forests

Article

Morphological and Physiological Responses of *Pinus massoniana* Seedlings to Different Light Gradients

Haoyun Wang [1,2,3], Feng Wu [1,2,3,*], Min Li [1,2,3], Xiaokun Zhu [1,2,3], Changshuang Shi [1,2,3] and Guijie Ding [1,2,3]

1 Institute for Forest Resources and Environment of Guizhou, Guizhou University, Guiyang 550025, China; hyunwangat@gmail.com (H.W.); minruili0105@gmail.com (M.L.); xkun.zhugz@gmail.com (X.Z.); cshuang.shigz@gmail.com (C.S.); gjding@gzu.edu.cn (G.D.)
2 Key Laboratory of Forest Cultivation in Plateau Mountain of Guizhou Province, Guizhou University, Guiyang 550025, China
3 College of Forestry, Guizhou University, Guiyang 550025, China
* Correspondence: fwu@gzu.edu.cn

Abstract: Light intensity is a critical factor regulating photosynthetic capacity in plants. However, the effects of varying light intensity on morphological and photoprotective mechanisms in *Pinus massoniana* seedlings have not been explored in depth, especially those in the first seedling growing season. We measured the growth, photosynthetic physiology, biochemistry, and chlorophyll fluorescence of *P. massoniana* seedlings at four light gradients: 100% relative irradiance (RI, full sunlight), 70% RI, 50% RI, and 20% RI. The seedling height at 70% RI was 9.27% higher than that at 100% RI. However, seedling height was inhibited under low light intensity; at 20% RI, all seedlings died. The decreasing light intensity inhibited ground diameter growth but increased the height-diameter ratio. The secondary needle emergence rate was 53.4% higher at 70% RI than at 100% RI but was only 2% at 50% RI. The chlorophyll and carotenoid contents increased significantly with decreasing light intensity. The increased Chl b and Car contents promoted the photoreceptor potential of the violet (400~420 nm), blue (440~480 nm), and yellow-orange (597~655 nm) regions in leaves. Among the chlorophyll fluorescence parameters, Fv/Fm, Fv′/Fm′, Y(II), q_p, and ETR all reached maximum values at 70% RI but were significantly lower at 50% RI than at 100% RI. However, decreasing the light intensity caused a reduction in NPQ. The 70% RI level increased POD and SOD activity and the contents of osmotic regulation substances and slowed MDA accumulation. Seedlings at 70% RI had a higher growth rate, higher photosynthetic activity and potential, and significantly greater stress resistance than the other seedlings. Therefore, appropriate shading measures were beneficial to the cultivation of vigorous seedlings. Furthermore, spectral reflectance indexes were found to be a suitable tool for monitoring the photosynthetic physiological characteristics, stress resistance characteristics, and growth status of *P. massoniana* seedlings in real time.

Keywords: *Pinus massoniana*; light gradient; chlorophyll fluorescence; spectral index; photosynthetic physiological; biochemical attributes

check for updates

Citation: Wang, H.; Wu, F.; Li, M.; Zhu, X.; Shi, C.; Ding, G. Morphological and Physiological Responses of *Pinus massoniana* Seedlings to Different Light Gradients. *Forests* **2021**, *12*, 523. https://doi.org/10.3390/f12050523

Academic Editor: Cate Macinnis-Ng

Received: 18 March 2021
Accepted: 22 April 2021
Published: 24 April 2021

1. Introduction

Light is the environmental factor that most directly affects seedling survival and early growth [1] and is also an important ecological factor in forest community succession [2]. The complexity of forest ecosystems results in the creation of light gradients when vertical light passes through the forest canopy, and the differences in the physiological and ecological traits of seedlings under different light environments explain niche differentiation along the succession axis to a large extent [3]. Nevertheless, the adaptation of plants to light gradients limits not only the regulation of the photosynthetic capacity of individual leaves but also the overall photosynthetic characteristics of seedlings. In both saplings and mature trees, the light adaptation strategy of the leaves will directly determine the carbon acquisition and energy distribution modes [4,5]. At present, light intensity regulation is considered an effective means to promote carbon transfer (organ construction or nutrient storage) in nursery management for most crops and ornamental plants [6]. A change in light intensity causes differences in the plant growth environment that have a significant influence on plant morphology, photosynthetic characteristics, and development [7]. Therefore, species tolerance to light gradients in early ontogenetic development has important guiding significance for forest management, ecosystem restoration and horticultural cultivation. Many species have been evaluated extensively in terms of the characteristics of plant responses to different light conditions [8,9].

In forest nurseries, superior seedlings are cultivated for use as high-quality forest saplings in the construction development of artificial forests and desertification control projects [10]. However, the habitat changes caused by seasonal light environment conditions result in high mortality in the seedling stage [11]. Therefore, seedling establishment is a demographic bottleneck for plant regeneration, and the real-time monitoring of seedling adaptability and plasticity under different ecological environments is conducive to cultivating excellent seedlings and achieving precise forest management [12]. Plants are organisms that are composed of a variety of modularized tissues. When the external light environment poses a threat, plants activate defence responses in only the most valuable modules and focus their captured defence resources on this par; however, not all tissue modules are equally important [13]. Hence, the induction of plant defences against external attacks is systematic, and changes in photoreceptor signals are helpful for understanding the adaptability of plants under different light conditions. Summer is the peak period of growth and development for most plants, and hot climate conditions are conducive to the accumulation of biomass and vegetative growth [14]. For example, the differentiation of bud organs in temperate species usually takes place in summer [15]. However, the higher light intensity in summer is far beyond the capacity limits of plant photosynthetic organs, and excessive light energy induces the activation of plant light protection mechanisms that intensify thermal energy dissipation or increase reactive oxygen species (ROS) accumulation [16]. Light stress can cause damage to the photosynthetic organs, membrane lipid peroxidation, and even plant death in extreme cases [17]. Therefore, proper shading can protect photosynthetic organs and facilitate photochemical reactions in plants [18].

Shading, an effective cultivation technique, reduces sunlight exposure, air temperatures, and evapotranspiration while increasing relative humidity, and is widely used in agricultural ecosystem management [19,20]. For forestry applications, shading significantly improved the photosynthetic efficiency of photosystem II for subtropical trees [21], promoted the germination and elongation of new leaves [22], and reduced the sunburn rate in pitaya fruit [23]. Therefore, shading can be used as an effective method to evaluate the potential physiological response of seedlings to light energy utilization. However, a prolonged period of insufficient light inhibits photosynthesis and reduces net carbon gains [24]. In contrast, under weak irradiance conditions, insufficient carbon accumulation severely restricts plant growth [25]. Some studies have indicated that pioneer tree species cannot adapt to weak irradiance environments [26], and seedling mortality increases significantly when the light intensity is below 15% [27]. Currently, plant light stress response mechanism is understood as follows: (1) Increasing non-photochemical quenching (NPQ) at high light intensities uses too much light energy to release through thermal dissipation [28]. (2) Plants adjust their photosynthetic pigment content to absorb more light quanta and increase their photosynthesis rate at low light intensities [29,30]. (3) Under adverse light conditions, excessive ROS can be removed by increasing antioxidant enzyme activity (superoxide dismutase (SOD) and peroxidase (POD)), reducing malondialdehyde (MDA) accumulation, and avoiding membrane lipid peroxidation and cell damage [31,32]. (4) Osmotic regulation substances (soluble sugars (SS), soluble protein (SP) and RPO) are used to maintain the level of cell osmotic potential in plants and improve the protective effect of biofilms [33,34]. Among these protection systems, the NPQ mechanism and ROS elimination capacity are often used to detect plant adaptation levels under light stress [35,36]. The measurement of conventional biochemical indexes is not conducive to the long-term monitoring of plant growth in the field [37]. As a consequence, it is imperative to accurately and effectively evaluate the physiological status of seedlings.

