fig. 1 A). This was specially the case for plants grown under fixed light and fixed temperature conditions, with about 26% lower plant heights across species compared with the outdoor treatment. No interaction between the temperature and light treatment was found for the indoor treatments, were just the light treatments had a significant effect on height (Table 1). Almost all species follow the same trend of lower height under fixed light conditions, independent of the temperature regime. The only exception was *Ocimum* which have taller plants under fixed light conditions; even taller than in the outdoor control treatment.

For the specific leaf area (SLA), similar trends were visible (Fig. 1 B), with higher SLA (thinner leaves) under variable light compared with the control and the fixed light conditions. Interestingly, the temperature dynamics did not have an additional effect across species under fixed or variable light conditions, between the indoor treatments (Table 1). Similar treatment's tendency was visible across all species.

The total dry biomass was affected in an opposite direction to plant height, where on average across species, only the treatment with fixed light and variable temperature had significant higher values than the field control (+28%, Fig. 1 C). All other treatments did not differ from the field control and when this one was not included, an interaction between the light and temperature treatments was found (Table 1). The response was similar across species, except for *Triticum* that showed exceptionally high biomass at variable light and fixed temperatures (Fig. 1C). A comparison of investment into shoots and roots revealed higher R:S ratios compared to the field control plants across all species (Fig. 1 D). The slightly higher R:S values at fixed light - variable temperature and variable light - fixed temperature conditions were largely driven by *Raphanus* and *Triticum* in the former, and *Triticum* in the latter case. This was confirmed by the interaction between the light and temperature treatments between the indoor treatments (Table 1).

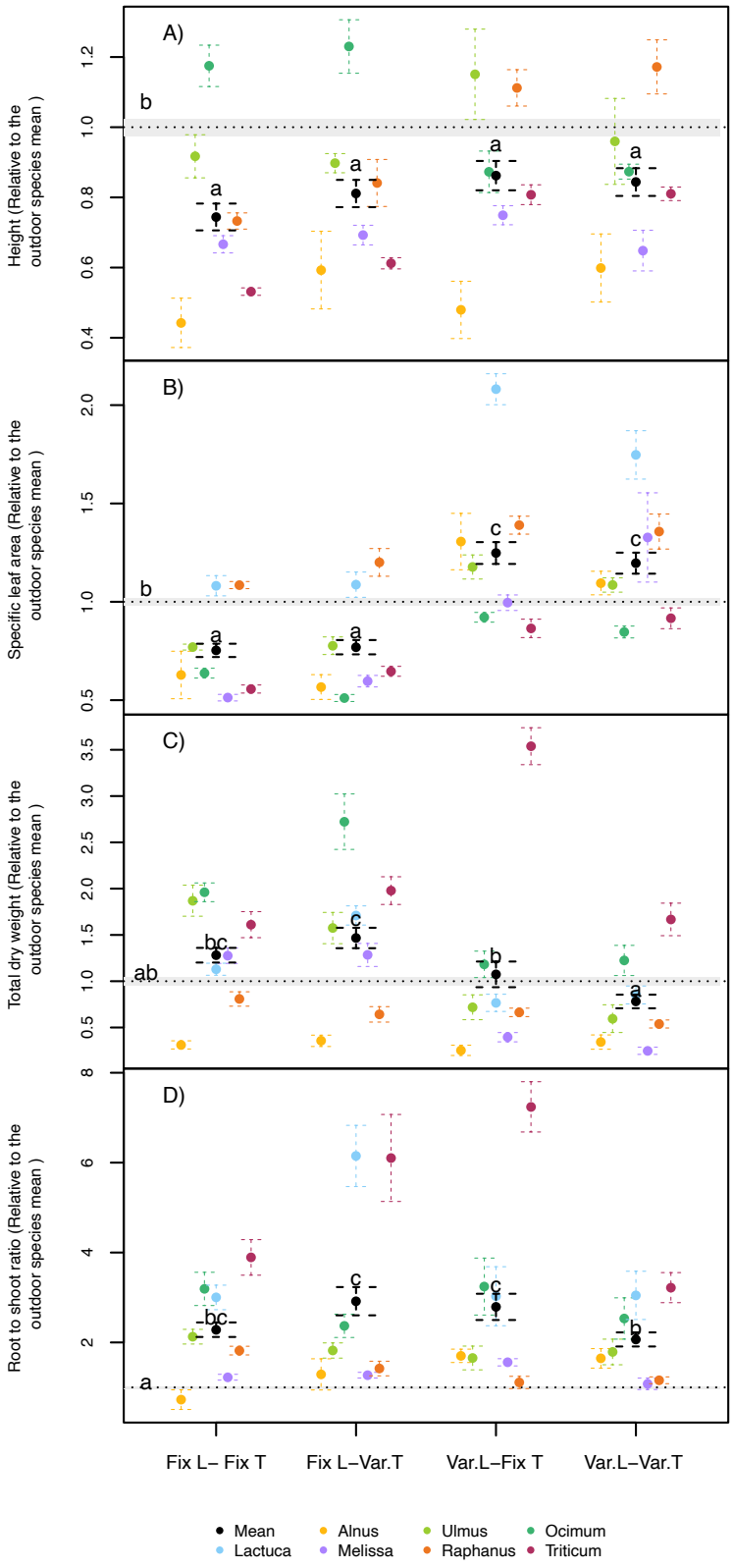

Figure 1: A) Plant height, B) specific leaf area, C) total plant dry weight and D) root to shoot ratio of 7 different species normalized to an outdoor control treatment under four combinations (fixed or variable) of two environmental factors (light and air temperature). Error bars correspond to the standard error. N=9 for each species, where N for the average = 63. Letters indicate significant differences (P<0.05, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect.

Table 1: P-values of the two ways ANOVA excluding the field trial and considering different treatments as a fix factor and species as random effect. Bolts numbers correspond to significant values (α = 0.05)

	Variable	Temperature	Light	Temperature x Light
Biomass and Morphology	Height*	-	**0.004**	-
	SLA	-	**< 2.2e-16**	-
	Dry weight shoot**	-	**3.84E-08**	-
	Dry weight roots	0.188	**7.96E-05**	**4.46E-05**
	Total dry weight	0.196	**0.001**	**6.21E-05**
	Root to shoot ratio	**0.034**	**0.010**	**4.19E-05**
Chloropyll	Chlorophyll A (mg g-1)	0.347	**< 2.2e-16**	0.06
	Chlorophyll b (mg g-1)	-	**< 2.2e-16**	-
	Chl A:B ratio	-	**< 2.2e-16**	-
	Carotenoids(mg g-1)	-	**< 2.2e-16**	-
	FvFm	9.08E-10	**0.0003**	**0.005**
	Pi	**0.001**	**5.71E-08**	-
Light parameters	Max photosynthesis	**0.041**	0.808	0.082
	Compensation point	**0.001**	0.946	**0.025**
	Quantum yield for CO2 fixation	**0.002**	0.995	**0.007**
	Dark respiration	-	-	-

* Lettuce was not included in this analysis

** Lettuce, Melissa and Triticum were not included in this analysis.

-: The variable was removed from the analysis due non statistically significant

Chlorophyll and photosynthetic parameters.

Exceptionally strong reactions were found in the leaf chlorophyll content among treatments. Averaged across all species and independent of temperature dynamics, the variable light treatments reached 45 % higher Chl a leaf concentrations compared to the field control plants, while both treatments with fixed light led to significantly reduced Chl a concentrations (-37 %) than in the field (Fig. 2A) The ANOVA revealed a significant treatment interaction (Table 1), where all species reacted similar to the different treatments.

While the treatments had strong effects on Chl a and a similar but smaller effect on the leaf Chl b concentrations (data not shown), the Chl a:b ratio showed the opposite trend in response to the phytotron treatments than Chl a (Fig. 2B). Independent of the temperature dynamics, the fixed light treatments produced plants with significantly higher Chl a:b ratios (+ 15 % on average) than both variable light treatments and the field trial. Interestingly similar Chl a: b levels were reached between the field control and the variable light treatments in the

phytotrons (Fig. 2B), where only the light treatments had a significant effect between the indoor treatments.

F_v/F_m values were generally similar across the different treatments and species with a maximum variation of *circa* 10%, indicating no extensive stress in response to all treatment. However, when fixed light was combined variable temperatures, F_v/F_m values decreased significantly across all species compared to the field trial (-8 %, Fig. 2C), with significantly stronger declines in both tree species (*Alnus* and *Ulmus*). An interactive effect between the light and temperature treatments was found for the indoor treatments, which was not present on Pi.

Between the indoor treatments, many of the light parameters present an interactive effect between the temperature and light treatments (Table 1). In contrast to the other investigated plant traits, the average values for A_{max} were not significantly different in among all indoor treatments when the field trial was included (Fig. 3 A), but all phytotron treatments had significant higher values than the field control plants (+ 64%). Although all species showed similar trends, there were large differences in the size of the A_{max} reactions between species. Up to 2.5 times higher values that in the field control were reached by phytotron-grown *Ocimum* and *Lactuca*, while other species, like *Alnus* and *Melissa*, deviated significantly less from the outdoor treatment (Fig. 3A). Interestingly, there was a tendency for lower A_{max} values under fixed light and variable temperature conditions, corresponding to the lower F_v/F_m values in this treatment (Fig. 2C). The light compensation point of photosynthesis was not significantly different from the field control in any phytotron treatment, but it was significantly higher for the fixed light - variable temperature compared to both treatments with variable light conditions (Fig. 3 B). This was specially the case for *Melissa* and *Raphanus*. Finally, when analysed across all species, the CO_2 yield of all indoor treatments was in average significantly higher compared with the field control plants (Fig. 3 C), with the fixed light - variable temperature treatment showing significantly lower values than the other phytotron treatments. Almost every species followed this trend, where only *Triticum* under fixed light and variable temperature did not seems to be affected as strong as the other species.

4. Discussion

Several studies demonstrated the effects of light and temperature fluctuations on plants, where a big amount of specific trait characteristics was attributed to daily differences between day and night temperatures (DIF). *E.g.* Myster *et al.,* (1995) reviewed the topic, where positive DIF, as continuous difference between the day and night temperature or as temperature drops over the night, can stimulate cell elongation due to changes in the gibberellin (GA) concentrations that

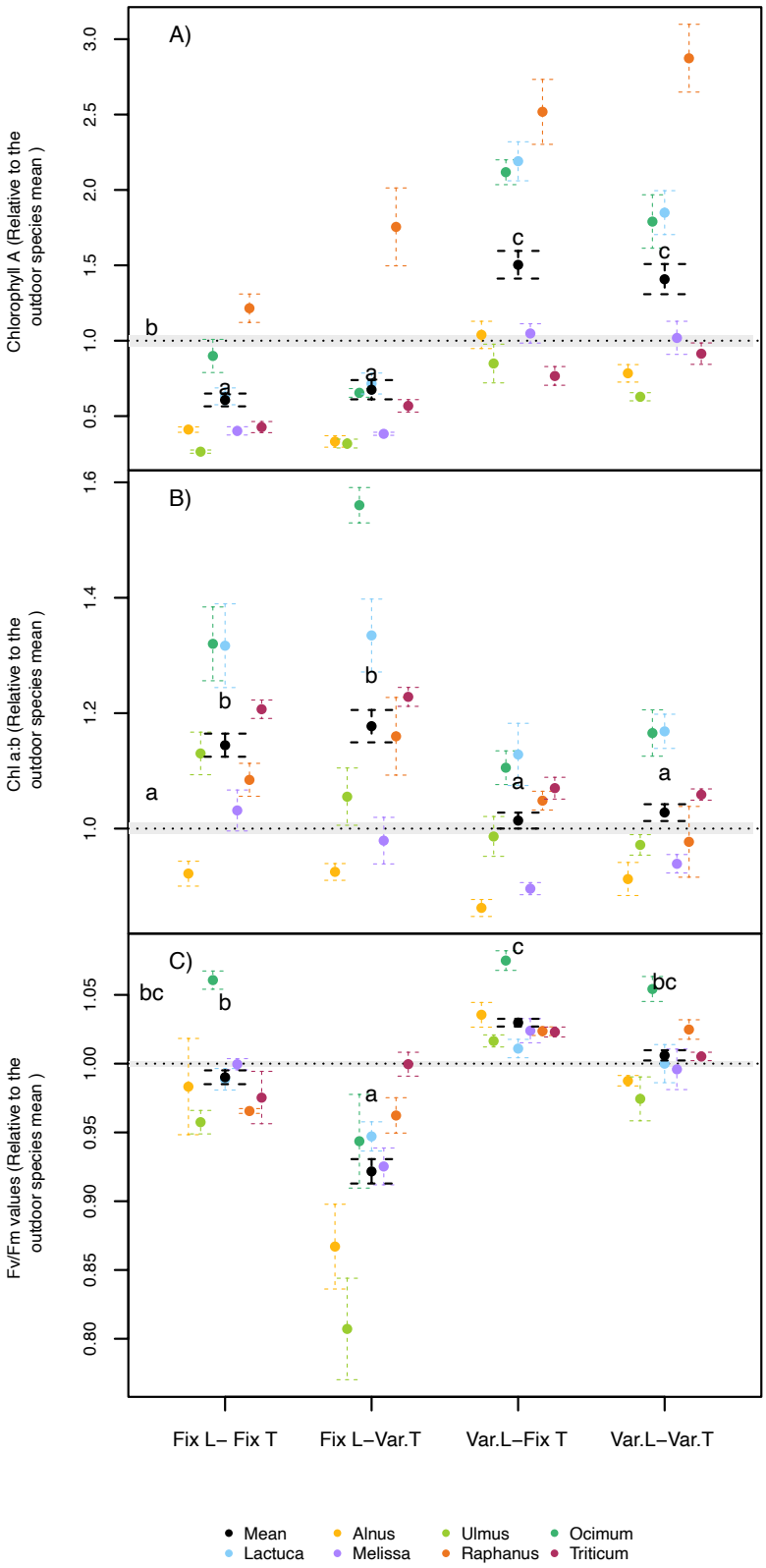

Figure 2:A) Chlorophyll a concentration, B) chlorophyll a to b ratio and C) F_vF_m values of 7 different species normalized to an outdoor control treatment under four combinations (fixed or variable) of two environmental factors (light and air temperature). Error bars correspond to the standard error. N=3 for each species, where N for the average = 21. Letters indicate significant differences ($P<0.05$, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect.

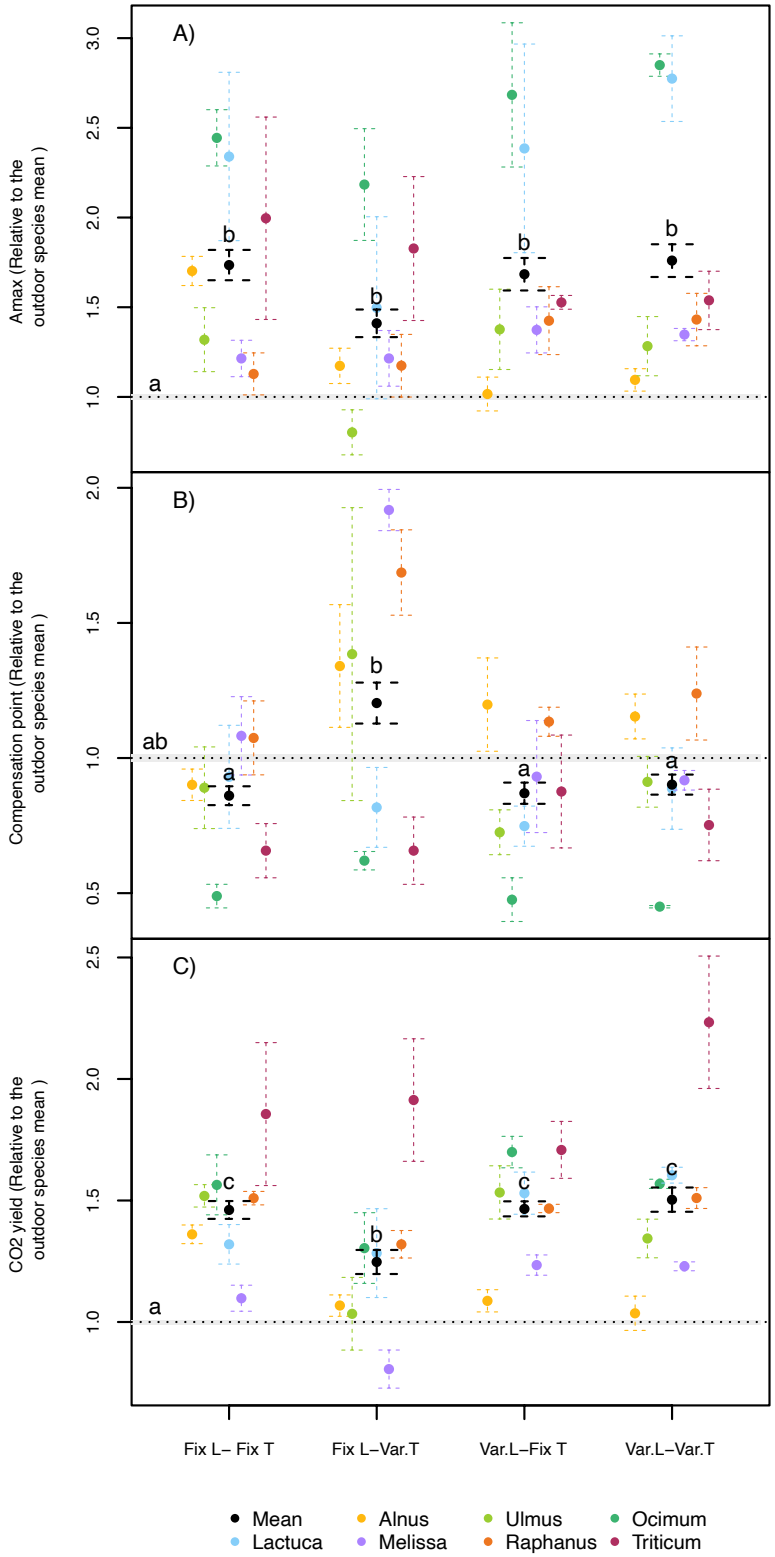

Figure 3:A) Maximum photosynthesis (A_{max}), B) light compensation point of photosynthesis (CP) and C) CO_2 yield of 7 different species normalized to an outdoor control treatment under four combinations (fixed or variable) of two environmental factors (light and air temperature). A_{max} and CP were measured under a standard light source (70%R and 30%B). Error bars correspond to the standard error. N=3 for each species, where N for the average = 21. Letters indicate significant differences ($P<0.05$, by Tukey post-hoc tests) among treatments (incl. the outdoor trials) using species as random effect.

promotes shoot elongation, leaf orientation, chlorophyll content and lateral branching among others. Yuan (2016) demonstrated in tomato that not only these factors can be affected, but also the net photosynthesis rate, stomatal conductance, F_v/F_m, quantum yield of PSII chemistry (Φ_{PSII}) and photochemical quenching (q_p) increased under positive DIF, meanwhile Chl a: b ratio and non-photochemical quenching (NPQ) were reduced. However, despite these significant influences of diurnal temperature changes on plant development, the majority of greenhouse experiments are currently still keeping temperature relative constant with small variations between day and night, either for practical reasons or technical limitations.