Chlorophyll fluorescence measurement uses modulation and saturation pulse technology to continuously monitor plant photosynthetic activity on a long-term basis [38]. Chlorophyll fluorescence parameters are used mainly to measure the potential quantum yield under high light intensity and shaded conditions to reflect the performance of plant photosynthetic organs [39]. Because of its sensitivity and convenience, chlorophyll fluorescence measurement is often used for real-time plant growth monitoring in the field [40]. Optical remote sensing (RS) is a technique used in the rapid assessment of ecosystem status and function [41]. Spectral reflectance measurements are rapid and non-invasive, which gives them great application potential as an RS technology. Spectral reflectance has been widely applied to assess plant responses to environmental changes [42,43] and is used as a parameter to reflect the effects of long-term plant stress [44]. Vegetation indexes (VIs) based on spectral reflectance ratios at different wavelengths as well as the water band index (WBI) have been used to monitor indicators of the initial damage to a plant; as indicators of green biomass (NDVI), the chlorophyll concentration ('Red Edge' NDVI), the structure-independent pigment index (SIPI), and the carotenoid and xanthophyll pigment contents (CRI); and to evaluate plant physiology or stress responses (PRI) [45,46]. Spectral reflectance, as a key light response feature, can be used to monitor plant responses to light stress [47] and to further characterize the basic biological functions of plants [48].

Pinus massoniana Lamb. is the main pioneer tree species used for afforestation in South China and is a typical heliophyte [49]. In the seedling stage, primary (juvenile) and secondary (adult) needles are the main photosynthetic organs developed in their growth cycle and play an adaptive role in responding to complex environments through seedling ontogeny and species' ecological niches [50]. After seed germination, with the elongation of the cotyledon, the primary needles grow gradually from young stems that develop from the growth point of the epicotyl; then, secondary needles grow in the axils of the primary needles [51]. We found that current-year *P. massoniana* seedlings with secondary needles exhibited less energy and thermal dissipation, more dry matter accumulation, stronger drought resistance under severe drought stress, and faster response ability after rehydration than those without secondary needles [52,53]. In the early stage of seedling growth, especially during leaf morphological development, seedlings are easily affected by the heterogeneity of the light environment caused by seasonal changes [54]. In the present study, we focused on the seedling stage, which is highest to respond pressure. We investigated the initiation of secondary needles in current-year seedlings under a light gradient and performed quantitative analyses of the pigment contents, photosynthetic characteristics, protective enzyme activity, and osmotic regulation substance contents during seedling growth to test the following hypotheses: (1) during the whole growth season, from cotyledon germination to the end of the seedling growth period, the seedlings would show different responses and regulatory mechanisms in morphology and metabolic systems along the light gradient, and (2) the change trend between the vegetation index and the measured values would capture the intrinsic differences among seedlings, indicating the feasibility of using spectral reflectance measurement technology to monitor the physiological status of seedlings in real time. We foresee that spectral reflectance measurement may be a useful tool to provide a theoretical scientific basis for seedling selection and precise management in various habitats.

2. Materials and Methods

2.1. Plant Materials and Light Treatments

P. massoniana seeds were obtained from seed orchard in national base of improved tree variety (26°9′ N, 107°18′ E, 920 m a.s.l.), located in Duyun, Guizhou Province, China. The experiments were carried out in an outdoor field (26°26′ N, 106°39′ E, 900 m a.s.l.) in Guizhou University, Guiyang, China. Uniform current-year seedlings were transplanted into nonwoven fabric containers (8.0 cm diameter, 12.0 cm height). The substrate mixture was a topsoil:humus:vermiculite mixture (2:1:1, $v/v/v$; pH 5.3) combined with manure. The substrate mixture had an organic matter content of 76.84 $g \cdot kg^{-1}$, a total N content of 0.78 $g \cdot kg^{-1}$, a total P content of 0.74 $g \cdot kg^{-1}$, and a total K content of 11.51 $g \cdot kg^{-1}$; the alkali-hydrolysed nitrogen, available phosphorus, and available potassium contents were 47.81, 40.00 $g \cdot kg^{-1}$, and 80.00 $mg \cdot kg^{-1}$. During the experiment, the average temperature was 23.2 °C, and the total precipitation was 500 mm. To prevent drought from affecting seedling growth, seedlings were watered to field capacity once a day.

The experiment was carried out under a randomized completed block design consisting of three blocks, with four light levels: 100% full sunlight (100% RI), 70% of full sunlight (70% RI), 50% of full sunlight (50% RI), and 20% of full sunlight (20% RI). The 70% RI, 50% RI and 20% RI treatments were implemented with the use of neutral shade netting. Shade nets of different densities were used to build shade shelters (2 m × 2 m × 1.5 m, length × width × height) that were placed with the seedlings in the central area (n = 50). A total of 600 seedlings were treated. Spectral distribution of light under the shade nets was measured for several days, during partly cloudy weather using a fiber-optic spectrometer (Maya 2000 Pro, Ocean Optics Inc., Dunedin, FL, USA). The relative light intensity (%) was measured at multiple random sites under the shade nets using an illumination metre (LI-250A, Li-Cor Inc., Lincoln, NE, USA). The maximum light intensity in the natural field was approximately 1382 or 188 $\mu mol \cdot m^{-2} s^{-1}$ on clear or overcast days from May to September 2017, respectively. The shading experiments were conducted during the summer (1 June through 31 August) in 2017. For the analyses of pigment contents and photosynthetic characteristics, the topmost fully expanded needles were sampled from different individuals in each treatment after exposure to the light treatments for 150 days. All photosynthetic measurements were taken from 9:00–11:00 a.m. to avoid the midday reduction in photosynthetic capacity.

2.2. Plant Growth Measurement

After 150 days of treatment, the number of seedlings with secondary needles was counted. The height (cm) and ground diameter (mm) of seedlings with different foliage types were measured with a ruler and Vernier calliper.

2.3. Spectral Reflectance

For the non-destructive leaf spectral reflectance measurements, a CI-710 (CID-Bio-Science Inc., Camas, WA., USA) was used, which rapidly estimates plant spectral absorbance properties using published and accepted vegetation indexes (Table 1) [55]. The CI-710 consists of two modules, a leaf probe and a linear CCD-based spectrometer, and it has a wavelength range of 400–1000 nm, an optical sample size of 7.6 mm, and an optical resolution of 1.5–2.0 nm full width at half maximum (FWHM). A computer program, Spectra Snap, is used to set measurement parameters and display the spectral data. The leaf probe is equipped with a tungsten-LED dual light source that provides a broad range of wavelengths of light that are suitable for visible and near-infrared spectroscopy. The light then passes through a bifurcated fibre optic cable and connects to one of two sampling light ports on the side of the leaf probe for transmission or reflectivity measurements. Six seedlings were randomly selected for measurement in each treatment, and the current-year needles were arranged side by side in order to cover the whole leaf chamber for the measurements.

Table 1. Optimization of spectral parameters and calculation formula.

Spectrum Parameters	Definition	Reference
Carotenoid Reflectance Index	CRI = $(1/R_{510}) - (1/R_{550})$	[56]
Structure Intensive Pigment Index	SIPI = $(R_{800} - R_{445})/(R_{800} + R_{680})$	[45]
Red Edge Normalized Difference Vegetation Index	RENDVI = $(R_{750} - R_{705})/(R_{750} + R_{705})$	[57]
Normalized Difference Vegetation Index	NDVI = $(R_{800} - R_{680})/(R_{800} + R_{680})$	[45]
Modified Red Edge Simple Ratio Index	MRESRI = $(R_{705} - R_{445})/(R_{705}/R_{455})$	[56]
Photochemical Reflectance Index	PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$	[58]
Water Band Index	WBI = (R_{970}/R_{900})	[58]

In the formula, R stands for reflectivity, and the subscript number stands for spectral wavelength.