In contrast to temperature, the possible influence of light fluctuations during the day has gained stronger attention only over the last years (*e.g.* Annunziata *et al.,* 2017; Vialet-Chabrand *et al.,* 2017; Kaiser *et al.,* 2018; Kaiser *et al.,* 2020). Literature agree that across species light fluctuations are generally considered as a source of stress at either leaf or the whole plant level, mainly due to the asynchronies between the different processes during photosynthesis (*e.g*. light reaction, rubisco activity, stomatal conductance), where these effects have been previously quantified in several levels, *e.g.* also at metabolic level (Annunziata *et al.,* 2017). Although light fluctuations are basically a stress, fluctuating light within physiological unproblematic ranges can also strengthen a plant, make it more resistant and lead to more natural plant growth. Today, recommendations for more natural climatic conditions in indoor experiments that incorporate these fluctuations are thus getting more frequent (Poorter et al., 2016; Matsubara, 2018; Kaiser et al., 2020).

Interestingly, Annunziata *et al.* (2018), incorporated temperatures fluctuations, in a controlled greenhouse environment, and concluded that no further effects were found at metabolic nor genetic level due the inclusion of temperature fluctuations. This is in line with the present study, conducted under a temperature range that was not growth-limiting (Supplementary Fig. 1) light fluctuation dynamics had a bigger effect than temperature dynamics on almost all measured traits. Also, the results suggest that more natural values can be reached when both variables are applied in synchrony. The lack of an additional effect of the temperature's dynamic in several of the measured parameter is controversial due the previously mentioned effects, but could be explained due the interaction between irradiance and DIF, previously reported by Myster *et al.,* 1995)

A clear effect of light fluctuations on SLA was reported by Vialet-Chabrand *et al.,*(2017), where light fluctuations produced thinner leaves compared with a square treatment (*circa* 25% thinner leaves in average, independent of the level of light), and in the same study also a decrease of the Chl a: b ratio was found with fluctuating light (*circa* -12% higher under

variable light). Interestingly, Yuan (2016) reported 23% higher Chl a and a 9% reduction of Chl a:b under +16 DIF compared with 0 DIF. Our results, together with these previous studies, suggest that the effect of temperature and light variation is not additive, and interactions must exist at leaf level. This is most obvious at the photosynthetic level, where although higher net photosynthesis and photosynthetic efficiencies associated to lower Q_p and NPQ have been found under positive DIF (Yuan, 2016), this was not the case in the current study where less efficient and lower photosynthetic levels were recorded under the combination of variable temperature and fixed light. This is in line with the results previously summarized by Kaiser *et al.,* (2018), where to avoid the formation of active oxygen species trade-offs need to be done. Thus the the lifetime of exited state of chlorophyll a is reduced what reduce the potential of the electron transport, therefore the levels of photosynthesis. Annunziata *et al.,* (2018) suggested that an asynchronous application of light and temperature variations has a negative effect on plants, which in our case was especially visible under the fixed light - variable temperature combination. Under this condition, especially A_{max} and F_v/F_m decreased, what might indicate a slight photo-stress when light fluctuates to high values at constantly lower temperatures., while there was no negative effect on total biomass production was observed. However, if biomass allocation was considered, it revealed an increase in the R:S ration under fixed light - variable temperatures. The higher investment in root growth might be a signal of higher stress levels at this specific condition.

5. Conclusion

In the previous study (Chapter 3), it was demonstrated how environmental fluctuations can help to reach more natural growth in indoor experiments, while the present study is empathized the significance of applying environmental fluctuations also in synchrony. This experiment demonstrated that not only processes at the molecular level can get affected (*e.g.* Annunziata *et al.,* 2018), but that this effect also translates to morphological traits at the level of the whole plant. Within a non-growth-limiting range of light and temperature/humidity conditions, as applied in the current study, light dynamics had a bigger effect on several of the measured parameters compared with temperature dynamics, and indications of higher plant stress were observed when fixed light conditions were applied together with variable temperatures. The results suggest that it can be beneficial to accompany changes in temperature within the photoperiod with suitable and synchronous changes in light intensity.

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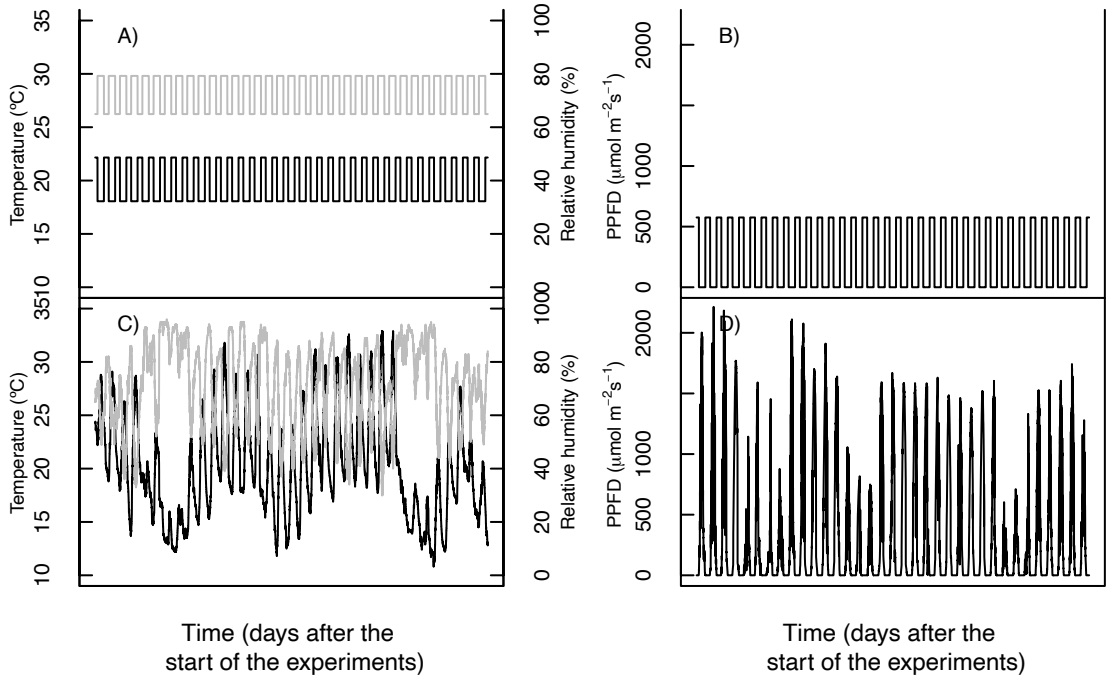
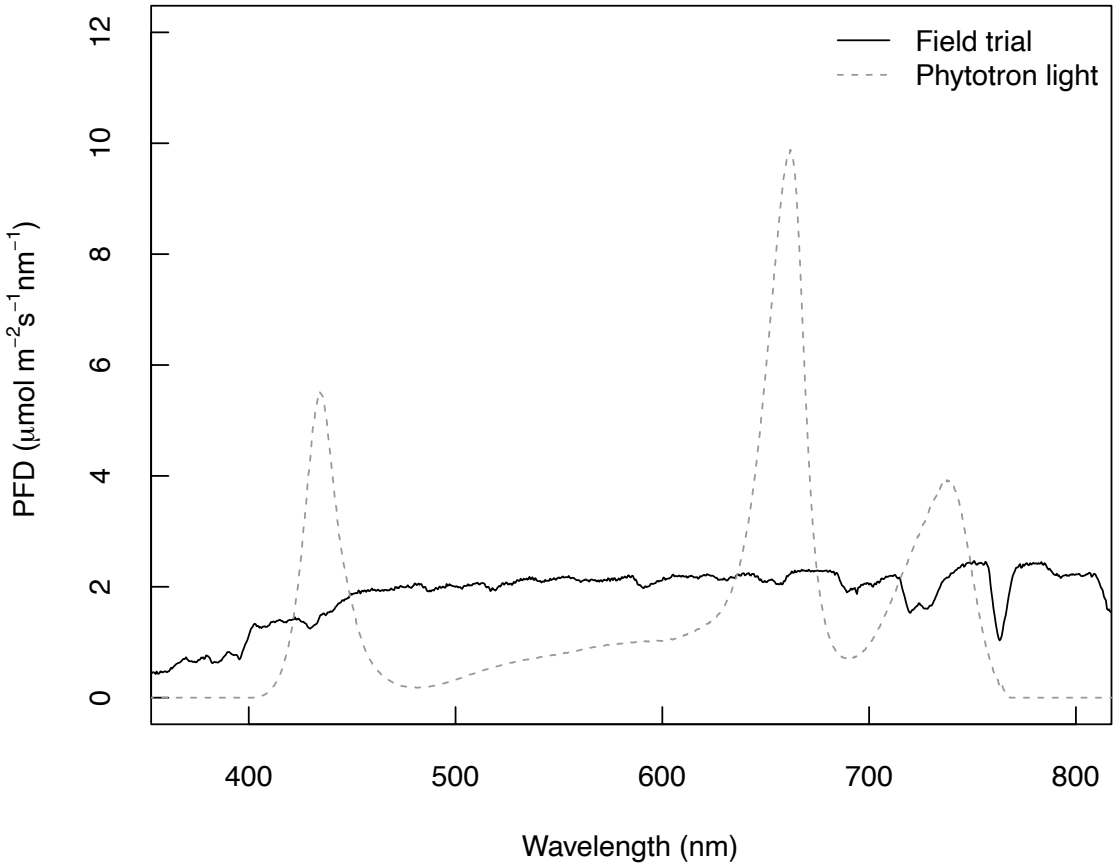
Supplementary materials:



Supplementary figure 1: Applied temperature and light conditions for the phytotron treatments. The upper panels correspond to the applied fixed temperature/humidity (A, temperature = black line, relative air humidity = grey line) and photosynthetic active photon flux density (B). The lower panels correspond to the applied variable temperature/humidity (C, temperature = black line, relative air humidity = grey line) and light (D), corresponding to the climatic records during the 35 days of the natural field trial and applied under the variable conditions.



Supplementary figure 2: Spectrum examples of the applied light. The field trial example corresponds to a sample of the sun spectra (28% Blue light, 36% Green, 36% Red and R:FR 1.1 in average), meanwhile the phytotron light quality corresponds to the used spectra in the phytotrons (25%B, 16%G, 59%R and R:FR 1.8). The integrated area between 400 and 700 nm corresponds to an approximately 575 µmol $m^{-2}s^{-1}$ of photosynthetic photon flux density in each case

Supplementary table 1: Absolute values of all measured traits for each species and treatment. Values are means ± s.d., N=3 to 9 (see methods for details).

Species	Alnus					Lactuca				
Trial\Treatment	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T
Biomass and Morphology										
Height	22.32±3.6	9.88±4.71	13.23±7.41	10.7±5.46	13.37±6.5	-	-	-	-	-
SLA	34.76±3.73	21.83±12.58	19.7±6.56	45.41±14.95	38.07±6.25	25.14±5.32	27.19±3.88	27.32±4.86	52.3±5.94	43.91±9.28
Dry weight shoot	0.51±0.15	0.17±0.12	0.15±0.13	0.09±0.05	0.16±0.13	-	-	-	-	-
Dry weight roots	0.52±0.15	0.11±0.11	0.2±0.14	0.2±0.14	0.24±0.14	3.15±0.93	6.86±1.69	13.72±3.32	4.66±2.85	5.28±3.13
Total dry weight	2.09±0.57	0.65±0.28	0.75±0.38	0.53±0.35	0.72±0.48	11.94±2.47	13.48±2.33	20.42±3.71	9.15±3.33	10.17±3.46
Root to Shoot ratio	0.34±0.08	0.25±0.23	0.44±0.36	0.59±0.15	0.57±0.22	0.35±0.06	1.06±0.29	2.18±0.72	1.07±0.7	1.08±0.57
Chlorophyll										
Chlorophyll a (mg g-1)	7.35±1.93	3.02±0.26	2.44±0.56	7.63±1.33	5.76±0.85	2.51±1.06	1.59±0.28	1.8±0.35	5.5±0.65	4.65±0.73
Chlorophyll b (mg g-1)	1.45±0.5	0.64±0.09	0.52±0.12	1.74±0.34	1.24±0.26	0.57±0.23	0.28±0.06	0.31±0.06	1.12±0.2	0.91±0.15
Chl a: b ratio	5.13±0.45	4.72±0.22	4.74±0.15	4.42±0.15	4.68±0.29	4.38±0.66	5.76±0.63	5.84±0.55	4.94±0.47	5.11±0.26
Carotenoids (mg g-1)	1.94±0.36	1.89±0.2	1.31±0.43	2.9±0.52	2.09±0.3	0.68±0.27	0.72±0.12	0.96±0.19	1.74±0.14	1.57±0.24
Fv/Fm	0.78±0.01	0.77±0.05	0.68±0.04	0.81±0.01	0.77±0.01	0.85±0.01	0.84±0.01	0.81±0.02	0.86±0.01	0.85±0.02
Pi	1.16±0.47	1.13±1.39	0.18±0.14	1.66±0.44	0.71±0.09	3.5±1.21	3.29±1.26	1.7±0.67	3.6±0.95	3.23±1.58
Photosynthesis										
Max photosynthesis**	8.37±0.5	14.25±1.18	9.82±1.43	8.51±1.37	9.17±0.91	5.77±1.13	13.5±4.69	8.63±5.07	13.76±5.81	16±2.38
Initial slope	30.33±4.62	27.33±3.06	40.67±11.93	36.33±9.07	35±4.36	38.33±10.6	35.67±12.66	31.33±9.81	28.67±4.93	34±10
Dark respiration	0.04±0	0.06±0	0.05±0	0.05±0	0.05±0.01	0.04±0	0.05±0.01	0.05±0.01	0.06±0.01	0.06±0
Compensation point	-1.74±0.12	-1.77±0.14	-2.01±0.54	-1.91±0.53	-1.72±0.24	-1.85±0.29	-1.88±0.61	-2.01±0.56	-1.92±0.17	-2.15±0.61

Supplementary table 1: (continued)

Species	Melissa					Ocimum				
Trial\Treatment	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T
Biomass and Morphology										
Height	25.91±2.44	17.27±1.91	17.94±2.17	19.41±2.11	16.8±4.5	21.16±4.13	24.86±3.74	26.02±4.84	18.47±3.75	18.48±1.35
SLA	40.16±7.73	20.59±2.01	23.96±3.51	39.98±4.76	53.31±27.34	21.37±3.56	13.62±1.61	10.92±1.14	19.68±1.58	18.09±1.93
Dry weight shoot	-	-	-	-	-	0.38±0.13	0.54±0.11	0.78±0.29	0.3±0.12	0.27±0.08
Dry weight roots	2.1±1.16	3.01±0.64	3.07±0.82	1.07±0.41	0.55±0.29	0.37±0.16	1.62±0.45	1.86±0.66	1±0.58	0.91±0.62
Total dry weight	5.72±3.13	7.29±1.43	7.34±2.13	2.26±0.86	1.42±0.66	2±0.65	3.92±0.61	5.45±1.8	2.37±0.86	2.45±0.98
Root to Shoot ratio	0.58±0.06	0.71±0.11	0.74±0.11	0.9±0.14	0.63±0.22	0.23±0.07	0.73±0.25	0.54±0.18	0.74±0.44	0.58±0.32
Chlorophyll										
Chlorophyll a (mg g-1)	8.04±3.07	3.23±0.44	3.08±0.16	8.43±1.03	8.2±1.76	2.19±0.59	1.97±0.48	1.43±0.13	4.64±0.36	3.92±0.78
Chlorophyll b (mg g-1)	1.82±0.61	0.72±0.11	0.73±0.07	2.17±0.29	2.02±0.49	0.54±0.15	0.37±0.11	0.23±0.03	1.03±0.08	0.84±0.23
Chl a: b ratio	4.35±0.37	4.49±0.31	4.26±0.35	3.9±0.09	4.09±0.14	4.08±0.1	5.38±0.52	6.36±0.25	4.51±0.24	4.75±0.33
Carotenoids (mg g-1)	1.94±0.78	1.08±0.16	1.31±0.13	2.28±0.22	2.57±0.46	0.55±0.16	0.64±0.15	0.66±0.03	1.23±0.1	1.18±0.16
Fv/Fm	0.81±0.01	0.81±0.01	0.75±0.02	0.83±0.01	0.8±0.02	0.77±0.03	0.82±0.01	0.73±0.05	0.83±0.01	0.82±0.01
Pi	2.66±0.28	2.59±0.1	0.79±0.19	3.07±1.13	1.98±1.04	1.93±1.32	6.28±0.44	0.77±0.45	7.81±1.6	3.73±0.96
Photosynthesis										
Max photosynthesis**	11.08±0.56	13.45±1.95	13.46±2.98	15.22±2.46	14.93±0.66	6.73±0.52	16.46±1.83	14.7±3.63	18.07±4.69	19.19±0.73
Initial slope	24.33±2.08	26.33±6.11	46.67±3.21	22.67±8.74	22.33±1.53	76.33±5.86	37.33±5.77	47.33±4.51	36.33±10.69	34.33±0.58
Dark respiration	0.05±0.01	0.06±0	0.04±0.01	0.06±0	0.06±0	0.04±0	0.06±0.01	0.05±0.01	0.06±0	0.06±0
Compensation point	-1.63±0.16	-1.67±0.25	-2.01±0.31	-1.49±0.54	-1.51±0.08	-2.8±0.37	-2.18±0.12	-2.3±0.41	-2.29±0.7	-1.97±0.03

Supplementary table 1: (continued)

Species	Raphanus					Triticum				
Trial\Treatment	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T
Biomass and Morphology										
Height	5.94±0.59	4.36±0.41	5±1.19	6.61±0.92	6.97±1.37	53.96±3.63	28.68±1.72	33.03±2.58	43.58±4.57	43.71±3.12
SLA	23±2.76	24.95±1.24	27.61±4.86	31.96±3.14	31.21±6.15	30.38±1.89	16.91±1.87	19.64±2.3	26.28±4.27	27.83±4.82
Dry weight shoot	0.53±0.16	0.22±0.06	0.2±0.16	0.32±0.12	0.24±0.1	-	-	-	-	-
Dry weight roots	4.7±1.59	4.54±1.33	3.31±1.39	3.18±0.77	2.66±0.76	21.6±7.46	47.88±13.68	61.08±16.3	111.5±20.28	48.41±17.43
Total dry weight	7.47±2.05	6.04±1.74	4.79±1.87	4.95±1.02	4.02±0.97	33.89±7.57	54.6±14.3	67.06±15.2	119.93±20.2	56.52±17.88
Root to Shoot ratio	1.69±0.39	3.07±0.49	2.39±0.82	1.87±0.69	1.95±0.38	1.86±0.86	7.24±2.21	11.36±5.4	13.47±3.12	5.99±1.87
Chlorophyll										
Chlorophyll a (mg g-1)	2.72±1.6	3.3±0.51	4.77±1.4	6.84±1.17	7.81±1.22	7.12±0.87	3.04±0.52	4.04±0.59	5.46±0.88	6.51±1.01
Chlorophyll b (mg g-1)	0.67±0.33	0.78±0.13	1.03±0.22	1.67±0.29	2.09±0.62	1.89±0.14	0.67±0.11	0.88±0.15	1.37±0.23	1.64±0.25
Chl a: b ratio	3.93±0.47	4.26±0.22	4.55±0.53	4.12±0.13	3.84±0.48	3.75±0.24	4.53±0.12	4.61±0.12	4.01±0.14	3.97±0.07
Carotenoids (mg g-1)	0.63±0.35	1.04±0.04	1.55±0.42	1.83±0.3	2.22±0.39	1.45±0.11	1.23±0.28	1.78±0.33	1.54±0.22	1.93±0.25
Fv/Fm	0.83±0.01	0.8±0	0.8±0.02	0.85±0	0.85±0.01	0.83±0.01	0.81±0.03	0.83±0.01	0.84±0.01	0.83±0
Pi	7.88±1.93	3.39±0.36	3.04±1.31	8.79±0.53	10.82±2.18	4.13±0.47	3.83±1.82	3.86±0.85	5.36±0.88	3.61±1.1
Photosynthesis										
Max photosynthesis**	13.77±3.41	15.53±2.79	16.16±4.16	19.62±4.51	19.7±3.49	9.12±0.82	18.19±8.91	16.66±6.34	13.92±0.61	14.02±2.57
Initial slope	22.33±2.31	24±5.29	37.67±6.11	25.33±2.08	27.67±6.66	35±0	23±6.08	23±7.55	30.67±12.66	26.33±8.02
Dark respiration	0.05±0	0.07±0	0.06±0	0.07±0	0.07±0	0.04±0	0.08±0.02	0.08±0.02	0.07±0.01	0.1±0.02
Compensation point	-1.05±0.02	-1.76±0.4	-2.35±0.27	-1.74±0.14	-1.95±0.48	-1.9±0.09	-2.12±0.86	-2.35±0.39	-2.38±0.63	-3.44±2.16

Supplementary table 1: (continued)

Species	Ulmus				
Trial\Treatment	Outdoor	Fix L - Fix T	Fix L - Variable T	Variable L - Fix T	Variable L - Variable T
Biomass and Morphology					
Height	26.67±9.16	24.46±4.92	23.93±2.18	30.69±10.31	25.59±9.8
SLA	28.71±2.78	22.1±1.3	22.3±3.86	33.8±5.28	31.16±3.14
Dry weight shoot	0.71±0.46	1.26±0.44	1±0.35	0.52±0.4	0.38±0.26
Dry weight roots	0.63±0.42	1.91±0.62	1.46±0.54	0.6±0.37	0.59±0.54
Total dry weight	2.77±1.69	5.19±1.39	4.37±1.41	1.99±1.12	1.65±1.25
Root to Shoot ratio	0.28±0.05	0.59±0.14	0.51±0.14	0.46±0.22	0.5±0.24
Chlorophyll					
Chlorophyll a (mg g-1)	6.8±0.86	1.8±0.15	2.16±0.4	5.78±1.75	4.28±0.37
Chlorophyll b (mg g-1)	1.54±0.19	0.36±0.04	0.46±0.08	1.35±0.49	1±0.07
Chl a: b ratio	4.43±0.1	5±0.33	4.67±0.44	4.37±0.31	4.3±0.16
Carotenoids (mg g-1)	1.15±0.18	0.64±0.07	0.91±0.05	1.62±0.41	1.26±0.15
Fv/Fm	0.81±0.01	0.77±0.01	0.65±0.05	0.82±0.01	0.79±0.02
Pi	3.46±0.8	1.37±0.32	0.3±0.17	3.59±0.42	1.63±0.55
Photosynthesis					
Max photosynthesis**	7.3±0.68	9.62±2.25	5.86±1.59	10.04±2.83	9.36±2.09
Initial slope	30.33±1.53	27±7.94	42±28.48	22±4.36	27.67±4.93
Dark respiration	0.03±0	0.05±0	0.04±0.01	0.05±0.01	0.05±0
Compensation point	-1.27±0.26	-1.59±0.24	-1.71±0.47	-1.33±0.26	-1.48±0.24

Supplementary table 2: Statistical results considering the different treatments as a fix factor and species as random effect.

	Variable	P value - Treatment (Fix)	P value - Specie (Random)
Biomass and Morphology	Height*	1.97e-07	< 2e-16
	SLA	< 2e-16	< 2e-16
	Dry weight shoot	< 2e-16	< 2e-16
	Dry weight roots	< 2e-16	< 2e-16
	Total dry weight	1.76e-10	< 2e-16
	Root to shoot ratio	< 2e-16	< 2e-16
Chloropyll	Chlorophyll A (mg g-1)	< 2e-16	< 2e-16
	Chlorophyll b (mg g-1)	< 2e-16	< 2e-16
	Chl A:B ratio	1.53e-14	< 2e-16
	Carotenoids(mg g-1)	< 2e-16	< 2e-16
	FvFm	< 2e-16	< 2e-16
	Pi	2.62e-09	< 2e-16
Stand. light	Max photosynthesis	4.39e-08	< 2e-16
	Compensation point	5.47e-04	< 2e-16
	Quantum yield for CO2 fixation	2.10e-15	< 2e-16
	Dark respiration	0.0261	< 2e-16

Chapter 5

Additional experiments: Exploring the application potential of light quality and variability treatments for indoor crop production

Abstract: Plants that are growing under natural environments need to cope with continues changes of environmental factors. Due to adaptation to marked changes in light, air humidity and temperature, plants increase their resilience to environmental stress at an expense of productivity. In contrast, moderate fluctuations of climatic factors along the day can also have a positive effect on plant development compared with completely fixed environmental conditions (Chapter 2, 3 and 4), as they largely prevail in indoor growth facilities. Previous studies have shown the benefits of mildly fluctuating environments for plant performance mainly for temperature and air humidity. In these cases, *e.g.* an increase of stomatal time response and plant height has been identified. This chapter aims to explore the possibilities of using light quantity or quality treatments to increase either productivity or shelf life (*i.e.* the durability of crops after harvest) of different crops in two different series of experiments. In a first experiment, stomatal conductance responsiveness was investigated under different light quantities and qualities at the beginning of the day. It was further tested, if a stimulation of gas exchange at the beginning of the day does lead to increased productivity in indoor farming. Four different morning light strategy treatments were applied to basil (*Ocimum basilicum* L.) and melissa (*Melissa officinali*s L.). Enriched blue light spectrum allowed for a faster stomatal opening during the morning, but this effect did not translate into higher total biomass at harvest. Additionally, independent of the used spectra no changes in height were reported. The possible effect in plants with longer crop cycles is discussed.

A second series of experiments aimed to stimulate biomass production and stomatal responsiveness using fluctuating day light conditions throughout the cultivation period in four crop species, including two different varieties of lettuce (*Lactuca sativa* L.). A tendency to lower biomass and height was detected under high light fluctuations, either in quantity or frequency of the light fluctuation, whereas a tendency for longer shelf life was found in both varieties of lettuce treated with variable light conditions. This experiment thus suggests that light quantity fluctuations in an otherwise climatically stable environment tends to negatively affect the total biomass production but could potentially be used as a tool to induce a faster stomatal responsiveness which increases shelf life of crops.

1. Introduction

As sessile organism, plants need to adapt to the prevailing environmental conditions at their site of growth. Whenever possible, plants will adapt to the environment and will successfully colonize and survive in a specific area. However, in view of the ongoing climate crisis, it is expected that the amount of extreme weather events will increase in future (IPCC, 2013), thereby threatening conventional food production and agriculture in many regions world-wide. Modern agriculture can use sophisticated technology that decoupled natural environments from food production (Poorter *et al.,* 2016), by using greenhouses or - even more extreme - indoor growth facilities which climates are almost completely independent from the surrounding environment. However, such extremely controlled conditions have been shown to have potentially negative effects on crops with respect to productivity and food quality, but also shelf life (*e.g.* Myster and Moe*.,* 1995, Arve *et al.,* 2017). In this respect, several authors have proposed and demonstrated that a more natural scenario in indoor growth facilities that include fluctuation environmental conditions can help to improve crop quality and even productivity (*e.g.* Poorter *et al.*, 2016; Arve *et al.,* 2017; Matsubara, 2018; chapter 2, 3 and 4 of this thesis).

The effect of specific environmental fluctuations in plant development has been previously looked in detail (*e.g.* Myster and Moe, 1995, Barlow *et al.,* 2015, Kaiser *et al.,* 2020), were specially today, with an increased prevalence of controlled environments in commercial plant production, the positive effect of incorporating climatic variability on crop performance has been suggested (*e.g.* Arve *et al.,* 2017, Annunziata *et al.,* 2017, Annunziata *et al.,* 2018, Matsubara, 2018).

While light variations in quantity and/or quality, can also have an important effect on plants, light variations for crop production have gained much less attention compared to temperature and humidity variations, as a tool to improve plant performance, contrary to the case of microalgae (*e.g.* Abu-gosh *et al.,* 2016). Although it is known that under fluctuating light conditions there is a reduction in plant growth due to the required adaptations to a changing environment (*e.g.* Kaiser *et al.,* 2018; Kaiser *et al.,* 2020), other studies (Annunziata *et al.,* 2018, chapter 2 and 4 of this thesis) have demonstrated that under indoor conditions with a mild range of temperatures (10-30°C), fluctuations of light quantity can significantly change plant traits (*e.g.,* SLA), indicating that the application of variable light quantity conditions can potentially help to increase crop quality in controlled environments. McAusland *et al.,* (2016) demonstrated in several species that there is a discoordination between photosynthesis and stomatal conductance if there is an abrupt change from darkness to light at the start of day in growth chambers, mainly due to a longer response time of stomatal opening. It is possible that

a slow light increase at the beginning of the photoperiod could help to couple photosynthesis and stomatal opening at the beginning of the day, instead of an abrupt change in light conditions, which is standard in indoor production. In addition, light quality could also play an important role here. Interestingly, even with the mentioned evidence, the interception point where benefits of fluctuating light quantity and losses in plant biomass intersect have not been exploited.

Up to date, effects of light quality changes on plant performance have gained little attention, with exception of growth effects related to red to far red (R:FR) ratios, specially at the end of the day, which has been broadly documented due its important role in plant signalling and morphology (*e.g.* chapter 1 and references therein). However, with the development of new and affordable LED lamps, light quality conditions are been further investigated as *e.g.* the B:R ratio (Furuyama *et al.,* 2014, Gautam *et al.,* 2015, Chapter 2 of this thesis). Meanwhile new LED lamps can provide fluctuations of light spectra for plant growth, light recommendations for plant growth are centred on avoiding these conditions, even though a specific benefit could come *e.g.,* from a higher blue spectrum with lower energy consumption or desired plant morphology.

Using the knowledge acquired in previous experiments (Chapter 2, 3 and 4), the present studies aimed to test different methods of light manipulation, inspired from natural conditions, to enhance either biomass production or shelf life in plants grown under indoor conditions. Two different hypotheses were proposed. First, that a slow increase of light in the beginning of the photoperiod would lead to a higher stomatal conductance, independent of the used light spectra, compared with the control treatment. This would lead to higher biomass at the end of the crop cycle, specially at higher levels of blue light ("blue morning" experiments). Second, that a slowly fluctuation of light quantity during the day along the crop period would enhance stomatal responsiveness, and therefore the shelf life after harvest without affecting the final biomass yield ("fluctuating light" experiments).

2. Material and methods

2.1 Germination conditions

Four different species were used in these experiments. Seeds of Basil (*Ocimum basilicum* L. var Aroma 2, Johnsons-seeds), Lettuce (*Lactuca sativa* L. var Kimenoz RZ referred later as green lettuce and var Galiano referred later as red lettuce. Riz zwann and Impecta respectively), Melissa (*Melissa officinalis* L., Impecta) and Shiso (*Perilla frutescens* L., Atariya) were pre-grown for 7 days before the start of the experiments. 10-15 seeds were germinated per pot (7 x 7 x7 cm, Impecta) for basil and melissa, while for lettuce and shiso a single seed per plot was

sown. A 2:1 mix of turf (Emmaljunga Torvmull AB, Sweden) and vermiculite (HydroGarden, England) was used as substrate. The plants were germinated under 160 μmol $m^{-2}s^{-1}$ (spectrum available as supplementary Fig. S1, model SIERA – Propagation spectrum, Heliospectra, Sweden), 28/20 ºC and 47.5 /62.5 % relative humidity (RH) for 18 hours day and 6 hours night, respectively.

2.2 General growing conditions during the experiments

Along a period of four weeks, the different species were grown in an approximate density of 20 plants per square meter, separated in 4 different trays with 6 pots of each species per tray (45 x 30 cm) without mixing the species. To account for space variability, the trays were homogenously distributed within the growth units. The plants were watered as required with a nutrient solution of PlantProd 20:20:20 (1gL^{-1}, PlantProd, United states) mixed with $Ca(NO_3)_2$ (226.8 mg L^{-1}) and $MgSO_4$ (156.76 mg L^{-1}). The average room temperature was 24°C during the day and 20°C during the night.

2.3 Experimental treatments

Two different experiments were performed at the PlantLab of Heliospectra (Gothenburg, Sweden). In each experiment, the light treatments were applied in individual growth units (1.2 m^2) surrounded by reflecting material to avoid light contamination in the main room and cross-contamination among units. A single LED lamp with 4 individually controlled LED types/channels (blue, 450 nm; red, 660 nm; far-red 720 nm; and white, 5700K) from Heliospectra (ELIXIA LX601C, Heliospectra, Sweden) was used as light source in each growth unit.

2.3.1 'Blue morning' experiment:

With the aim of increasing biomass production through a faster stomatal response over the morning, 4 different light treatments were applied for a period of four weeks to seven days-old plants of basil and melissa. The experiment was replicated four times, with 12 plants of each species in each experiment distributed on two trays for each species. A **control treatment (CT)**, was done with a photoperiod of 16 hours with constant light (150 μmol $m^{-2}s^{-1}$; spectrum shown in supplementary Fig. S2). A second treatment was stablished to evaluate the effect of a **morning rise (MR)**, by extending the photoperiod by 30 minutes and increasing light intensities by 2.5 μmol $m^{-2}s^{-1}$ per minute until the same light intensity and spectrum as the

control treatment were reached (Fig. 1). A third and fourth treatment were applied to evaluate the effect of a morning rise enriched in blue light. Like the MR treatment, a slow increase of intensity was applied until the control light intensity was reached, but with a different light spectrum during this 'morning rise'. The third treatment had a morning rise with a high blue content (80%; **MR-HB**) until the last 10 minutes of the rise, when upon a slow change in light quality transformed the blue enriched spectrum to the control light spectrum. In the fourth treatment, a morning rise with decreasing blue (**MR-DB**), the light during the morning rise was enriched in blue light (80%), but the change in spectrum from blue enriched to the control light spectrum was continuous and linear over the first hour of daylight (Fig. 1). All treatments had the same daily light integral (DLI) and the same light spectrum was used during the main part of the day (17.5-18 hours depending on the treatment, supplementary Fig. S2). The same green to red ratio and red to far red (R:FR) ratio were kept along all the treatments, including the period during the morning rise.

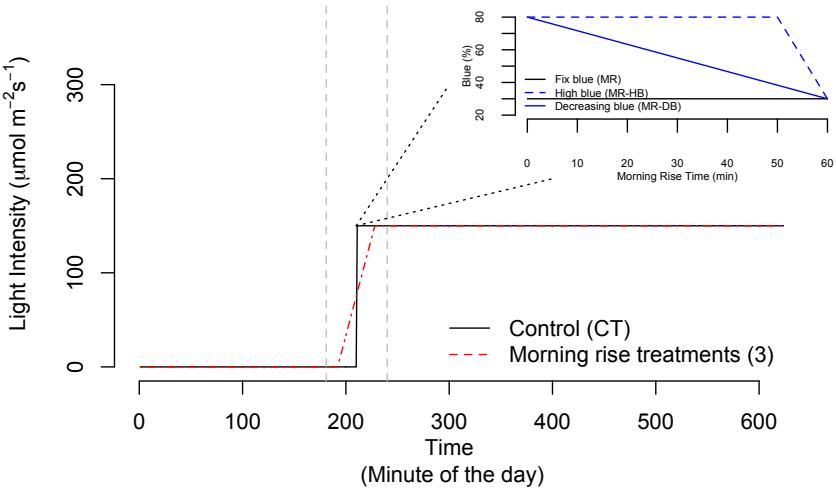


Figure 1. Light treatments in the blue morning experiment. CT corresponds to the control treatment with typical light conditions in indoor experiments; MR corresponds to a slow increase of light for a period of 1 hour with the same light spectrum as the control treatment, which is also applied throughout the main daylight period in all treatments; MR-HB corresponds to a morning rise treatment enriched with 80% of the total light in blue until the last 10 minutes where the spectrum is gradually changing to the spectrum of the main light period (with a blue proportion of 30%); MR-DB corresponds to a morning rise enriched in blue, starting with 80% blue, that slowly changes over the first hour to the light spectrum of the main light period.