2.4. Chlorophyll Fluorescence Parameters

According to the method of Guo et al. [59], the chlorophyll fluorescence parameters of needles under different light intensities were measured using a MONITORING-PAM (Heinz Walz GmbH, Effeltrich, Germany), and the spectral parameters were obtained at the same time. The dark adaptation period before the measurement was 35–40 min, and then a PIN-photodiode with an extended pass filter and the MONI-HEAD/485 of the integrated optical quantum sensor were connected with the sample clamp equipped with a scattering disk. The needles were fully spread throughout the sample clamp with PAM modulation, and the measurement data were transmitted to the computer through MONI-IB4 and MONI-DA. Finally, the fast chlorophyll fluorescence curves and the rapid optical response curves (RLCs) were simulated in WinControl-3 software. The maximum photochemical efficiency (Fv/Fm) [60], effective photosynthetic quantum yield (Fv′/Fm′) [61], actual quantum yield [Y(II)] [62], photochemical quenching coefficient (q_p), non-photochemical quenching coefficient (NPQ) [63], and maximum relative electron transfer rate (ETRmax) were obtained from OJIP curves and RLCs.

2.5. Pigment Analysis

The photosynthetic pigment contents of the needles were measured using spectrophotometry (UV-2100, UNICO, Princeton, NJ, USA). Fresh, south-facing needles were collected and quickly packed into a self-sealing bag and brought back to the laboratory in an icebox. The photosynthetic pigment contents were determined by acetone extraction [64]. The chlorophyll extract was subsequently injected into a spectrophotometer using the energy spectrum from 470 to 662 nm to assess the photosynthetic pigment content ($mg \cdot g^{-1}$); each treatment had six replicates. The chlorophyll a (Chl a), chlorophyll b (Chl b), Total chlorophyll (Chl c), and carotene (Car) contents were calculated according to the following formulas:

$$\text{Chl a (mg/mL)} = 11.75\ A_{662} - 2.35\ A_{645} \tag{1}$$

$$\text{Chl b (mg/mL)} = 18.61\ A_{645} - 3.96\ A_{662} \tag{2}$$

$$\text{Chl c (mg/mL)} = \text{Chl a} + \text{Chl b} \tag{3}$$

$$\text{Car (mg/mL)} = (1000\ A_{470} - 2.27\ \text{Chl a} - 81.4\ \text{Chl b})/227 \tag{4}$$

$$\text{Pigment contents (mg/g)} = (C \times V)/(W \times 1000) \tag{5}$$

where A_{662}, A_{645}, and A_{470} are the light absorption at 662, 645, and 470 nm, respectively; V (mL) is the volume of the extract, and W (g) is the weight of fresh needles.

2.6. Determination of Osmotic Regulation Substances and Lipid Peroxidation

The treatment material collection method was the same as that used for the determination of pigment content, and then, the SS content was determined by the anthrone colorimetric method [65]. Fresh samples (0.2 g) were weighed out, cut into pieces, and blended in a graduated test tube. Distilled water (10 mL) was added to the cylinder, the cylinder was sealed, and the mixture was boiled for 30 min. After cooling, the reagent was filtered into a 25 mL volumetric flask, and the volume was adjusted. Then, 0.5 mL of the reagent was transferred into a 10 mL test tube, as well as 5 mL anthrone solution, mixing with shaken. The mixture was placed in a boiling water bath for 10 min and absorbance read at 620 nm. The SS content calculation formula was

$$SS\ (\%) = [(C \cdot Vt)/(V \cdot W \cdot 10^6)] \times 100\% \quad (6)$$

where C represents the SS content of the extract in μg, which is derived from the standard curve; Vt represents the total volume of the extract, mL; V represents the volume absorbed during the determination, mL; and W is the sample weight, g.

The content of SP was measured by the Coomassie brilliant blue G-250 staining method and was measured spectrophotometrically at 595 nm [66]. The content of free proline (PRO) was measured by the ninhydrin colouration method and was measured spectrophotometrically at 520 nm [67]. MDA accumulation was determined by a thiobarbituric acid reactive substances assay and was measured spectrophotometrically at 532 and 600 nm [68].

2.7. Activities of Antioxidant Enzymes

POD activity was determined according to a previously described method [69]. First, 0.3 g of fresh sample was weighed, and 0.1 mol/L phosphate buffer (pH = 6.0) was added and then ground into a homogenate on an icebox. After centrifugation at 10,000 r/min for 10 min, the supernatant was extracted for preservation. The enzyme solution (1 mL) was absorbed into the colourimetry tube, and the control was 1 mL 0.1 mol/L phosphate buffer (pH = 6.0). Then, 3 mL reaction mixture was added. The reaction mixture contained 50 mL 0.1 mol/L phosphate buffer (pH = 6.0) and 28 μL guaiacol dissolved into the buffer mixed evenly with 19 μL 30% hydrogen peroxide. The OD value was measured at 470 nm every minute for 5 times. The calculation formula was

$$\text{POD activity } [U/(g \cdot min)] = (\Delta A_{470} \cdot Vt)/(W \cdot Vs \cdot 0.01 \cdot T) \quad (7)$$

where ΔA_{470} represents the change in the OD value during the reaction time, Vt represents the total volume of the extracted enzyme solution, mL; W is the sample weight, g; Vs is the extracted enzyme liquid volume, mL; and T is the reaction time, min.

SOD activity was measured spectrophotometrically at 560 nm [70].

2.8. Statistical Analysis

Microsoft Excel (2016) was used for preliminary statistics. The significance of the results was checked by using the least-significant difference (LSD) test and Tukey–Kramer comparison at least at the 5% level with SPSS 20.0 (SPSS, Chicago, IL, USA) via one-way analysis of variance (ANOVA). Origin 8.5 (OriginLab Corporation, Northampton, MA, USA) was used to plot the data.

3. Results

3.1. Effects of Light Conditions on Morphological Characteristics

As shown in Table 2, there were significant differences in the morphological indexes of *P. massoniana* seedlings, all of which were affected by the RI treatments. The seedlings treated with 20% RI all died. The seedling height at 70% RI was significantly higher than that at 100% RI, while the seedling height at 50% RI was significantly lower than that at 100% RI. At 70% RI and 50% RI, the ground diameters of seedlings were significantly lower than at 100% RI, decreasing by 12.33% and 35.62%, respectively. Compared with that at 100% RI, the height-diameter ratios at 70% RI and 50% RI were 1.25 and 1.31 times higher, respectively. The results showed that an appropriate light intensity was beneficial to seedling height growth, while long-term exposure to low light intensity inhibited the longitudinal growth and diameter of the seedlings. The percentage of seedlings with secondary needles at 100% RI was 24.33%, that at 70% RI was 37.33% (53.43% higher), and that at 50% RI was only 2.00%.

Table 2. Comparison of *P. massoniana* seedling growth under different light treatments (means ± SD).

Treatment	Seedling Height/cm	Ground Diameter/mm	Height/Diameter Ratio	Seedlings with Secondary Needles/%
100% RI	12.41 ± 0.82 b	1.46 ± 0.11 a	8.50 ± 0.56 b	24.33 ± 3.78 b
70% RI	13.56 ± 0.88 a	1.28 ± 0.06 b	10.59 ± 0.74 a	37.33 ± 4.62 a
50% RI	10.49 ± 0.49 c	0.94 ± 0.06 c	11.16 ± 0.37 a	2.00 ± 0.01 c
20% RI	–	–	–	–

Different letters indicate significant differences ($p < 0.05$) between different light intensities according to ANOVA and an LSD test, n = 30; "–" means all seedlings had died. Abbreviations: RI, relative irradiance.

3.2. Effects of Light Conditions on Pigment Contents

There were significant differences ($p < 0.05$) in pigment content under the different light treatments (Figure 1). The Chl a contents at 70% RI and 50% RI were 59.02% and 80.33% higher, respectively, than that at 100% RI. The content of Chl b increased significantly at 70% RI, to 1.69 times that at 100% RI. At 70% RI and 50% RI, the total pigment contents of the needles were significantly higher than that at 100% RI. The variation trend of Car content under different light treatments was consistent with that of Chl b, i.e., 70% RI > 50% RI > 100% RI. The results showed that the decrease in light intensity promoted the accumulation of photosynthetic pigments.