After four weeks, at the end of the experiment above ground biomass was measured as fresh weight together with plant height, in both species. Dry biomass was weighted after 14 days at 70°C in a drying oven. In the case of basil, the biomass was divided between stems and leaves, while for melissa only total biomass was collected. In the last replicate of the experiment,

the stomatal conductance was measured during the morning rise (7:00 AM to 8:00 AM) in all species and treatments: during the last 3 days of the experiment a random leaf from the top 2 pair of leaves was measured in a random plant in each treatment every 10 minutes using a leaf porometer (SC-1, Meter environment, Germany) daily calibrated under the actual measuring conditions.

To evaluate the effect of the different treatments, a one-way analysis of variance (ANOVA) was performed on each replicate and species, considering the different treatments as fixed factors. *Posteriori*, a one-way ANOVA was realised for each species, considering the different replicates as a random effect and the treatments as fix factors. An additional analysis using normalized data (normalizing with respect to the average of the control treatment in each replicate), between replicates was done without significant differences in the statistical output of the ANOVA with non-normalized data (data not shown). All data were checked for normal distribution, independence and homogeneity of variance. Finally, as post hoc analysis, a Tuckey pairwise multiple comparison test was conducted to identify significant differences among treatments. Due to a small difference in air temperature between the growth units used in the experiment (supplementary Fig. S3) an additional analysis was performed after a linear temperature correction, without further effects (data not shown).

2.3.2 'Fluctuating light' experiments:

With the aim to evaluate the possibility of using controlled light fluctuations on indoor grown plants as a tool for improving plant shelf-life, different light treatments were given to 7-day old plants of basil, melissa, lettuce and shiso for a period of four weeks. Three different experiments were performed, where different scenarios of variable light during the photoperiod were tested. In all experiments, a control treatment (CT) was included with a photoperiod of 18 hours with constant light intensity (200 - 250 μmol $m^{-2}s^{-1}$) and constant light spectrum (see supplementary Fig. S2 for light quality information). In an initial experiment (Exp 1) the effect on biomass production was investigated, whereas in a second and third experiment (Exp 2 and 3), the shelf-life in terms of plant water retention after harvest was evaluated. The applied light conditions of the different experiments are shown in Table 1 and Fig. 2. In situ light conditions were measured and controlled with a PAR sensor (Li-190R, Li-cor, United States) connected to a central unit (HelioCore, Heliospectra, Gothenburg, Sweden). The light levels were measured in the center of each growth unit during the first two days of the experiments. Changes in light intensity were applied every minute through http communication with the different lamps using a python script.

Table 1: Light treatments: number of applied fluctuations along the photoperiod and their relative amplitude, given as percentage compared with the average light intensity.

Experiment – Light intensity	Treatments	Species
Exp. 1 – 250 µmol $m^{-2}s^{-1}$	Control (CT): continuous light F05-P20: 5 fluctuations at 20% of light intensity F20-P05: 20 fluctuations at 5% of light intensity	basil, melissa
Exp. 2 – 200 µmol $m^{-2}s^{-1}$	Control (CT): Continuous light Variable (FL): 5 fluctuations at 20% of light intensity Mixed (CT-FL): 21 Day of Control + 7 Days Variable	basil, red lettuce, green lettuce, shiso
Exp. 3 – 200 µmol $m^{-2}s^{-1}$	Control (CT): Continuous light Variable (FL): 5 fluctuations at 50% of light intensity Mixed (CT-FL): 21 Day of Control + 7 Days Variable	basil, melissa, red lettuce, green lettuce

At the end of each experiment, after four weeks under the light treatments, the following plant traits were measured. In Exp 1, replicated 3 times, the above ground biomass and plant height were measured in both species (n=12). Dry biomass was taken after 14 days at 70°C in a drying oven. In the case of basil, the biomass was divided between stems and leaves. In Exp 2 (not replicated), at the end of the experiment, the specific leaf area (SLA) of all 4 species was measured (n=3) together with the water losses in a dark cold environment (8°C) for up to 160 hours post-harvest (n=3). For this, the different species were divided in two harvesting categories depending on the more practical selling strategies. Basil and shiso are generally sold as potted plants, thus the water loss was determined in potted plants stored in the dark cold environment. Lettuce is generally sold as harvested above ground biomass, whereby the water loss was measured in cropped heads of lettuce. For shiso, in addition to water loss from potted plants, also the top pair of leaves was harvested, and water loss was determined in these leaves as well. In experiment 3 (not replicated), chlorophyll content of mature leaves (n=5) and dry biomass (n=3-6) was measured in all species (see Table 1). Water loss of the two lettuce varieties was measured on cropped heads of the two lettuce varieties (n=3) during three days in a dark cool environment (4°C).

To evaluate the effect of the different treatments in Exp. 1, a one-way analysis of variance (ANOVA) was performed for each species and replicate, considering the different treatments as fixed factors. *Posteriori*, a one-way ANOVA was realised for each species,

considering the replications of the experiment as a random effect and the treatments as fixed factor. An additional analysis with normalization between replicates, normalizing respect to the average of the control treatment in each replicate, was done without significative changes in the output (data not shown). In Exp. 2 and 3, a separate one-way ANOVA for each species, with treatment as fixed factor was used. In all experiments, post hoc Tuckey pairwise multiple comparation tests were conducted to identify significant differences among treatments. All data were checked for normal distribution, independence and homogeneity of the variance before analyses.

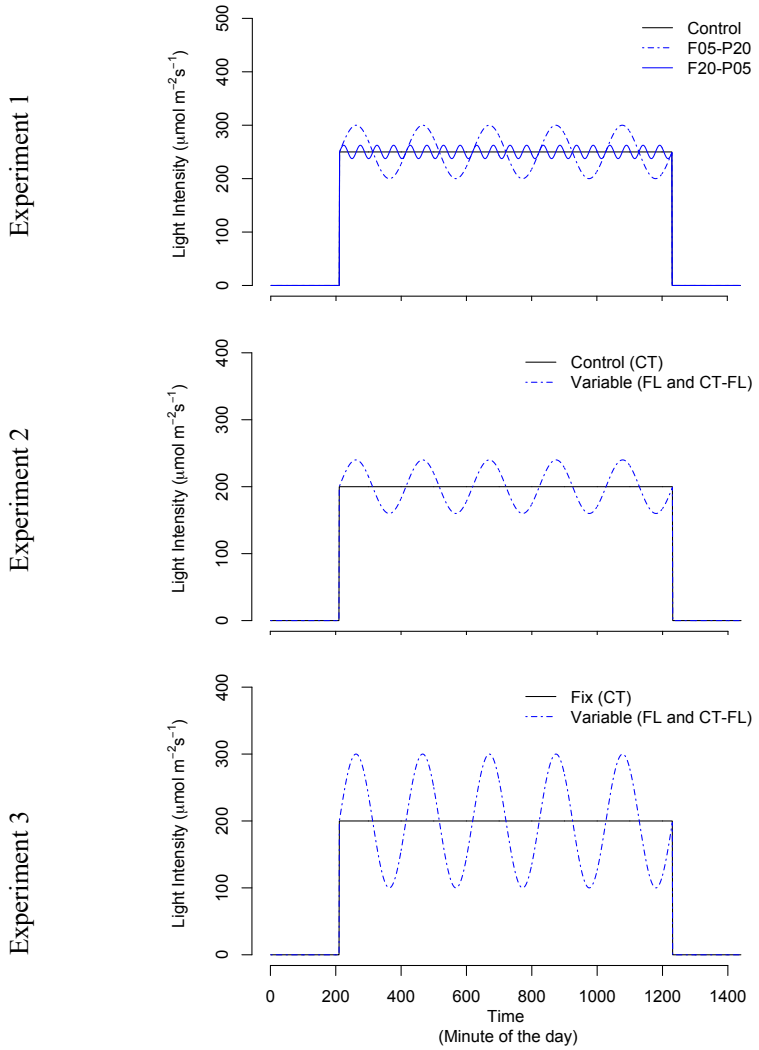


Figure 2. Light treatments applied in the three different experiments. All experiments had a control treatment (CT) together with different light fluctuation treatments. In Exp. 1, two different light strategies were tested: 5 % change of the average light intensity fluctuated 20 times along the photoperiod (F05-P20), and 20% change fluctuated 5 times (F20-P05). In Exp. 2, the F20-P05 treatment was applied throughout the crop cycle (FL) as well as just at the end of the crop cycle (CT-FL). In Exp. 3 the same treatment combination as in Exp. 2 was applied, but with a 50 % instead of the 20 % light fluctuation (F50-P05).

3. Results

3.1 Experiment 1: Blue morning experiment

When each replicate of the blue morning experiment was analysed separately, there were significant differences in plant height and biomass among the applied light treatments (supplementary Fig. S4). However, the direction of the treatment effects differed significantly among the four replicates of the experiments. Hence, when replicates were considered random factors, no significant differences between the light treatments were found for biomass and height in both species (*i.e.,* basil and melissa; Fig. 3). Slight temperature differences between the growth units might have influenced the treatments (supplementary Fig. S3), but still no significant treatment effects were found after linear temperature corrections between the used units (data not shown). Nevertheless, in two of the four replicates of the experiment, the blue enriched treatment (**MR-HB**) had significantly higher biomass, but not height, compared to the control treatment (**CT**) in basil (Fig. S4a and b). Similar results were found for melissa, where in two of the four replicates of the experiment, MR-HB induced higher plant heights and biomass at the end of the experiment (Fig. S4b). The treatment with only morning rise (**MR**) and with decreasing levels of blue **(MR-DB**) did not differ from CT in biomass and height in almost all replicates of the experiment, in both species (Fig. S4).

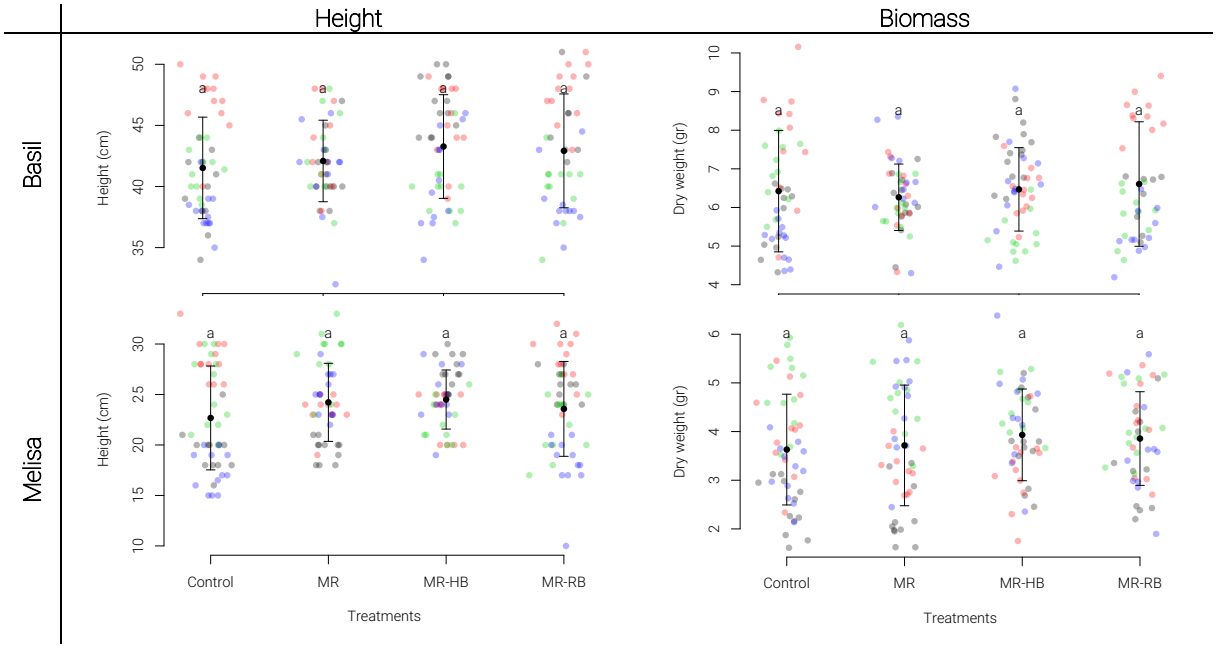


Figure 2. Height and biomass of basil and melissa at the end of the experiment with different morning light treatments. Each point correspond to an individual plant were the colours correspond to the different replicates of the experiment in the following order: grey, red, green and blue for replicate 1,2,3 and 4. Black dots correspond to the average value per treatment, black bars correspond to the standard deviation (n=48). Different letters would indicate statistically difference between groups with experiment replicate as a random effect, but no statistical significance ($p < 0.05$) was found for plant height or biomass in both species.

In the fourth run of the blue morning experiment, stomatal conductance (g_s) during the first hour of the morning (7:30-8:30 a.m.) was measured on the last three days of the experiment. When compared across all three days, g_s started and finished at similar values in all treatments (Fig. 3). However, there were significant differences in dynamics with respect to the early morning stomatal opening among treatments. In general, there was a stronger stomatal response to the treatments in melissa compared to basil, but the effect direction was the same in both species. CT took up to 30 minutes to reach stability and similar values as the other treatments. As expected, the MR treatments revealed an earlier increase in g_s, with MR-HB inducing the fastest increase followed by MR-DB and MR (Fig. 3).

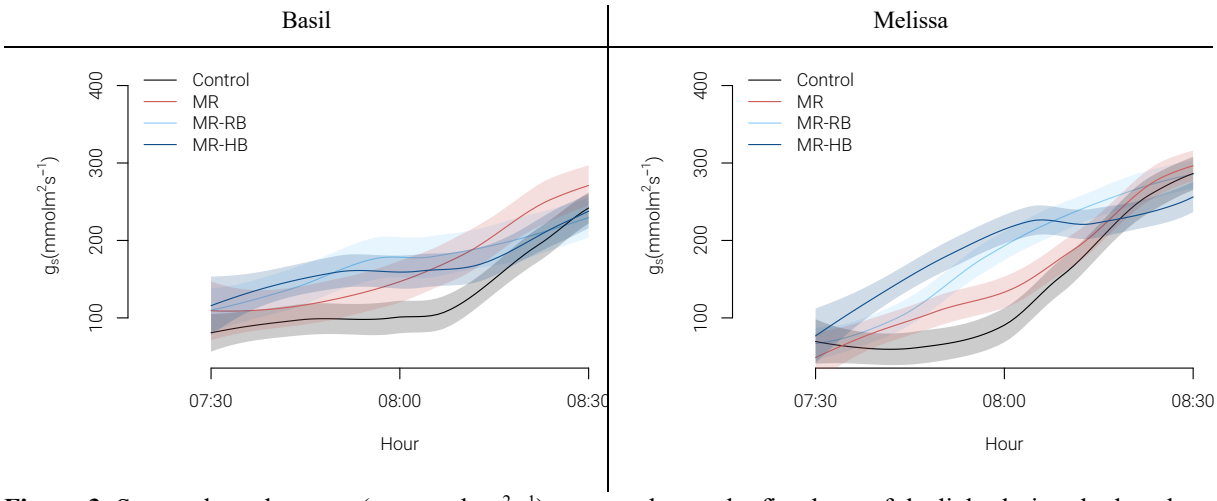


Figure 3. Stomatal conductance (g_s; mmol $m^{-2}s^{-1}$) measured over the first hour of daylight during the last three days of the fourth replicate of the experiment. The control treatment started at 8:00 meanwhiles the ramping treatments (MR, MR-HB and MR-DB) started at 7:30 AM and reached the target light intensity at 8:30 AM. A loess regression was fit to the data for easier graphical representation

3.2 Fluctuating light experiment

3.2.1 Experiment 1

Similar results were found between the three replicates of the first experiment (supplementary Fig. S5), and the trends across all three runs is shown in Fig. 4. Overall, the effects were rather weak but, independent of the species, the treatment with 20 light fluctuations along the day (**F20-P05**) tended to yield equally tall or taller plants compared with the control treatment (**CT**). This was not the case for the light treatment with 5 light fluctuations along the day (**F05-P20**), which in the case for melissa resulted in shorter plants on average. The biomass production was significantly increased under F20-P05 in basil, while in melissa both fluctuating light conditions induced lower biomass compared to CT (Fig. 4).

3.2.2 Experiment 2

Note that experiment 2 was not replicated due to time constraints, and therefore only descriptive statistics are presented. Since it is not possible to measure water loss and dry biomass on the

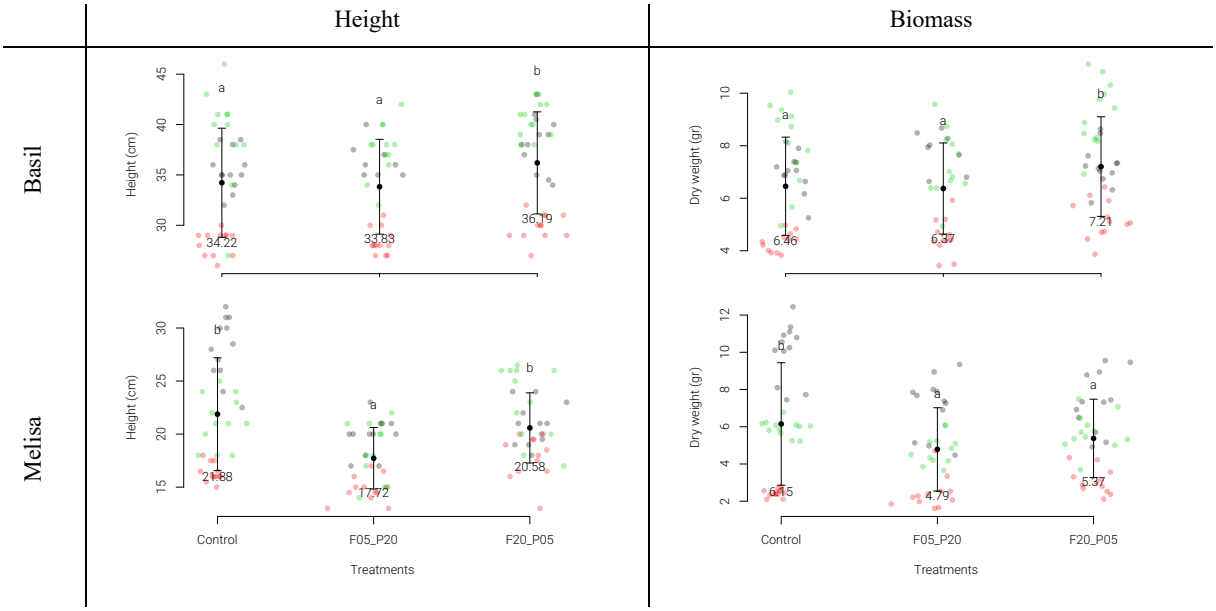


Figure 4. Height and biomass of basil and melissa at the end of the experiment with different light fluctuations treatments (Exp. 1). Each point correspond to an individual plant were the colours correspond to the different replicates of the experiment in the following order: grey, red and green for replicate 1,2 and 3. Black dots correspond to the average value per treatment and the black bars show the ± standard deviation (n=36). Different letters indicate statistically difference between groups with experiment replicate as a random effect.

same individual plant, the plants were divided in two groups with respect to the measured variable.