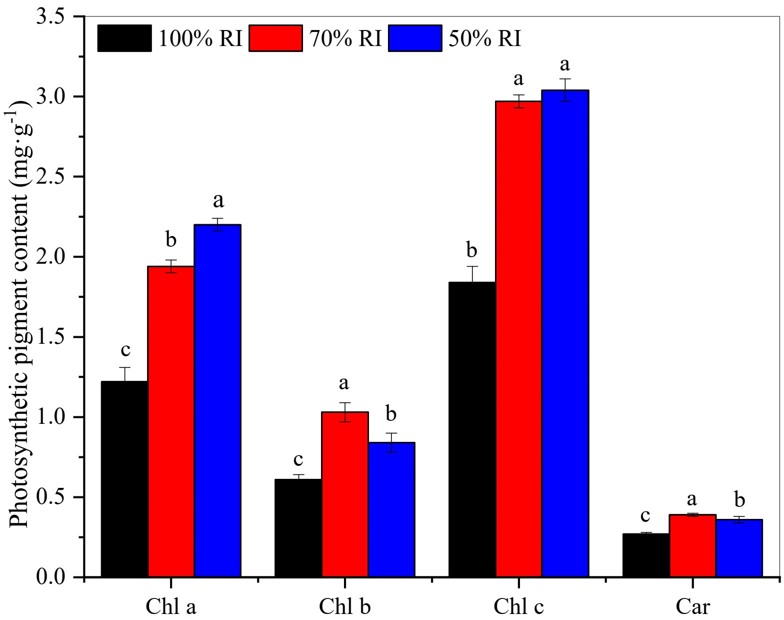

Figure 1. Photosynthetic pigment content in needles under different light treatments (means ± SDs), $n = 6$. Abbreviations: RI, relative irradiance; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl c, total chlorophyll; Car, carotene. Significant differences are indicated by different lowercase letters: $p < 0.05$.

3.3. Spectral Reflectance Analyses

The percentage of each wavelength range of the total photon flux from 400 to 800 mm was counted, and the results showed no significant differences between the shade treatments (Table 3). The overall variation trends of the spectral reflectance curves of *P. massoniana* seedling needles under different light treatments were consistent (Figure 2A), but there were significant differences in single wavelengths within the visible light spectrum (Figure 2B). The spectral reflectance characteristics of *P. massoniana* seedling needles were as follows: there were two high absorption wavelengths, one in the blue-violet region from 400 to 480 nm and one in the red region near 680 nm, exhibiting a trough shape; the spectral reflectance values were lower than 0.1 under the different light treatments. Near the green region from 500 to 600 nm, a wave crest appeared due to the enhanced reflection effect. There was a higher peak in the near-infrared region extending from 750 to 1000 nm due to the occurrence of less pigment absorption and increased reflection. The visible region extended from 400 to 700 nm at 70% RI and 50% RI, which resulted in reduced reflectance values in the violet (400–420 nm), blue (440–480 nm), and yellow-orange (597–655 nm) regions. In addition, the spectral reflectance of the green region from 492 to 577 nm and the near-infrared region from 700 to 1000 nm were significantly lower at 70% RI and 50% RI than at 100% RI. In addition, the shade net did not change the proportion of different wavelength ranges when the light through it (Table 3). The results suggest that exposure to these light conditions for a long time would lead to changes in the ability of seedlings to respond to parts of the light spectrum.

Table 3. The proportion of different light wavelength ranges in the light treatments.

Treatment	400–500 nm	500–600 nm	600–700 nm	700–800 nm	660–680 nm/720–740 nm
	Blue	**Green/Yellow-Orange**	**Red**	**Far-Red**	**Red/Far-Red**
100% RI	32.41	22.48	20.87	24.24	0.899
70% RI	32.41	22.48	20.87	24.24	0.899
50% RI	32.39	22.46	20.89	24.26	0.899

The values represent the percentage of different light wavelength range of natural light from 400 to 800 nm ($\mu mol \cdot m^{-2} \cdot s^{-1} \cdot nm^{-1}$). Abbreviations: RI, relative irradiance. Follow the spectral described method of Riikonen et al. [71].

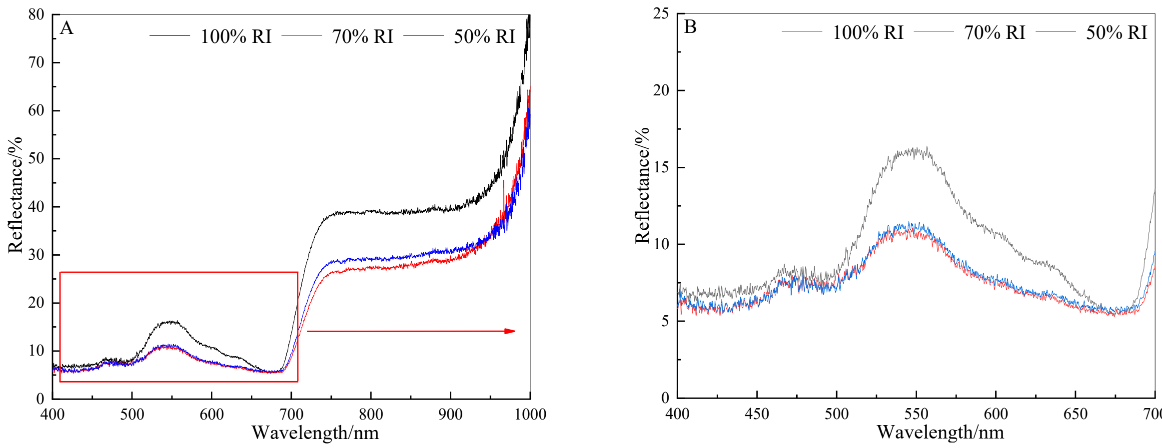

Figure 2. The mean spectral reflectance curves for *P. massoniana* seedling needles at 400–1000 nm (**A**) and within the visible light spectrum at 400–700 nm (**B**) under different light treatments (n = 6). Abbreviations: RI, relative irradiance.

3.4. Effects of Light Conditions on Spectral Reflectance Indexes

Spectral reflectance parameters reflect the spectral characteristics of plants responding to natural light, and vegetation indexes estimated by spectral reflectance parameters can represent the current growth status of plants (Table 4). The different light conditions had significant effects on the spectral reflectance indexes of *P. massoniana* seedlings ($p < 0.05$). The CRI, SIPI, MRESRI, and WBI showed the same change trend, with significant differences among light treatments; the values of these indexes at 70% RI were the highest, and those at 100% RI were the lowest. With the decrease in light intensity, NDVI and PRI first increased and then decreased, while RENDVI significantly increased.

Table 4. Comparison of the spectral parameters of leaves under different light treatments (means ± SDs).

Treatment	CRI	SIPI	RENDVI	NDVI	MRESRI	PRI	WBI
100% RI	0.048 ± 0.003 c	0.787 ± 0.012 c	0.434 ± 0.018 b	0.815 ± 0.019 b	4.362 ± 0.625 c	0.045 ± 0.002 b	1.026 ± 0.035 c
70% RI	0.081 ± 0.006 a	0.854 ± 0.009 a	0.589 ± 0.028 a	0.942 ± 0.056 a	5.269 ± 1.158 a	0.071 ± 0.009 a	1.317 ± 0.076 a
50% RI	0.064 ± 0.007 b	0.819 ± 0.008 b	0.594 ± 0.035 a	0.723 ± 0.041 c	4.859 ± 0.985 b	0.038 ± 0.011 c	1.172 ± 0.102 b

Different letters indicate significant differences ($p < 0.05$) between different light intensities according to ANOVA and the LSD test, $n = 6$. Abbreviations: RI, relative irradiance; CRI, Carotenoid Reflectance Index; SIPI, Structure Intensive Pigment Index; RENDVI, Red Edge Normalized Difference Vegetation Index; NDVI, Normalized Difference Vegetation Index; MRESRI, Modified Red Edge Simple Ratio Index; PRI, Photochemical Reflectance Index; WBI, Water Band Index.