On average, the mixed treatment (CT-FL) induced higher SLA values (thinner leaves) compared with the other two treatments (Fig. 5). When water loss (used as a proxy of shelf life) was measured during a period of 170 hours post-harvest, a clear difference was visible depending on if whole potted plants or harvested plants are considered (Fig. 6). Potted plants

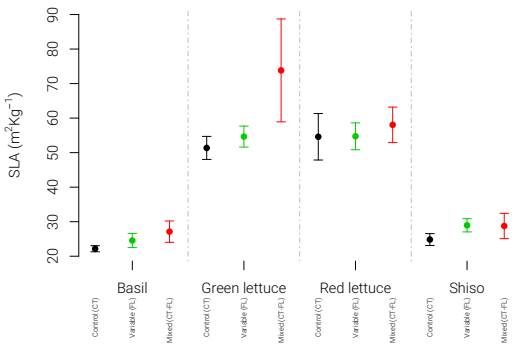


Figure 5. Specific leaf area (SLA) of four different species under the three light conditions in Exp. 2. Black dots correspond to the average value per treatment, bars represent ± standard deviation (n=3).

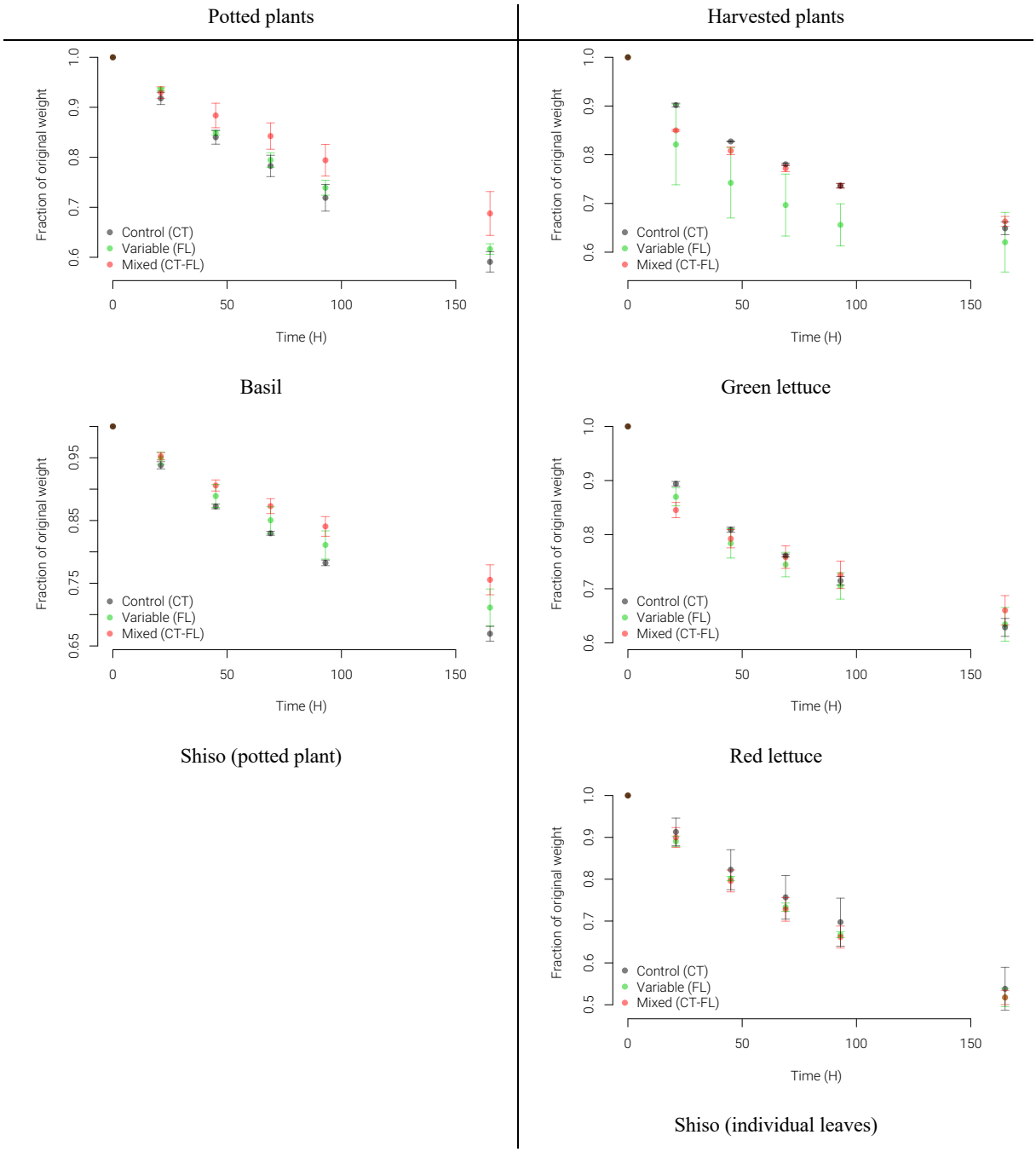


Figure 6. Post-harvest water loss of four different species grown under three different light treatments in Exp. 2. According to the commercial selling strategy (see Material and methods for details) the water loss was measured either in potted or harvested plants. The three light treatments correspond to a control treatment with continuous light (CT, black dots), a variable light treatment (FL, green dots) with 20% amplitude of the average light intensity and a combination of both (CT-FL, red dots), were just during the 5 final days of the experiment light fluctuations were applied. Dots correspond to the average value per treatment and bars are ± standard deviation (n=3).

under the mixed treatment tended to maintain higher levels of water, meanwhile the different light treatments did nod induce any trend in harvested plants. It is important to mention that there was a time gap between the harvest of the plants and the start of the cold treatment of

circa 2 hours, due to transport (in closed boxes), which may have influenced stomatal closure and thereby the results.

3.2.3 Experiment 3

CT-FL tended to induce higher levels of relative chlorophyll content, independent of the species and with the strongest effect in red lettuce (Fig. 7). Similar to Exp. 2, a biomass reduction was visible on the plants grown under the variable treatment (FL). This was especially the case for basil (although with considerable variability among replicates), followed by green lettuce.

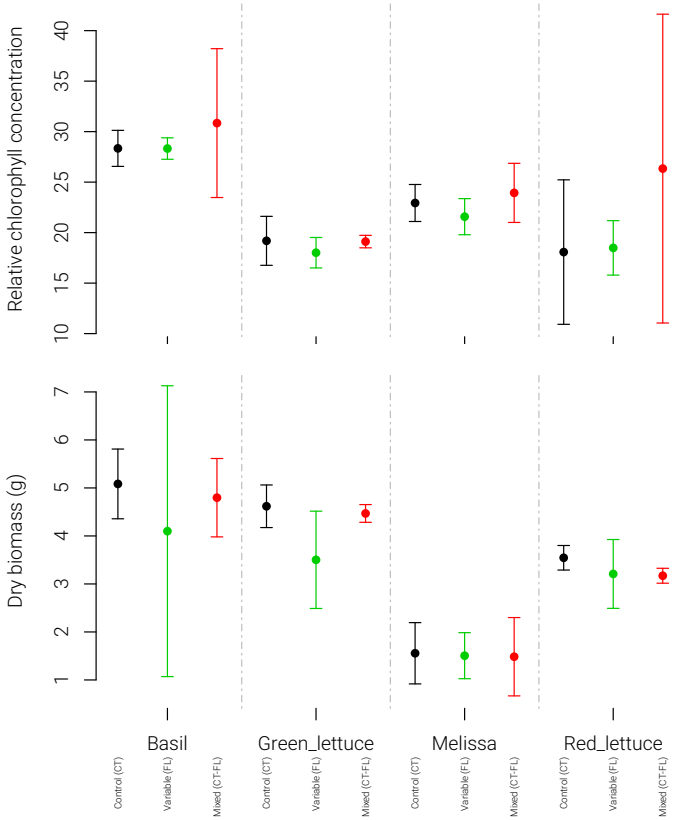


Figure 7. Relative chlorophyll concentration (A) and Dry biomass (B) of four different species grown under three different light treatments in Exp. 3: A control treatment with continuous light (CT), a fluctuating light treatment (FL) with 50% amplitude of the average light intensity and a combination of both (CT-FL) were just during the 5 final days of the experiment light fluctuations were applied. Dots correspond to the average value per treatment and the bars indicate ± standard deviation (n=5).

In Exp. 3 the plants were transferred immediately from harvest to a cold dark environment (in less than 5 minutes). Water losses were on average slightly lower under the FL treatment compared with the CT and CT-FL treatments. All treatments present a larger standard deviation, mainly associated with the low number of replications of the experiment (n=3).

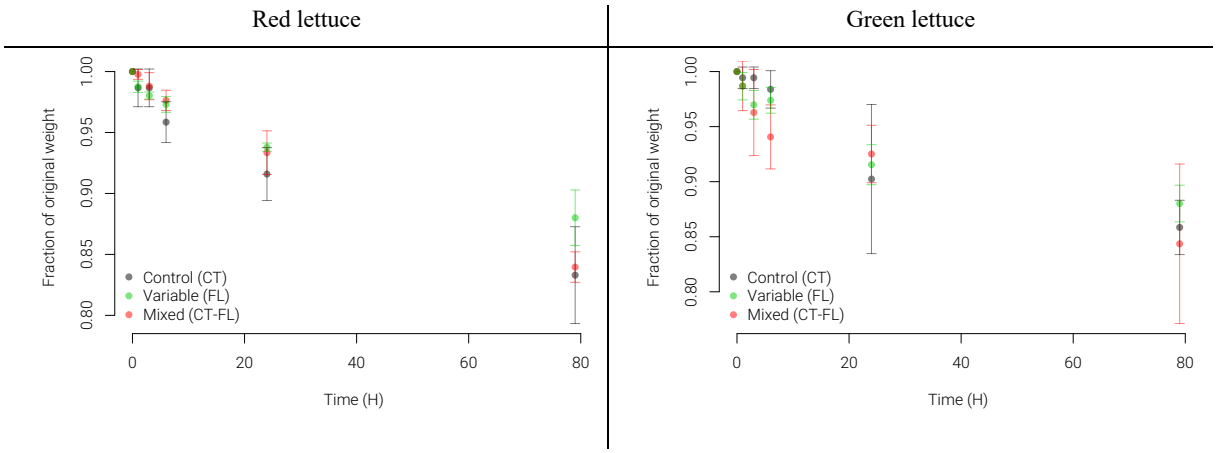


Figure 8. Post-harvest water losses of two different lettuces varieties grown under three different light treatments in Exp. 3: A control treatment with continuous light (CT, black dots), a variable light treatment (FL, green dots) of 50% amplitude of the average light intensity and a combination of both (CT-FL, red dots) were just during the 5 final days of experiment light fluctuations were applied. Dots correspond to the average value per treatment and the bars indicate ± standard deviation (n=3).

4. Discussion

Light quality treatments for higher productivity

The use of specific light quality treatments along the day is a rather new area of research and most of the few publications are just a couple of years old. Several general strategies have already been proposed (*i.e.* Jishi *et al.,* 2016; Chen *et al.,* 2017), but a common denominator is clear: species-specific responses to the different light treatments. Additionally, the lack of adjustment for other related parameters (*e.g.* R:FR ratio) makes comparisons and conclusions challenging from a biological point of view. Jishi *et al.,* 2016 demonstrated in lettuce that applying only red and blue light at different timings can affect plant morphology and plant growth. Plants that started the day with only blue light with a posterior addition of red light had higher biomass than plants exposed to both lights simultaneously. Kaiser (2019) suggested that increases in light intensity during the morning should be done slowly due to an imparity between the photosynthesis machinery and stomatal conductance response. In the current experiments, the benefit of using this morning rise was clear at stomatal conductance level but no effect was present in harvest biomass and plant morphology. Other studies have also seen that enhancements on photosynthetic level do not always yield a significant increase in final biomass, especially if plant growth is depending on other limiting factors than assimilated C (Kirschbaum, 2011).

Another reason for using specific light treatments along the day could be to improve energy efficiency, since different LEDs exhibit different energy and photon efficiencies. A blue heavy spectrum is generally less favourable compared with a red heavy spectrum from a photon

efficiency point of view (*e.g.* Poulet *et al.,* 2014). This was also the case in the present study, in which the blue (MR-HB) spectrum had an 8% higher electric power consumption for the same photon output compared to the control light spectrum (data not shown).

Light quantity fluctuations

The effect of light quality fluctuations has been well documented and reviewed during the last years (Kaiser *et al.,* 2016, Kaiser *et al.,* 2018; Kaiser *et al.,* 2020). The negative effects of these fluctuations, *e.g.* biomass reduction, oxygen radical increases, were also indicated in this study (see Exp. 1). Interestingly, continuous small variations (F20_P05) induced higher height and biomass in basil, but not in melissa. Less documented, as far as the authors know, are the potential benefits of applying light fluctuations. Chapter 3 and 4 of this thesis, together with Vialet-Chalbrand *et al.,* (2017) identified some positive effects, such as higher chlorophyll concentrations and higher F_v/F_m values, in addition to the above mentioned negative effects on productivity. The increase of water retention documented in this chapter in either potted or harvested plants, has been previously linked to bigger stomata and reduced stomatal responsiveness due to a continuous environment compared with fluctuating environments. Arve *et al.,* (2017) demonstrate that changes in the water vapour pressure (VPD) for up to two hours are enough to improve stomatal responsiveness, were plants growth at high level of humidity have between 8-16% higher stomatal opening compared with plants growth at lower humidities. Although leaf temperature was not measured in the presented experiments, we recorded air temperature effects due to light fluctuations (Supplementary Fig. 6). I can be expected that changes in light irradiance may have led to changes of leaf temperature. The resulting changed VPD at leaf level (Jones, 1993), could have led to a stimulation of stomatal responsiveness along the day. Overall, the explanatory power of these experiments is diminished due to the limited replication. Clearly, the potential use of fluctuating light application for plant production needs to be further investigated in more detail, and experiments with higher replications are recommended.

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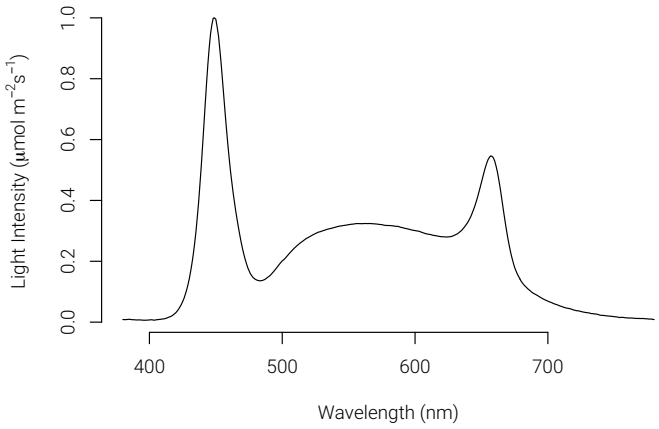
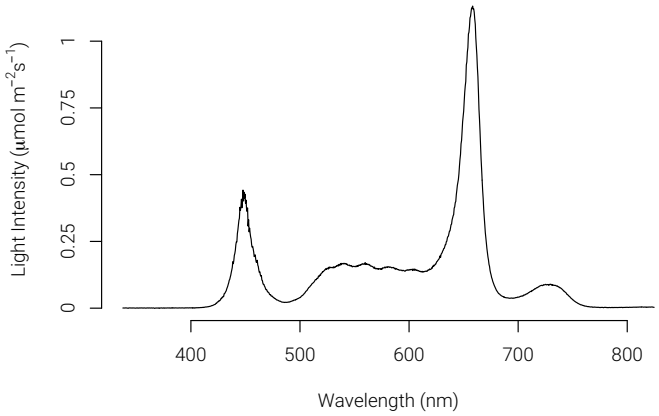
Supplementary materials:

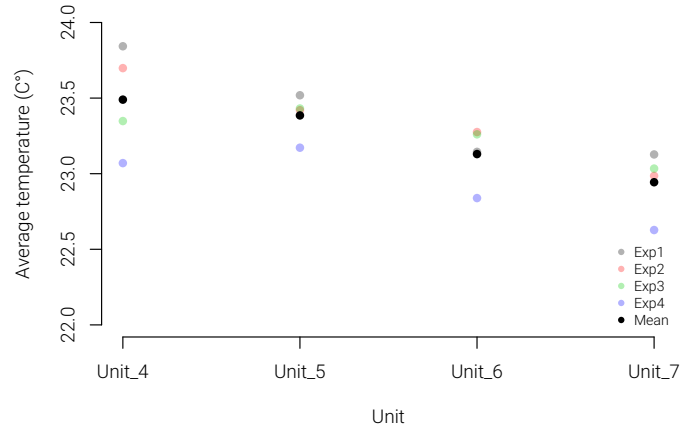
Supplementary fig. S1: pre-growing spectra