3.5. Chlorophyll Fluorescence Parameters under Different Light Conditions

The fluorescence induction and light response parameters of *P. massoniana* seedlings differed significantly under the different light treatments (Figure 3A,B). Fv/Fm reflects the potential maximum photosynthetic capacity of plants; it reached 0.82 at 70% RI, which is significantly higher than that at 100% RI but significantly lower than that at 50% RI. Fv′/Fm′ and Y(II) presented a similar change trend. These values indicate that moderate light intensity could improve the primary light capture efficiency of the PS reaction centre for needles, but that inhibition would occur under weak light. q_p represents plants' photosynthetic activity, and NPQ reflects the capacity for thermal dissipation by plants in response to high light intensity. Compared with 100% RI, 70% RI improved the photosynthetic activity of seedlings. Moreover, 70% RI caused a reduction in the thermal dissipation of energy (Figure 3A). The ETRmax at 70% RI reached 136.9 μmol·m^{-2}·s^{-1}, which was 1.30 and 2.19 times that at 100% RI and 50% RI, respectively. At 70% RI and 100% RI, the ETR tended to be stable when PAR > 825 μmol·m^{-2}·s^{-1}, while it tended to stabilize at 675 μmol·m^{-2}·s^{-1} at 50% RI. This indicated that an appropriate, moderate light intensity helped to improve the ETR in needles so that more electrons were used in photochemical reactions; in contrast, under low light, the seedlings easily reached a saturation level of light intensity under low light (Figure 3B).

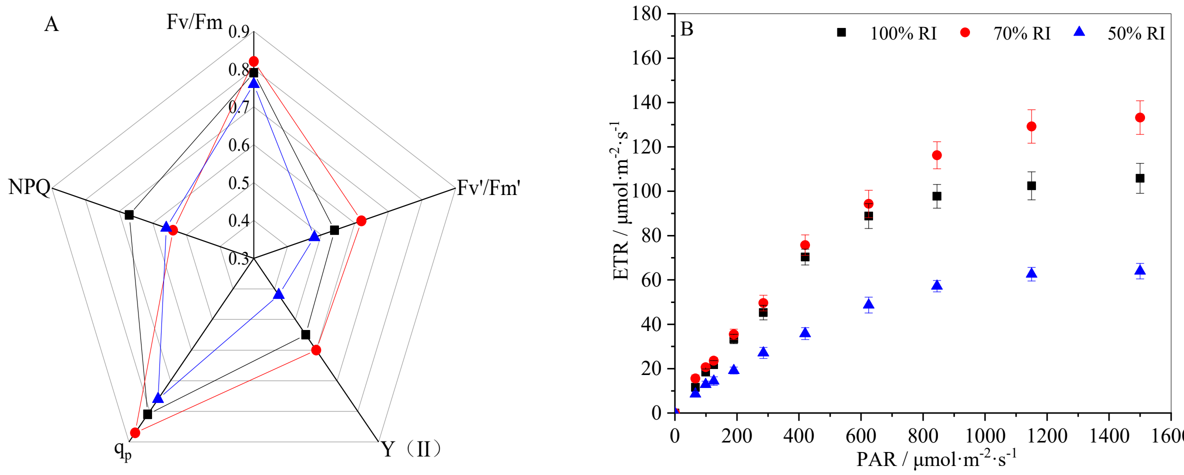

Figure 3. Comparison of chlorophyll fluorescence parameters of *P. massoniana* seedlings under different light treatments (means ± SDs), $n = 6$. The letters denote: (**A**) the slow induction kinetics parameters of dark-adapted leaves; (**B**) relative electron transfer rate of PSII at 0-1500 μmol·m^{-2}·s^{-1}. Abbreviations: RI, relative irradiance; Fv/Fm, maximum photochemical efficiency; Y(II), actual quantum yield; q_p, photochemical quenching; NPQ, non-photochemical quenching; ETR, relative electron transfer rate. Significant differences are indicated: $p < 0.05$.

3.6. Physiological and Biochemical Properties at Different Light Intensities

The POD and SOD activity in *P. massoniana* seedling needles first increased and then decreased with decreasing light intensity from 100% RI to 50% RI (Figure 4A,B). POD and SOD activity reached 107.22 U·g^{-1}·min^{-1} and 204.33 U·g^{-1} at 70% RI, which were 1.75 and

1.50 times that at 100% RI and 1.21 and 1.13 times that at 50% RI, respectively. Moreover, long-term illumination deficiency led to increased MDA accumulation (Figure 4C); this results in the accumulation of excessive oxygen free radicals that cannot be removed by protective enzymes, thus weakening the ability of seedlings to resist adverse conditions.