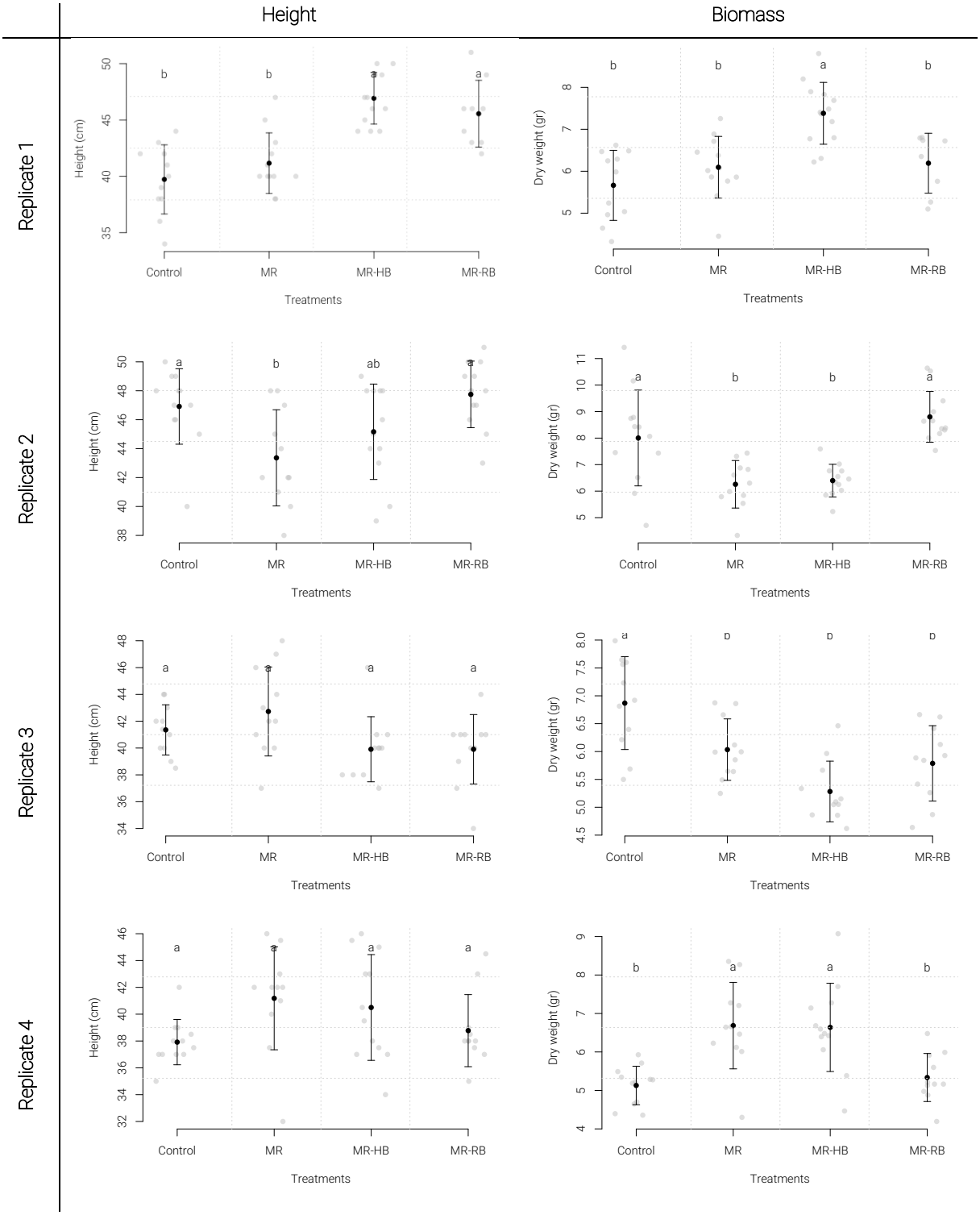
Supplementary fig. S2: Used growing spectra in the blue morning experiments.



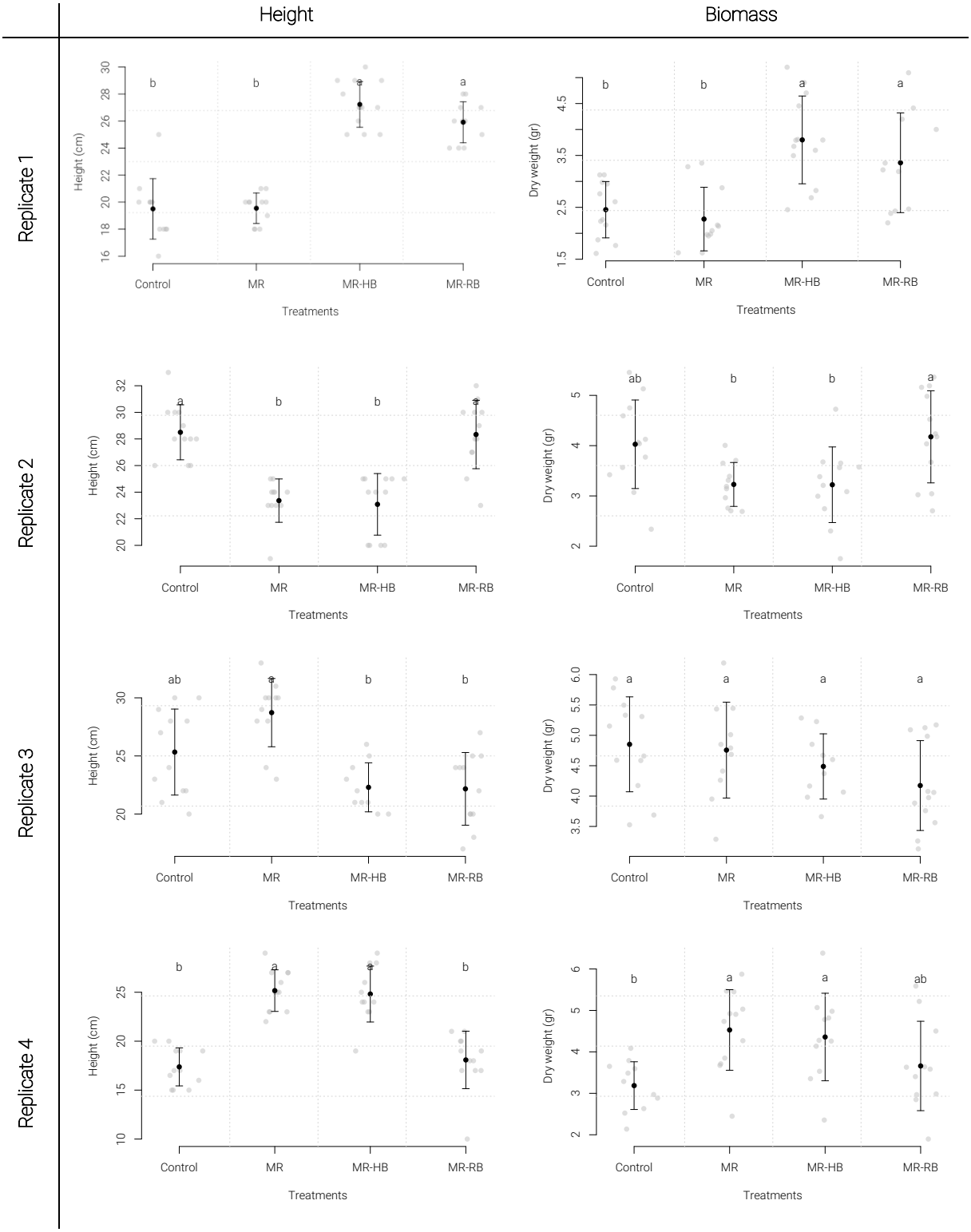
Supplementary fig. S3: Average temperature of the different used units during the 3 different replicates of the blue morning experiments



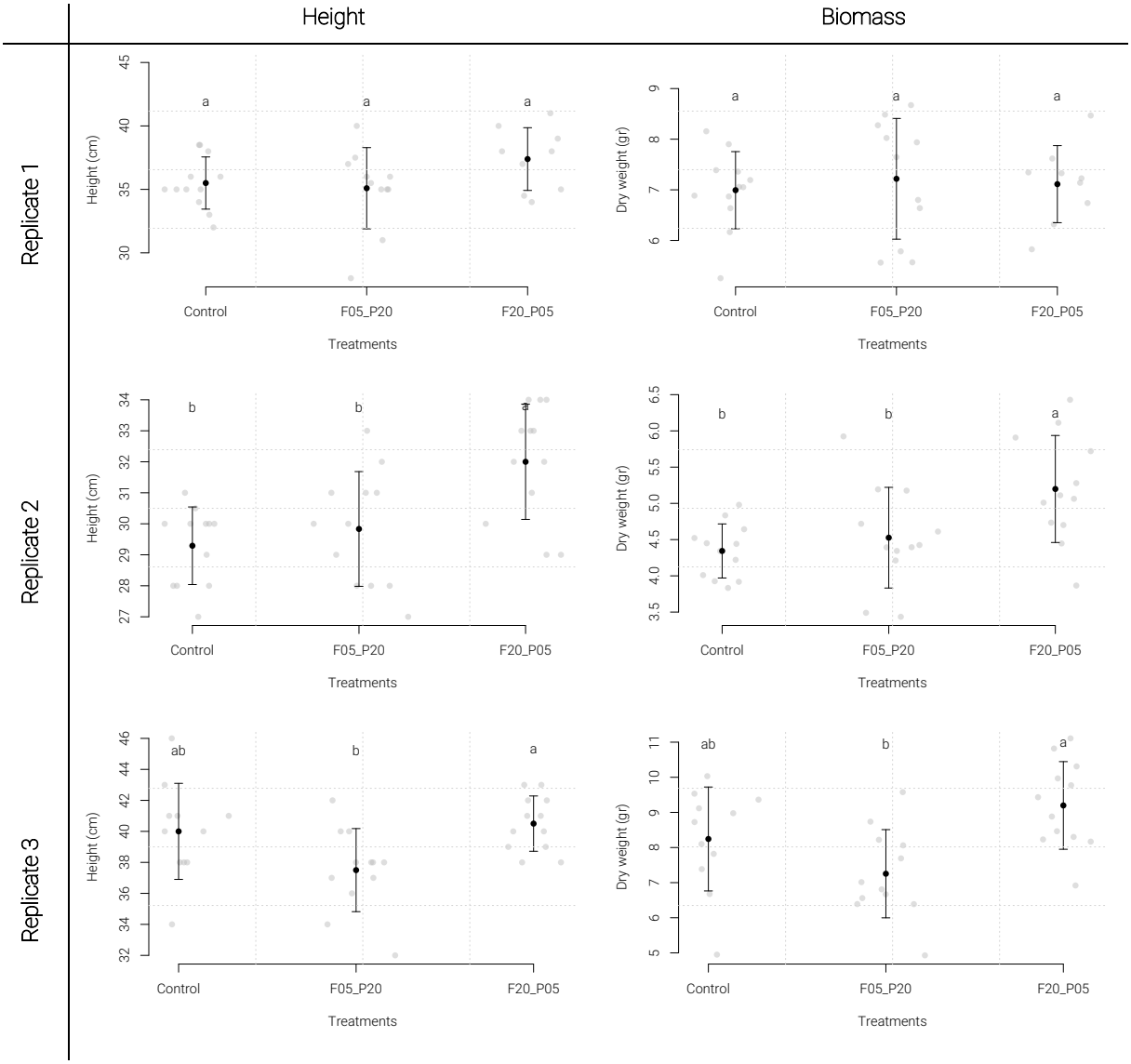
Supplementary Fig. S4 A: Replicates of basil in blue morning experiments



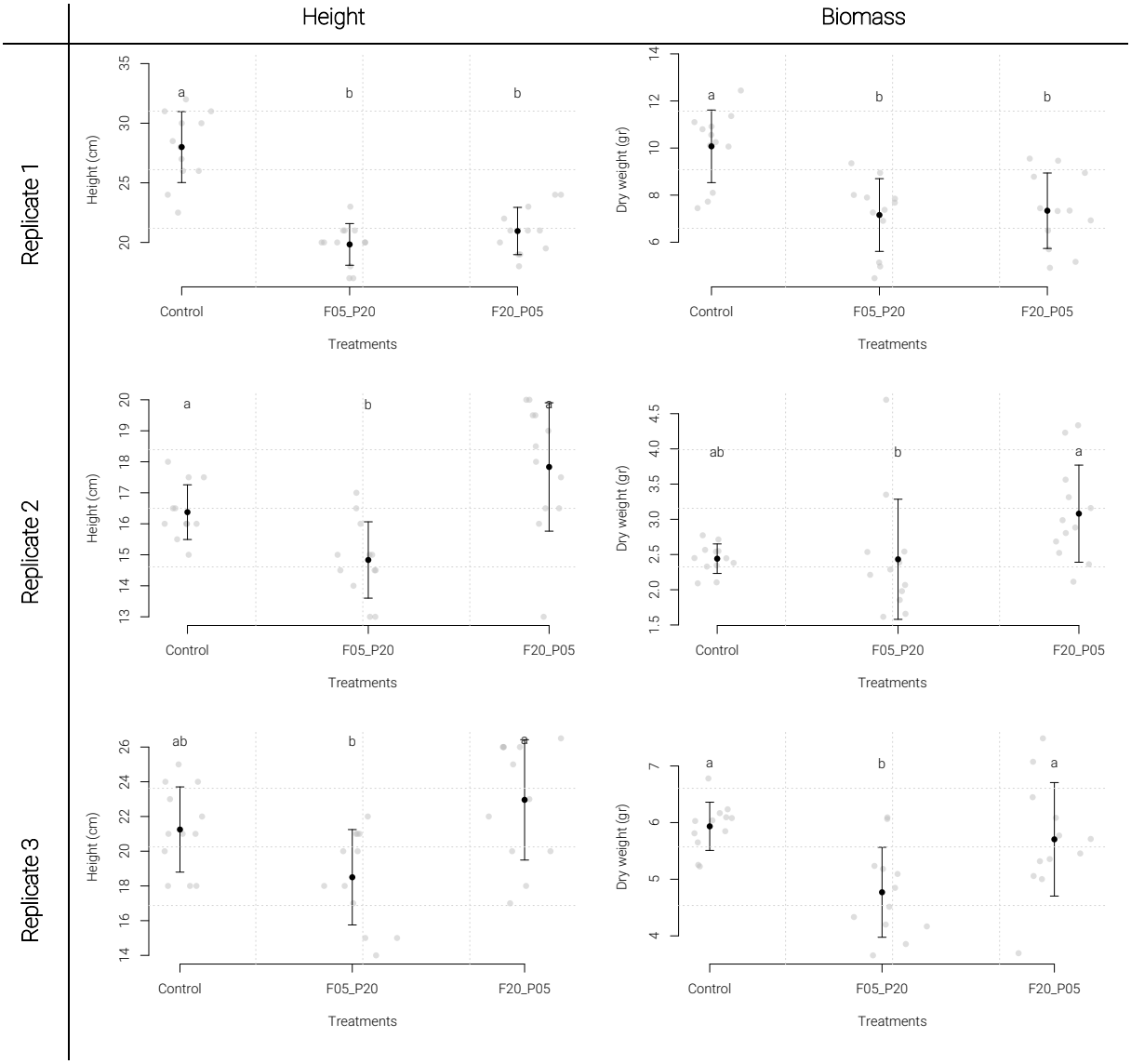
Supplementary Fig. S4 B: Replicates of melissa in blue morning experiments



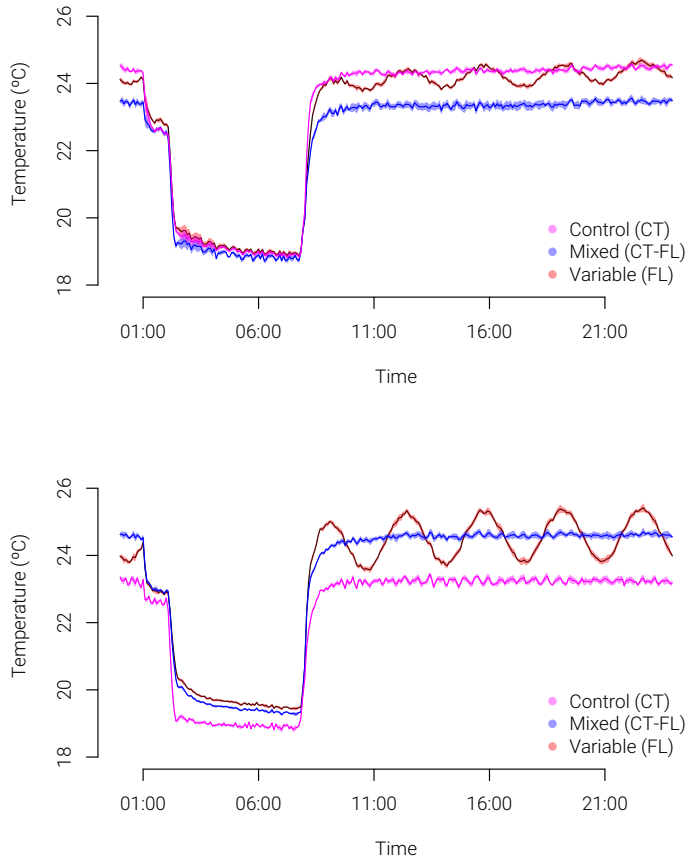
Supplementary Fig. S5: Replicates of Basil and melissa in first fluctuating light experiments



Supplementary Fig. S5: Replicates of Basil and melissa in first fluctuating light experiments



Supplementary Fig. S6: Temperature average 5 days second experiment



Appendix

Lamp simulator tool for optimizing light conditions and a natural light simulator tool for near-natural plant growth.

Summary: Natural changes in photoperiod, light quantity and quality play a key role in plant signalling, enabling daily and seasonal adjustment of growth and development. Today's LED technology enables mimicking of natural light climate scenarios, but our experience is that easy-to-apply knowledge and tools related to natural variation in the light spectrum are scarce, at least in the field of plant science. To that end, two different tools are presented here. First, a **lamp simulator tool**, including a lamp characterization protocol, that maps lamp intensity setting to light output (spectrum and intensity) for several lamps in Heliospectra's product portfolio. It allows the user to find what settings to use to reach a target light environment. The tool has a visual interface written in R. The tool is now based on Heliospectra products but can be translated to other lighting systems as well. Second, a **natural light simulator** that allow near natural plant growth recreating sunlight conditions, spectrum and intensity, at a given place, time and weather condition. It is based on conventional sunlight models, but also includes called environmental effects, based on the results of "Latitude and Weather Influences on Sun Light Quality and the Relationship to Tree Growth" (Chiang *et al.,* 2019). It requires information about the lighting system from the lamp simulator tool to calculate the lamp intensity settings that gives the target light environment. The control function focuses mainly on light properties that are known to be important for plant growth, such as intensity and quality.

Lamp simulator tool

What it is:

The lamp simulator tool is a program, coded in R using the shiny package, that recreates all possible spectrum combinations of the different Heliospectra lamps. The tool, available online at lightsimulator.heliospectra.com, predicts the light output of an average lamp considering the distance between the calculation point and the lamp taking in consideration the use of different channels. Although the tool is calibrated with a default setup, site-specific calibrations can be done for more precise simulations. Additionally, the tool is useful for finding lamp setting for desired intensities and spectral compositions.