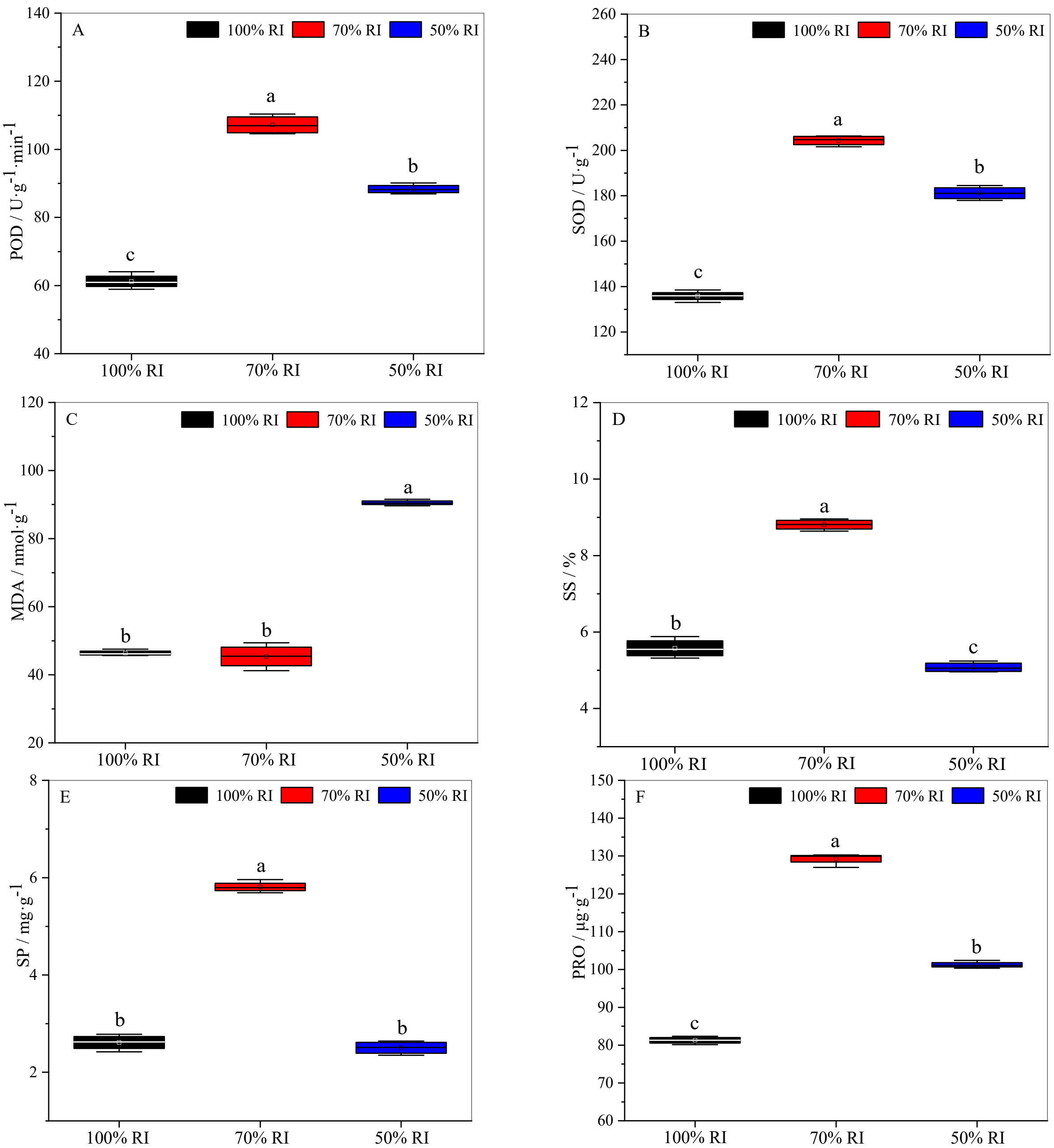

Figure 4. Comparison of protective enzyme activity, MDA content, and osmotic regulating substances in seedlings under different light treatments (means ± SDs), $n = 6$. Abbreviations: RI, relative irradiance. The letters denote: (**A**) POD, peroxidase (U·g^{-1}·min^{-1}); (**B**) SOD, superoxide dismutase (U·g^{-1}); (**C**) MDA, malondialdehyde (nmol·g^{-1}); (**D**) SS, soluble sugar (%); (**E**) SP, soluble protein (mg·g^{-1}); (**F**) PRO, proline (μg·g^{-1}). Significant differences are indicated by different letters (a, b and c): $p < 0.05$.

The different light treatments significantly influenced the SS, SP, and PRO contents in needles (Figure 4D–F). Among them, SS, SP, and PRO accumulated the most at 70% RI, to 1.58, 2.23 and 1.59 times higher than at 100% RI and 1.73, 2.33, and 1.28 times higher than at 50% RI, respectively. Compared with those at 100% RI, the SS content at 50% RI was significantly lower ($p < 0.05$); the SP content decreased slightly but not significantly; and the PRO content increased significantly ($p < 0.05$).

4. Discussion

Light intensity is a crucial environmental factor that influences photosynthesis in green plants and is closely related to their growth and morphological formation [62]. Spectral reflectance data are indispensable for confirming the quantitative and qualitative relationships between optical properties and plant growth status [72]. To the best of our knowledge, this study is the first to correlate spectra with the responses of the pigment content and photosynthetic characteristics of *P. massoniana* seedlings to different light intensities. Our results show that *P. massoniana* seedlings differed in growth strategies across light intensities. Compared with that at 100% RI, ground diameter growth slowed with the decrease in light intensity, especially at extremely low light intensity; all the seedlings died at the lowest light intensity, indicating that *P. massoniana* seedlings cannot adapt to low light conditions. Some studies have suggested that appropriate shading can effectively prevent photoinhibition in seedlings caused by excess light, and *Torreya grandis* seedlings under moderate light intensity had the highest photosynthetic rate and substance accumulation in a previous study [73]. At 70% RI, the seedlings showed "slender" morphological characteristics, but the germination of secondary needles was accelerated. Studies of some tree seedlings have shown that partial shading is needed for plants to achieve higher survival rates, and shade tolerance in seedlings is connected with conservative carbon allocation patterns [74,75]. Under moderate light intensity, the plants can expand their light-receiving area in both the vertical and horizontal directions by increasing their height/diameter ratio and crown width [76]. We suspect that at 70% RI, the seedlings increased the amount of emerging secondary needles to increase the photosynthetic area of each layer of foliage. On the other hand, by reducing the energy distribution to the ground diameter accordingly, the seedlings used as much photosynthetic product as possible for vertical growth to obtain more lights from their crown width; this could be interpreted as a growth strategy for rapid resource acquisition and consumption. However, long-term exposure to insufficient light directly inhibits the seedling growth rate and carbon accumulation; hence, seedlings tend to adopt conservative growth strategies for slow resource acquisition and consumption [77]. Our results demonstrate that moderate light intensity was beneficial to the production of secondary needles (mature foliage), which were conducive to seedling growth. This finding can be applied in seedling cultivation management. However, the secondary needles on seedlings have not yet formed into functional leaves.

Leaf spectral reflectance is an apparent bio-optical property, and it varies largely in response to changes in plant internal metabolic indexes [78]. Studies have found that peak gains in shoot components were observed in *Pinus sylvestris* and *Betula* seedlings grown under red (660–680 nm) and blue light depletion, which was thought to be related to the efficiency with which Chl a and Chl b absorb the light spectrum [79]. In this study, we found that decreasing the light intensity reduced the spectral reflectance of needles in the violet (400–420 nm), blue (440–480 nm), yellow-orange (597–655 nm), and near-infrared (750–1000 nm) regions. The photosynthetic pigments of plants can selectively absorb spectra of certain wavelengths; Chl a absorbs mainly sunlight, in the red-orange region. Chl b absorbs short-wave light, such as that in the blue region, and a higher content of Chl b may enhance shade tolerance [80]. Carotenoids are accessory pigments that play an essential photoprotective role in chloroplasts [81]. Changing the proportions of pigment types constitutes an adjustment of foliage to the incident light conditions [40]. In this study, decreasing light intensity promoted pigment synthesis in leaves. We suspect that shaded foliage has higher, broader thylakoid stacks than sunlit foliage and invests in the accumulation of antenna pigments, which can change the blue photoreceptor (blue photon flux/far-red photon flux) and phytochrome potential (red photon flux/far-red photon flux). Simultaneously, the contents of Chl b and Car increased significantly at 70% RI, allowing the seedlings to absorb more blue-violet light to enhance the utilization ratio of shaded parts. However, in seedlings under lower light conditions for a long time, increasing the photosynthetic pigment contents may not be sufficient to acquire the ability to rapidly absorb light quanta. After analysing the variation tendency between pigment accumulation and the spectral reflectance index (Figure 1 and Table 4), we evaluated the accuracy of the spectral analysis technique. As expected, the change trend of NDVI was consistent with the seedling height and secondary needle emergence trends. The chlorophyll and carotenoid contents also matched the CRI, SIPI, MRESRI, and RENDVI trends, all of which can be used to estimate the photosynthetic pigment concentration and the carotenoid-chlorophyll ratio. Besides, we found that the proportion of different wavelength ranges remained constant between treatments. It is consistent with previous studies demonstrating that quality will not be changed when the light through the shade net [82]. However, the reflectance values (around 550 and 720 nm) of seedlings under 70%RI and 50%RI treatments were relatively lower than those under 100%RI. It is indicated that the seedlings under shade treatments absorbed more green and far-red light. We speculated that the changes in photosynthetic pigment contents of seedlings affected the absorption capacity of light wavelengths. These results suggested that light quality, especially the proportion of the far-red world, will be changed due to the scattering and reflection of light in seedlings under shade treatments. Previous studies have confirmed that the far-red radiation scattered by the surrounding seedling is a signal of competition that promotes the elongation of stems [83]. In this study, we observed that seedlings under shade treatments showed a higher height/diameter ratio. Moreover, further studies in controlled LED light are needed to understand the response mechanisms of seedling growth regulated by light wavelength.

Chlorophyll fluorescence detection, a sensitive and simple technical method, can accurately reflect the absorption, transmission, and dissipation of light energy in the photosynthetic system, thus revealing the mechanism by which light environment factors influence the photochemical pathways in plant leaves [84]. This study found that Fv/Fm, Fv′/Fm′, Y(II), q_p, and ETRmax reached a maximum at 70% RI and that these parameters decreased with decreasing light intensity. The results show that an appropriate light intensity can increase the photochemical efficiency of the PSII reaction centre, the potential activity, and the ratio of antenna pigments absorbing light energy for photochemical electron transfer. This response allocates more energy for carbon accumulation and meeting the demands of rapid seedling growth. In addition, 70% RI led to a decrease in NPQ, which also demonstrated that the distribution ratio of light energy to thermal dissipation decreased under an appropriate light intensity and that there was a greater tendency to conduct photochemical reactions in *P. massoniana* seedlings at 70% RI. PRI was found to correlate strongly with photochemical reactions and the ETR. This finding suggests that spectral reflectance indexes can be used to monitor photochemical activity and thermal dissipation in *P. massoniana* seedlings grown in different habitats and to understand their acclimation to stress conditions, which will provide useful information for improving cultivation practices.