How to use it:

The application is accessible from anywhere in the world. The user has the option to choose between different Heliospectra's lamps, set the distance between the calculation point and the lamp and the intensity of the different lamp channels. Up to 9 channels can be selected depending on the chosen lamp model. These channels can be dimmed from 0 to 1000, and when changing the settings, the lamp simulator updates the displayed spectrum and tabulated lighting properties (*e.g.* photon flux density (PFD), photosynthetic photon flux density (PPFD), power consumption and color ratios) accordingly (Figure 1). The data can be downloaded for future references.

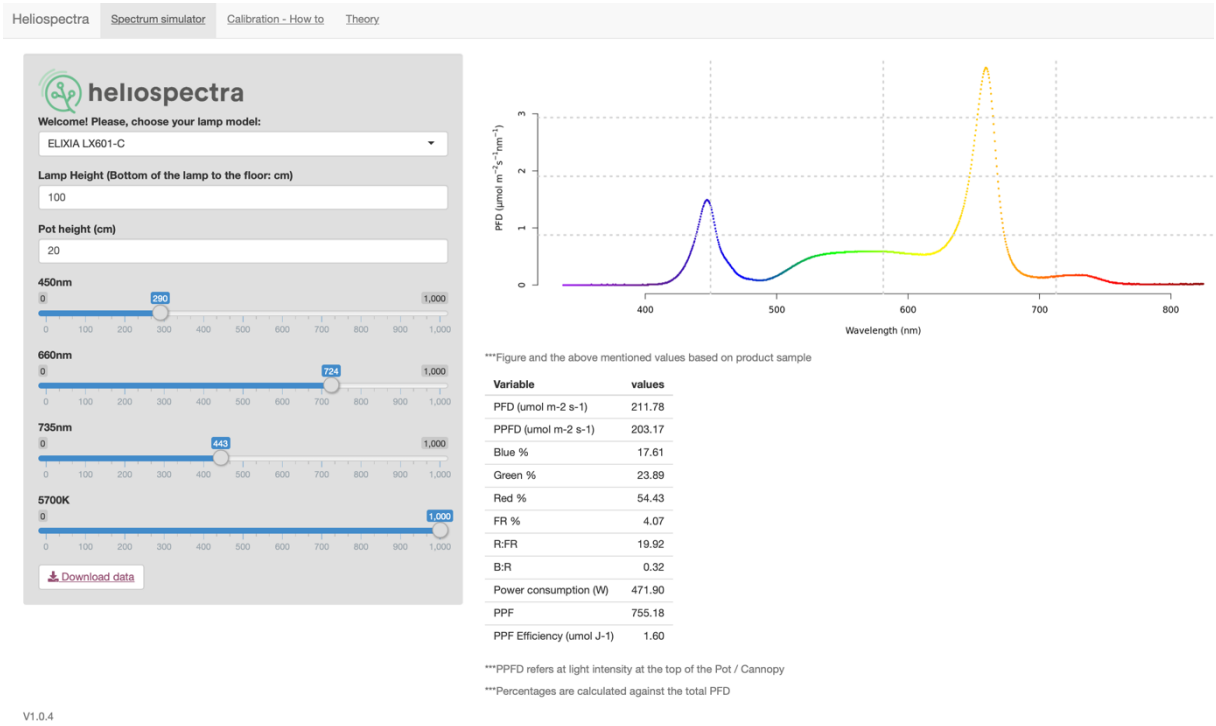


Figure 1: The lamp simulator tool designed for Heliospectra.

It is important to be aware that a light environment is typically site-specific, e.g. due to differences in reflectivity of the surrounding (wall materials and other present bodies), which is why a calibration on place is recommended. This can be done by measuring the light with a normal photosynthetic active radiation (PAR) sensor, and then replace the default calibration file (download and update it).

How light output is calculated:

As input to the application, lamp characterization data of the included lamps are needed. A lamp characterization procedure and protocol were developed for this purpose and most of the

controllable Heliospectra lamps were characterized. For each intensity setting in each LED channel, light output was measured automatically. *A posteriori*, different random combination of multiple channels was also measured, for later validation of the model. With this data, a lamp characterization file was created for each lamp, together with a model.

There are several factors influencing the light environment, which can roughly be divided into two groups: those affecting direct light and those affecting indirect light (*e.g.* reflectance). To simplify the model, the application focus on the factors that affect mainly direct light: the distance to the emitting object and the light output from the emitter (in this case depending on drive current). Other factors, such as light output distribution profile and light reflectance were excluded from the model for practical reasons and for simplicity

Light model: Height effect

As all radiation, light intensity follows an inverse-square law. As light intensity is dependent of the area where the light flux is distributed, the intensity is inversely proportional to the square of the distance from the light source. Therefore, we can see an exponential increase in light intensity when going closer to the lamp (Figure 2). It is good to be aware that the inverse square law applies when the light source can be approximated as a point source ("five times rule"). At short distances the LED lamp, with multiple diodes, does not behave as a point source but many.

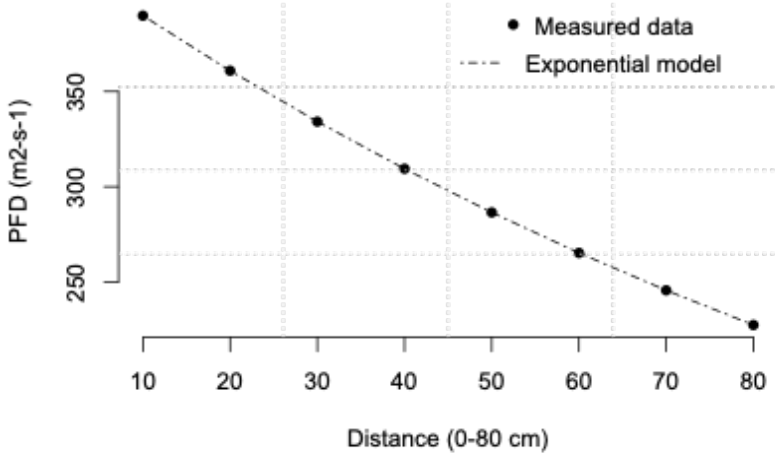


Figure 2: Measured light intensity in the center point at different distances from the lamp. Black dots correspond to measured data, meanwhiles the dashed line corresponds to a fitted exponential model

Light model: Drive current

LED light intensity can be regulated mainly through two different procedures: increasing the drive current through the LED strings (analog dimming) or increasing the duty cycle ("on

time"; PWM dimming). Heliospectra lamps are capable of using both techniques and the light output is set by the lamp intensity setting ranging from 0 to 1000. The standard correlation between intensity setting and light intensity is shown in figure 3, where PWM and analog dimming is applied in the ranges 0-100 and 100-1000, respectively.

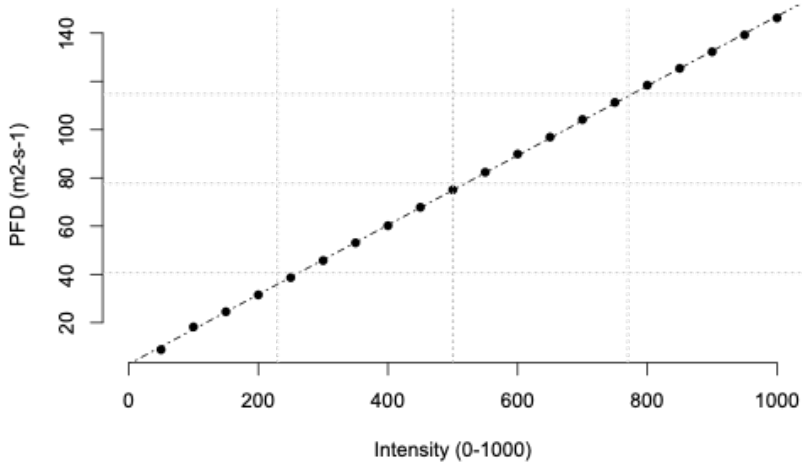


Figure 3: Measured light in the center point at different lamp intensity settings. Black dots correspond to measured data, meanwhiles the dashed line correspond to a fitted exponential model

Although there is a small difference between the pulse wide modulated (PWM) region, and the rest of the curve, this different is not significant compared with the full effect of setup intensity, which allows a linear relationship approximation between lamp intensity setting and lamp output (PFD).

The tool was validated internally at Heliospectra with good correspondence between simulations and measurements (see table 1), which confirms that it is possible to model the different wavelengths combinations without measuring all possible combinations of the individual channels. The application has been up and running since November 2019 and can now be considered a relevant and useful tool internally as well as for customers.

Transfer to other facilities and lighting systems:

The application can easily be transferred to other facilities and lighting systems; R is flexible, and the physical laws of light are the same. This has been done at the facilities of terraXCube (EURAC, Bolzano, Italy) and the phytotrons in the University of Basel (Basel, Switzerland). In both cases the number of light channels were changed depending user specifications and a simple on place calibration was done with measurements at different heights and lamp intensities depending on each individual setup. In both cases the application was successful,

and further as well as new collaborations to this end, *i.e.* light environment characterization, modelling and simulation, are possible for parties that are interested in the tool.

Table 1: Internal validation of v. 1.04 of the lamp simulator tool show differences between measured and simulated values. The performance is good at longer distances (107 and 80 cm) with intensity and spectral differences of a few percentages, but slightly worse at shorter distances (47 cm). This is related to how the height model is calibrated and fitted. V. 1.04 only uses measurement data at 60 and 120 cm to fit the model.

LX601C										
Serial Number: 3119142146										
Measuring date: 2019-11-13										
Simulation date: 2020-01-14										
Simulation tool version: 1.04										
SETTING Nr.		1	2	3	4	5	6	7	8	9
Height (cm)		107			80			47		
Lamp Settings, per channel	450 nm	750	300	50	200	1000	450	70	850	1000
	660 nm	900	500	100	90	300	850	320	1000	80
	735 nm	600	1000	50	450	700	200	90	700	210
	5700K	950	50	400	700	80	300	1000	50	450
MEASURED										
Power (W)		536	227	139	240	218	338	322	379	220
Light intensity (µmol photons/m^2/s	PFD	135	52	36	101	85	153	399	439	252
	PPFD	129	45	35	93	76	149	389	411	241
	B	26	6.2	7.2	25	29	22	77	68	107
	G	27	2	13	36	5.6	17	134	9.8	67
	R	76	37	15	32	41	110	178	334	66
	FR	6	7.3	1	8.2	9.7	3.9	10	27	11
SIMULATED V1.04		0	-0.2	-0.2	0	0.4	0	0	-0.8	1
Power (W)		543	236	149	244	227	348	338	380	227
Light intensity (µmol photons/m^2/s	PFD	137	52	35	103	88	154	439	464	272
	PPFD	131	44	34	95	79	150	428	438	260
	B	28	6	8	26	32	23	88	77	121
	G	27	2	12	36	5	16	145	10	68
	R	76	36	15	33	42	111	194	352	71
	FR	6	7	1	8	9	4	12	27	12
Diff										
Power W		7.43	9.36	10.05	4.34	8.57	9.91	16.31	0.95	7.31
Light intensity (µmol photons/m^2/s	PFD	1.65	-0.48	-0.92	1.87	3.23	0.80	40.05	25.42	19.97
	PPFD	1.96	-0.62	-0.91	2.28	3.03	1.08	38.79	26.89	19.35
	B	1.67	-0.12	0.38	1.43	2.96	0.55	11.48	8.55	14.44
	G	0.23	-0.11	-1.12	-0.18	-0.21	-0.66	11.10	-0.14	0.81
	R	0.06	-0.59	-0.37	1.03	0.68	1.19	16.21	17.68	5.10
	FR	-0.29	-0.16	-0.01	-0.55	-0.50	-0.18	1.52	-0.15	1.02
% Diff										
Power (%Diff)		1.4%	4.1%	7.2%	1.8%	3.9%	2.9%	5.1%	0.3%	3.3%
Light intensity (% diff)	PFD	1.2%	-0.9%	-2.6%	1.9%	3.8%	0.5%	10.0%	5.8%	7.9%
	PPFD	1.5%	-1.4%	-2.6%	2.5%	4.0%	0.7%	10.0%	6.5%	8.0%
	B	6.4%	-1.9%	5.3%	5.7%	10.2%	2.5%	14.9%	12.6%	13.5%
	G	0.9%	-5.5%	-8.6%	-0.5%	-3.8%	-3.9%	8.3%	-1.4%	1.2%
	R	0.1%	-1.6%	-2.5%	3.2%	1.7%	1.1%	9.1%	5.3%	7.7%
	FR	-4.8%	-2.2%	-1.0%	-6.7%	-5.2%	-4.6%	15.2%	-0.6%	9.3%

Natural light simulator tool

What it is:

The "natural light simulator tool", is a program, coded in R using the shiny package, that simulates natural light conditions, spectrum and intensity, at a given time, place and weather condition. It is based on the work of chapter 1: Latitude and Weather Influences on Sun Light Quality and the Relationship to Tree Growth (Chiang *et al.,* 2019). Furthermore, with the addition of Heliospectra lamp models from the previous tool and corresponding physical lamps it is possible to create a natural light environment mimicking the simulated one as far as possible. This feature of the tool can be termed LED controlling software for near-natural plant growth, and together with the lamp simulator tool and the actual lamps it constitutes a complete system for near-natural plant growth.

The control function focuses on light properties that are known to be important for plant growth, such as intensity and light quality. An important notice is that in the case of most Heliospectra lamps, and several other commercially available setups, the amount of far red LEDs is relatively low implying that it is hard to reach natural light R:FR ratios, specially at high light intensities. Based on experience, a maximum light intensity of your setup 6 times the maximum light that will be applied in your experiment may be required to keep spectral properties close to natural.

How to use it:

To use this application, it is necessary to have a computer and Heliospectra lamps, all connected to the same network. The shinny app will directly talk to your lamps after setting the different inputs on the website (Figure 2). When the website is open, light quality and quantity is updated every 5 seconds. The website needs to remain open while light treatments are applied.

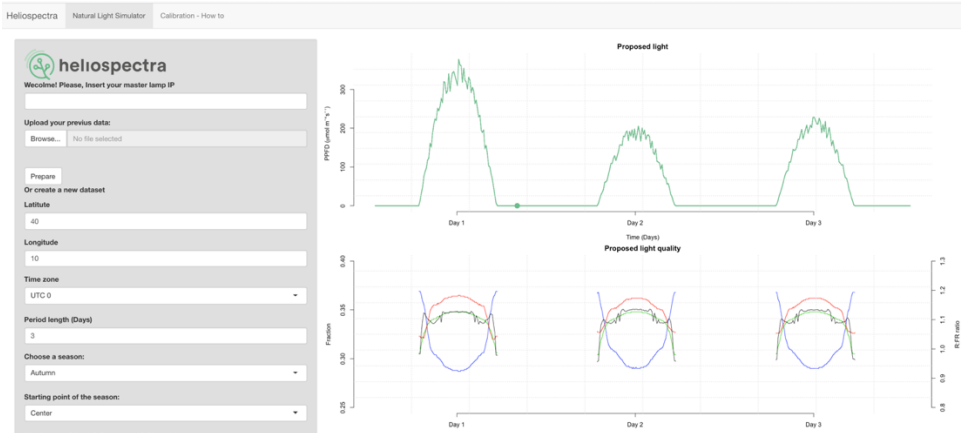


Figure 2: A first view of the running application.

How to set it:

Two different options are available:

- **First time use**: Creating new data set
- **Second time use:** Recreating an older data set

First time use:

Multiple inputs are required for simulation and lamp control. To start is necessary to detect the IP numbers of the used lamps. They can be identified using Heliospectra's HelioConnect software. Ones that the desired IPs have been identified (*e.g.* 192.168.XX.XX) a calibrating of the setup can be done, especially if several lamps are used at the same time. If this is the case, is necessary to set one lamp as a master and the other ones as followers. For calibration on place, download the calibration file on the tab "Calibration – How to" and using a PAR sensor refill the downloaded table at intensities 1000, 500, 200,100,50 and 0, measured in the center point at your plant height. With all this information, the application can be filled in the different fields of the website:

- **Calibration**: Necessary to calculate the maximum PFD of your setup. If calibration is not done, spectral composition will be correct and the fluctuations of intensity proportional to the required maximum values, but the absolute intensities of the system will most likely be inaccurate what may induce errors in the setup.
- **Lamp IP:** Necessary for communication between the website and the lamp. If several lamps are used, indicate the IP of the master lamp.
- **Latitude:** The latitude from where simulate data is desired (values from 90 to -90 are accepted with the north pole as positive values)
- **Longitude:** The longitude from where simulate data is desired (values from 90 to -90 are accepted were east of the Greenwich meridian have positive values)
- **Time zone:** It enables an easy shift of the schedule in time along the day. It is recommended to use it when experiments are starting at a different time of the day, to avoid any possible mistake. Changing the time zone to be able to see when the light will be applied may help to be present at the moment of sunrise and/or sunset. This will not change the values but just displace the shift the diurnal schedule.
- **Period length:** Corresponds to the desired amount of days to be simulated. Just positive whole numbers are accepted.
- **Season:** The period of the year to be simulated. Four options are available: Summer, Spring, Winter and Autumn.

- **Starting point:** When to start the experiment within the season. Three options are available: Beginning, Center and Ending.
- **Weather conditions:** Correspond to a linear approximation of the effect of different weather conditions in light quality and quantity. 10 steps are available with 0 as totally overcast and 10 as clear sky. For more details please refer to the next sections.
- **Weather variability within day:** Correspond to the fluctuation of light (intensity and spectrum) due to weather variations within a day. 10 steps are available with 0 as totally stable conditions and 10 as unstable conditions. For more details please refer to the next sections.
- **Weather variability between days:** Correspond to the fluctuation of light (intensity and spectrum) due to weather variations between days. 10 different steps are available with 0 as totally stable conditions and 10 as unstable conditions. For more details please refer to the next sections.

Once the data has been generated can be previously seen using the button "prepare" and download to be used later using the button "Download data". When pushing the button "load and run" the application will start controlling the lamps mimicking simulated data.

Since it is the web application that sends commands to the lamp, it **is required to keep the application open to keep it running.** It is possible to see the current settings being applied through the red vertical line in the figure. If there is an interruption in the application it is possible to re-upload the data, and it will start running immediately. For more information read the next section.

Second time use:

If for any reason an experiment was interrupted, it is always possible to reload the previously prepared data set and the software will start running it from the actual time. It is also possible to repeat an experiment, recreating the same light conditions; then edit the column "Applied date" in the downloaded data set, to change the starting date and time to any preference. This will replicate exactly the same weather conditions in a second experiment, something that is impossible in natural conditions. If outdoor conditions are sought to be replicated in indoor conditions, the user can do this using the solar angle and the different light properties. It is then recommended to contact Heliospectra for further guidance. For more details see supplementary material.

Log file

The log file will log every time that the application was not able to reach the required conditions or there was a problem to communicate with the lamp. 3 different spectral characteristics are available to flexible the log file. If one of them is ticket, if this condition is not reached for the current settings, a record will be done in the log file. This is possible to visualize through the color of the point who indicate which light has been applied:

- Green indicates that all the conditions have been reached
- Orange indicates that one or several spectral characteristics have not been reached
- Red indicates that the desired light intensity in your actual setup was not reached.

How it was calculated:

Sun light fluctuation:

Sun light fluctuation at extraterrestrial level is highly dependent of the sun activity and the distance between the sun and the earth. As changes in sun activity are almost neglectable for plant experiments, the application focus in the distance between the sun and the earth. As this distance change along the time of the year we can calculate the Earth's solar irradiance as:

$$H = Hconstant * (1 + 0.033 * \cos\left(\frac{360 * (n-2)}{365}\right))$$

Where $H_{constant}$ is equal to 1.353 kWm^{-2} and N the day of the year.

Solar radiation at the earth's surface

The solar radiation at surface level will be depended of several factor mainly related to time (Time of the day and time of the year), geography (Latitude, Longitude and Altitude) and Weather conditions. Using the Earth's solar irradiance previously calculated and the solar angle depending of the previous mentioned variables (Time and geography) it is possible to calculate the solar angle and therefore the "Clear Sky" radiation. Detailed information for calculation can be found on the NOAA solar calculator (https://www.esrl.noaa.gov/gmd/grad/solcalc/).

Weather effect

Once calculated the clear sky potential radiation for the desired place and time, it is possible to add the effect of fluctuations due specific weather conditions. If is wished, perfect clear sky conditions can be replicate using the setting "Weather condition" in 10 and both "Weather variability within days" and "Weather variability between days" at 0. To understand the effect of the weather in light quantity and quality, was necessary to analyze the data from the paper

Latitude and Weather Influences on Sun Light Quality and the Relationship to Tree Growth (Chiang *et al.,* 2019). As cloud cover was not measured during the previously mentioned work, a linear relationship was assumed (in 11 different steps). As is showed in Figure 1, even under totally cover days, the solar radiation is closet to 10 percent of the clear sky model. Additionally, in Figure 2 the effect of weather conditions in light quality depending of the sun angle can be extracted and applied in our model

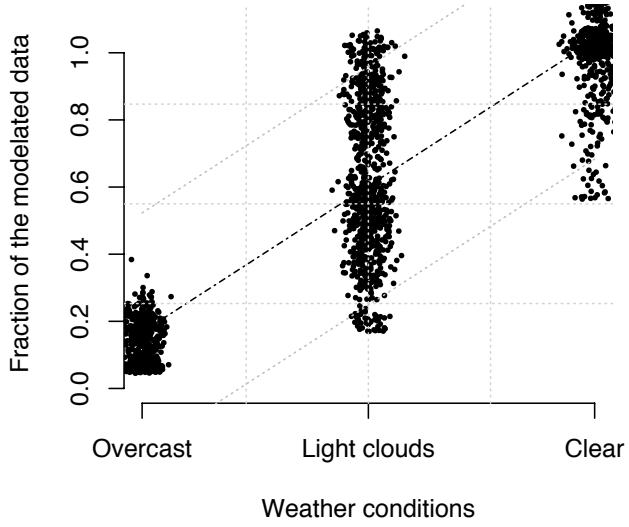


Figure 1. Effect of the cloud cover on the available total PPFD as fraction of the modeled data.

Additionally, to this is also possible to calculate the deviation of values within days. As is showed in Figure 1 and corroborate by Figure 3, partly cover days have higher variation in light quantity compared that cover days and clear sky days. This allow to model the fluctuation within days, assuming a linear relationship between the different weather conditions and the light quality. This same behavior has been reported independent of the geographic location

Finally, but not least important, due that there is a relationship between the place and the weather condition between days, simple statistics was used to create a linear relationship between days: with non-variation between days (value 0) to total variable between days (value 10), what allow the application to add variation between days. A strong limitation of the use of average statistical values is that the application is not able to recreate a day where *e.g.* half of this one was cloudy and the other half sunny, due that the average of this condition is a 50 percent reduction of the total solar radiation. In the other hand, this allow us to create trustable data with respect at the microvariations within days.

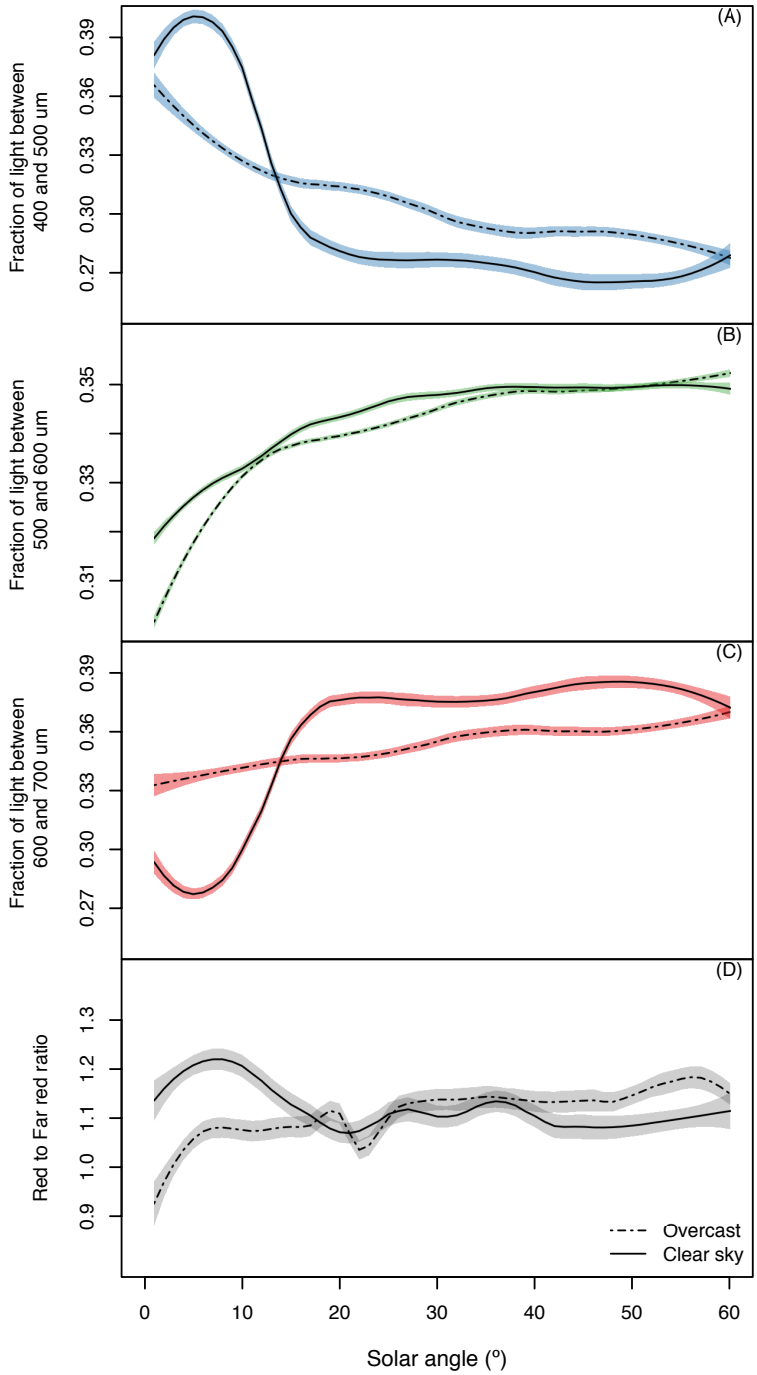


Figure 2.: Extracted from Latitude and Weather Influences on Sun Light Quality and the Relationship to Tree Growth (Chiang *et al.*, 2019). Changes in light quality as a fraction of the photosynthetic photon flux density (PPFD) depending of cloudiness (full line: clear sky, dotted line: overcast conditions) and the solar elevation angel. A) blue light fraction (from 400 to 500 nm), B) green light fraction (from 500 to 600 nm), C) red light fraction (from 600 to 700 nm). D) red to far red (R:FR) ratio. The lines represent the mean value of one day of each weather condition per month (n=12; see methods for detail). Shaded areas correspond to the standard error of a locally estimated scatterplot smoothing (LOESS) fitted model.

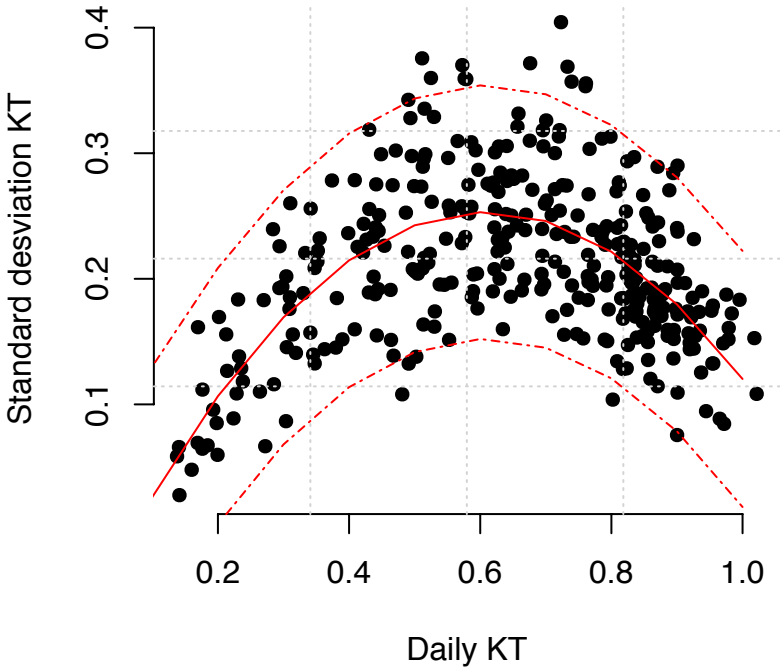


Figure 3. Effect of the daily light (KT) on the standard deviation of the light along the day. As observed in Figure 1. Middle KT values have a higher variance compared with totally cloudy (low KT) and clear sky days (high KT).

Supplementary material 1: Recreating outdoor experiments in indoor conditions

Recreating previously recorded outdoor conditions is possible. Of course, small variations in light quality will be dependent in the surrounding of the place where the measurement took place and therefore open spaces are recommended. For this, the following steps can be followed:

- At the time of your data collection, record light quantity with at least a resolution of every minute.
- Using the Heliospectra – Natural light simulator application, calculate the clear sky radiation for your place (Weather condition = 10; Weather variability within day = 0; Weather variability between days = 0). Download the dataset.
- Using the Heliospectra – Natural light simulator application, calculate the overcast radiation for your place and time (Weather condition = 0; Weather variability within day = 0; Weather variability between days = 0). Download the dataset.
- Interpolate your recorded data to get a resolution of every 5 seconds as the previously downloaded datasets.
- Compare your recorded dataset with the clear sky one. Even in a sunny day you will see differences. This is due that the NOAA model predicts just direct solar radiation. You can correct your clear sky data if you have enough sunny days fitting a model to the difference.
- Using the clear sky data set and the overcast dataset, compare your recorded values and classify these ones from 0 to 1 in a linear model. This will allow you small changes in light quality due to weather condition. You can follow the next example:

Clear sky output (μmol m^2s^{-1})	1800
Overcast output (μmol m_2s^{-1})	300
Recorded dataset (μmol m_2s^{-1})	600
Calculations	$\frac{600-300}{1800-300}=\frac{300}{1500}=0.2$

- Creating a copy of one of the data sets (either clear sky or overcast one) replace the column of light intensity with your interpolated data.
- At each measurement interpolate the different percentages of light quality depending on your previous calculation. You can follow the next example:

Bper clear sky	0.25
Bper overcast	0.15
Calculations	$0.2 * (0.25 - 0.15) + 0.15 = 0.152$

- As you see, variations due weather conditions are small, and the biggest variations are due the solar angle.
- Upload your data set to Heliospectra - Natural light simulator application and the application is ready.

General summary and conclusions

The presented work identified and quantified effects of what was previously mentioned as environmental sources of variation between indoor and outdoor experiments (*e.g.* Poorter *et al.,* 2016; Matsubara 2018). In chapter 1, is aimed to demonstrate how natural sunlight quality can change and how plants might adapt to site specific light conditions. In chapters 2-5, the applicability of more natural climatic conditions in indoor growth facilities (with different levels of complexity) was assessed in order to close the gap between indoor and outdoor plant growth and enable more natural-like plant performance in indoor experiments (Chapter 2-5).

In chapter 1, natural light spectra measurements along a year for different weather conditions, from a plant-biological point of view were reported. This make easier the reutilization of the obtained data, in modern LED technologies (see appendix). Additionally, this chapter discussed how trees might adapt evolutionary to the local light situations of their origin, and how such adaptations can be tested in indoor experiments. For example, we hypothesized that northern ecotypes may require higher levels of R or FR light to avoid premature budset due the significantly longer times under these conditions closer to the poles, especially in spring and autumn.

Chapter 2 summarized results of a study that aimed to identify blue and red light ratios in LED lamps that lead to the most natural plant growth in plant chambers (at a constant and close to natural red: far-red ratio). In contrast to previous studies that recommended a minimum proportion of 6 % blue light (*e.g.* Hogewoning *et al.,* 2010), was found that in different plant species, blue proportions of over 20 % are necessary in indoor growth chambers to reach near natural plant growth.

In chapter 3, the importance of applying fluctuating environmental conditions in indoor plant growth facilities to reach more natural-like plant growth was tested. In a series of experiments, it was demonstrated that plant growth and many physiological plant traits are behaving more natural if plants are not grown under constant mean day and night temperatures, but under sinusoidal daily climates that better reproduce natural maximum and minimum values of environmental factors. Surprisingly, a treatment that reproduced exactly the natural fluctuations of environmental factors (as recorded by a meteo station) did in general not lead to a further improvement over the sinusoidal conditions in terms of a more natural-like plant growth. On one hand, this study demonstrated that sinusoidal diurnal climate variations are preferable over static conditions, but on the other hand, it also showed that the exact replication

of natural plant growth cannot be reach in indoor growth chambers, even if natural temperature, humidity and light fluctuations are applied.

Chapter 4 presented data from an experiment that investigated possible negative effects on plants growing in indoor facilities, when fluctuations of environmental factors are not applied in synchrony (*e.g.*, Annunziata *et al.,* 2018). Such situations often occur for example in greenhouses, when plants are exposed to strong variations of sun light intensity at simultaneous constant temperatures. Under the specific conditions of our experiment where temperatures varied only within a physiologically benign range, the presence or absence of light variations had a stronger effect on most investigated plant traits than temperature. However, if fixed light conditions were combined with variable temperatures, we observed negative effects on the performance of some of the investigated species, indicating the importance to apply environmental variability in synchrony in closed growth chambers.

Finally, Chapter 5 and the appendix, show examples of how more natural lighting conditions could help to produce closer-to-natural plant products in indoor facilities. As was indicated in chapter 5, the use of blue light during the morning can induce a faster increase of the stomatal conductance (and thus photosynthesis), although, in the experiments this was not transferred to a larger biomass gain. The use of fluctuating light in indoor growth facilities, can also have a positive effect for commercial indoor farming, since plants grown under fluctuating light tended to have higher water retention after harvest. Certainly, additional and larger-scale experiments in this field will be necessary to assess the full potential of targeted light application for improved indoor plant production.

Conclusions

The present thesis demonstrates the importance of applying more natural growth conditions in indoor experiments. As previously proposed by several authors, the use of more dynamic climatic conditions promoted closer-to-nature plant responses. Near natural plant performance is important in indoor experiments that aim to be extrapolated later to outdoor conditions, as well as in commercial indoor plant production, where more natural-like phenotypes may be desired.

The simultaneous investigation of several, very different plant species in all experiments proofed to be relevant. This approach is in contrast to most previous studies in this field, that largely focused on one species (mostly *Arabidopsis thaliana*) or a view closely related species. The significant species effects in response to the applied phytotron treatments in the presented experiments revealed a high degree of species-specificity mainly in the reaction magnitude, but

not necessarily in the response direction. The multi-species approach allowed to find general patterns across species. Very generally spoken, more natural-like plant growth could be achieved when the blue proportion of the PPFD was around 30 %, when at least sinusoidal daily temperature and light variations were applied, and when light and temperature fluctuations were applied in synchrony.

Another important insight from the experiments was that although we were able to reproduce very closely the highly dynamic temperature, humidity and light conditions, that were recorded during the field trials, within our phytotrons, most of the measured plant traits were still significantly different from field grown plants. This corroborates previous comments (*e.g.* Poorter et al. 2016) that care has to be taken, whenever results from indoor plant experiments are extrapolated to natural ecosystems. Although many factors might have contributed to the persistent differences between field- and phytotron-grown plants in the experiments, two factors are likely most critical. First, the requirement of constantly high levels of air flow in plant growth chambers, in order to reach the required levels of air humidity and temperature, might significantly influence plant growth by reducing the plants' boundary layer resistance, as well as by direct mechanical impacts on aboveground plant organs. Second, the absence of UV light in plant growth chambers. This is especially a problem for LED lighting systems, since the currently available UV-LED diodes are energetically inefficient and have a very short life-time. However, these technical limitations might be overcome in the nearer future, which would allow to include UV diodes by default in commercial LED assimilation lamp systems.

In conclusion, this thesis could show that more natural-like plant growth can be reached in indoor growth facilities without an excessive complication of the experimental setup. The prudent application of dynamic changes in temperature and light quantity and quality can help to produce plants that are closer to fill grown plants in terms of productivity and physiology. The experiments presented here will hopefully contribute to the development of a new generation of software and hardware for plant growth chambers that will enable more natural growth conditions for plant research and commercial indoor plant production.

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