POD and SOD are key protective enzymes in the antioxidant enzyme system. When plants encounter stress, the activities of these enzymes are enhanced to remove excessive ROS in cells and maintain normal growth and metabolic activities in the plants [30]. Furthermore, MDA is the main product of membrane lipid peroxidation in plants under adverse conditions. In this study, the largest increase in protective enzyme activity occurred at 70% RI, and the MDA concentration was the highest at 50% RI. Under long-term exposure to low light intensity, membrane lipid peroxidation in *P. massoniana* seedlings intensified, which eventually led to the accumulation of free radical ions and MDA; as a result, the biofilm system and cell structure were damaged. As important osmotic regulation substances in plants, SS, SP, and PRO can regulate cell osmotic pressure to maintain the integrity of the cell membrane and eliminate reactive oxygen toxicity to improve plant stress resistance [85]. We found that the SS, SP, and PRO contents reached their maximum values at 70% RI. The SS content decreased significantly with decreasing light intensity, indicating that moderate light intensity was conducive to the accumulation of osmotic regulation substances. However, the cellular structure of *P. massoniana* seedlings was damaged to some extent under excessively low light. The results showed that WBI was positively correlated with the activity of protective enzymes. Thus, spectral reflectance sensing technology could be used to quickly and conveniently detect the growth status and anti-stress capabilities of *P. massoniana* seedlings in different ecological environments; such an approach would provide a certain theoretical basis for seedling cultivation and management as well as the prevention of seedling loss.

5. Conclusions

In this study, *P. massoniana* seedlings had different response mechanisms to different levels of light intensity during their rapid growth period, and they adjusted their growth and photosynthetic, physiological, and biochemical strategies to gradually adapt or respond to changes in the light environment. Our observations indicate that leaf spectral reflectance indexes can be used as sensitive indicators of light stress in *P. massoniana* seedlings by combining the vegetation indexes with biochemical analysis data. As this is the first report on the spectral reflectance response of *P. massoniana* leaves to different light intensities, we highlight how seedling growth conditions might be described with the help of spectral signals. Moreover, primary needles are more shade tolerant than secondary needles, and the emergence of secondary needles is beneficial to seedling growth. In summary, the seedlings had a high growth rate, high photosynthetic activity, and potential and significantly improved stress resistance under moderate light intensity. Therefore, it is suggested that appropriate shading measures that partially impede direct sunlight should be taken to ensure the rapid growth of seedlings during cultivation, especially during seasons with intense light. In plantations or natural secondary forest regeneration areas, pruning, weed removal, and other tending measures could be adopted to ameliorate the low-light environment caused by excessive canopy density to promote the natural regeneration and growth of *P. massoniana* seedlings.

Author Contributions: H.W.: methodology, field and lab experiments, formal analysis, writing—original draft, writing—review and editing. F.W.: methodology, conceptualization, project administration, writing—original draft, writing—review and editing, supervision. M.L.: lab experiments. X.Z. and C.S.: field experiments. G.D.: methodology, resources, revised and improved the manuscript, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Guizhou Science and Technology Department under Grant ([2018]5261); The National Natural Science Foundation of China under Grant (31660201); The first-class discipline construction project of Guizhou Province under Grant (GNYL[2017]007); and Postgraduate Research Project of Guizhou Province under Grant (YJSCXJH [2019]020).

Data Availability Statement: The data supporting the conclusions of this article will be made available by authors, without undue reservation.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Tang, H.; Hu, Y.-Y.; Yu, W.-W.; Song, L.-L.; Wu, J.-S. Growth, photosynthetic and physiological responses of Torreya grandis seedlings to varied light environments. *Trees* **2015**, *29*, 1011–1022. [CrossRef]
2. Rozendaal, D.; Poorter, V. Plasticity in Leaf Traits of 38 Tropical Tree Species in Response to Light; Relationships with Light Demand and Adult Stature. *Funct. Ecol.* **2006**, *20*, 207–216. [CrossRef]
3. Shi, J.G.; Cui, H.Y.; Zhao, B.; Dong, S.T.; Liu, P.; Zhang, J.W. Effect of light on yield and characteristics of grain-filling of summer maize from flowering to maturity. *Sci. Agric. Sin.* **2013**, *46*, 4427–4434.
4. Xie, X.; Yang, X.H.; Chen, X.Y. Effects of shading on leaf shape and photosynthetic characteristics of the transgenic lespedeza formosa with expressing BADH Gene. *Sci. Silvae Sin.* **2013**, *49*, 33–42.
5. Liu, Z.; Cheng, R.; Xiao, W.; Guo, Q.; Wang, N. Effects of shading on growth and photosynthetic characteristics of distylium chinense seedlings. *Sci. Silvae Sin.* **2015**, *51*, 129–136.

6. Pires, M.; Santos, H.A.; Santos, D.F.; Vasconcelos, A.S.; Aragão, C. Yield of muskmelon subjected to different water management with the use of polypropylene. *Hortic. Bras.* **2013**, *31*, 304–310. [CrossRef]
7. Brodersen, C.R.; Germino, M.J.; Johnson, D.M.; Reinhardt, K.; Smith, W.K.; Resler, L.M.; Bader, M.Y.; Sala, A.; Kueppers, L.M.; Broll, G. Seedling survival at timberline is critical to conifer mountain forest elevation and extent. *Front. For. Glob. Chang.* **2019**, *2*, 9. [CrossRef]
8. Dai, Y.; Shen, Z.; Liu, Y.; Wang, L.; Hannaway, D.; Lu, H. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of Tetrastigma hemsleyanum Diels et Gilg. *Environ. Exp. Bot.* **2009**, *65*, 177–182. [CrossRef]
9. Panigrahy, M.; Majeed, N.; Panigrahi, K.C.S. Low-light and its effects on crop yield: Genetic and genomic implications. *J. Biosci.* **2020**, *45*, 1–15. [CrossRef]
10. Santos, D.; Daniela, V.S.; Letícia, D.; Cristina, S.A.; Carla, D.; Schramm, M.M. Relationships between reflectance and absorbance chlorophyll indices with RGB (Red, Green, Blue) image components in seedlings of tropical tree species at nursery stage. *New For.* **2019**, *50*, 377–388.
11. Yamori, W. Photosynthetic response to fluctuating environments and photoprotective strategies under abiotic stress. *J. Plant Res.* **2016**, *129*, 379–395. [CrossRef]
12. Liu, Y.; Wang, T.; Fang, S.; Zhou, M.; Qin, J. Responses of morphology, gas exchange, photochemical activity of photosystem ii, and antioxidant balance in cyclocarya paliurus to light spectra. *Front. Plant Sci.* **2018**, *9*, 1704. [CrossRef]
13. Izaguirre, M.M.; Mazza, C.A.; Astigueta, M.; Ciarla, A.M.; Ballare, C.L. No time for candy: Passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. *Oecologia* **2013**, *173*, 213–221. [CrossRef]
14. Zhang, L.; Li, L.; Liu, J. Comparison of the photosynthetic characteristics of two Isochrysis galbana strains under high light. *Bot. Mar.* **2014**, *57*, 477–481. [CrossRef]
15. Torres, C.D.; Magnin, A.; Varela, S.A.; Stecconi, M.; Grosfeld, J.E.; Puntieri, J.G. Morpho-physiological responses of Nothofagus obliqua to light intensity and water status, with focus on primary growth dynamics. *Trees* **2018**, *32*, 1301–1314. [CrossRef]
16. Parker, W.C.; Mohammed, G.H. Photosynthetic acclimation of shade-grown red pine (Pinus resinosa Ait.) seedlings to a high light environment. *New For.* **2000**, *19*, 1–11. [CrossRef]
17. Bussotti, F.; Gerosa, G.; Digrado, A.; Pollastrini, M. Selection of chlorophyll fluorescence parameters as indicators of photosynthetic efficiency in large scale plant ecological studies. *Ecol. Indic.* **2020**, *108*, 105686. [CrossRef]
18. Stolárik, T.; Nožková, V.; Nosek, L.; Pavlovič, A. Dark chlorophyll synthesis may provide a potential for shade tolerance as shown by a comparative study with seedlings of European larch (Larix decidua) and Norway spruce (Picea abies). *Trees* **2018**, *32*, 951–965. [CrossRef]
19. Alarcón, J.; Ortuno, M.; Nicolás, E.; Navarro, A.; Torrecillas, A. Improving water-use efficiency of young lemon trees by shading with aluminised-plastic nets. *Agric. Water Manag.* **2006**, *82*, 387–398. [CrossRef]
20. Sevillano, I.; Short, I.; Campion, J.; Grant, O.M.; Grant, J.; O'Reilly, C. Comparison of photosynthetic performance of Fagus sylvatica seedlings under natural and artificial shading. *Environ. Exp. Bot.* **2018**, *152*, 90–96. [CrossRef]
21. Mditshwa, A.; Magwaza, L.S.; Tesfay, S.Z. Shade netting on subtropical fruit: Effect on environmental conditions, tree physiology and fruit quality. *Sci. Hortic.* **2019**, *256*, 108556. [CrossRef]
22. Wu, J.; Su, Y.; Wang, J.; He, Q.; Qiu, Q.; Ma, J.; Li, J. Morphological and physiological acclimation of Catalpa bungei plantlets to different light conditions. *Photosynthetica* **2018**, *56*, 537–548. [CrossRef]
23. Chang, P.-T.; Hsieh, C.-C.; Jiang, Y.-L. Responses of 'Shih Huo Chuan'pitaya (Hylocereus polyrhizus (Weber) Britt. & Rose) to different degrees of shading nets. *Sci. Hortic.* **2016**, *198*, 154–162.
24. Holland, N.; Richardson, A.D. Stomatal length correlates with elevation of growth in four temperate species. *J. Sustain. For.* **2009**, *28*, 63–73. [CrossRef]
25. He, Z.-s.; Tang, R.; Li, M.-j.; Jin, M.-r.; Xin, C.; Liu, J.-f.; Hong, W. Response of photosynthesis and chlorophyll fluorescence parameters of castanopsis kawakamii seedlings to forest gaps. *Forests* **2020**, *11*, 21. [CrossRef]
26. Zhang, Q.; Zhang, T.J.; Chow, W.S.; Xie, X.; Chen, Y.J.; Peng, C.L. Photosynthetic characteristics and light energy conversions under different light environments in five tree species occupying dominant status at different stages of subtropical forest succession. *Funct. Plant Biol.* **2015**, *42*, 609–619. [CrossRef] [PubMed]
27. Zhang, K.-M.; Yu, H.-J.; Shi, K.; Zhou, Y.-H.; Yu, J.-Q.; Xia, X.-J. Photoprotective roles of anthocyanins in Begonia semperflorens. *Plant Sci.* **2010**, *179*, 202–208. [CrossRef]
28. Deng, Y.; Li, C.; Shao, Q.; Ye, X.; She, J. Differential responses of double petal and multi petal jasmine to shading: I. Photosynthetic characteristics and chloroplast ultrastructure. *Plant Physiol. Biochem.* **2012**, *55*, 93–102. [CrossRef]
29. Fan, X.-X.; Xu, Z.-G.; Liu, X.-Y.; Tang, C.-M.; Wang, L.-W.; Han, X.-l. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Sci. Hortic.* **2013**, *153*, 50–55. [CrossRef]
30. Maruta, T.; Tanouchi, A.; Tamoi, M.; Yabuta, Y.; Yoshimura, K.; Ishikawa, T.; Shigeoka, S. Arabidopsis chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. *Plant Cell Physiol.* **2010**, *51*, 190–200. [CrossRef]
31. Kong, D.-X.; Li, Y.-Q.; Wang, M.-L.; Bai, M.; Zou, R.; Tang, H.; Wu, H. Effects of light intensity on leaf photosynthetic characteristics, chloroplast structure, and alkaloid content of Mahonia bodinieri (Gagnep.) Laferr. *Acta Physiol. Plant.* **2016**, *38*, 120. [CrossRef]
32. Kobayashi, K.; Amore, T.; Lazaro, M. Light-emitting diodes (LEDs) for miniature hydroponic lettuce. *Sci. Res.* **2013**, *3*, 74–77. [CrossRef]

33. Hogewoning, S.W.; Trouwborst, G.; Maljaars, H.; Poorter, H.; van Ieperen, W.; Harbinson, J. Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. *J. Exp. Bot.* **2010**, *61*, 3107–3117. [CrossRef]
34. Wang, Y.; Folta, K.M. Contributions of green light to plant growth and development. *Am. J. Bot.* **2013**, *100*, 70–78. [CrossRef] [PubMed]
35. Huang, D.; Wu, L.; Chen, J.; Dong, L. Morphological plasticity, photosynthesis and chlorophyll fluorescence of Athyrium pachyphlebium at different shade levels. *Photosynthetica* **2011**, *49*, 611–618. [CrossRef]
36. Tozzi, E.S.; Easlon, H.M.; Richards, J.H. Interactive effects of water, light and heat stress on photosynthesis in F remont cottonwood. *Plant Cell Environ.* **2013**, *36*, 1423–1434. [CrossRef] [PubMed]
37. Yu, W.; Liu, Y.; Song, L.; Jacobs, D.F.; Du, X.; Ying, Y.; Shao, Q.; Wu, J. Effect of differential light quality on morphology, photosynthesis, and antioxidant enzyme activity in Camptotheca acuminata seedlings. *J. Plant Growth Regul.* **2017**, *36*, 148–160. [CrossRef]
38. Basave Villalobos, E.; Alcalá Cetina, V.M.; Lopez Lopez, M.A.; Aldrete, A.; Del Valle Paniagua, D.H. Nursery practices increase seedling performance on nutrient-poor soils in Swietenia humilis. *iFor. Biogeosci. For.* **2014**, *8*, 552. [CrossRef]
39. Rascher, U.; Liebig, M.; Lüttge, U. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant Cell Environ.* **2000**, *23*, 1397–1405. [CrossRef]
40. Quevedo-Rojas, A.; García-Núñez, C.; Jerez-Rico, M.; Jaimez, R.; Schwarzkopf, T. Leaf acclimation strategies to contrasting light conditions in saplings of different shade tolerance in a tropical cloud forest. *Funct. Plant Biol.* **2018**, *45*, 968–982. [CrossRef]
41. Casale, M.; Bagnasco, L.; Giordani, P.; Mariotti, M.G.; Malaspina, P. NIR spectroscopy as a tool for discriminating between lichens exposed to air pollution. *Chemosphere* **2015**, *134*, 355–360. [CrossRef]
42. Couture, J.J.; Serbin, S.P.; Townsend, P.A. Spectroscopic sensitivity of real-time, rapidly induced phytochemical change in response to damage. *New Phytol.* **2013**, *198*, 311–319. [CrossRef]
43. Kumar, L.; Schmidt, K.; Dury, S.; Skidmore, A. Imaging spectrometry and vegetation science. In *Imaging Spectrometry*; Springer: Heideiberg, The Netherlands, 2002; pp. 111–155.
44. Kolhe, S.; Deshmukh, R. Detection of acid rain stress effect on plants using spectroradiometer—A review. *Int. J. Innov. Res. Comput. Sci. Technol.* **2016**, *4*, 13095–13100.
45. Sims, D.A.; Gamon, J.A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* **2002**, *81*, 337–354. [CrossRef]
46. Letts, M.G.; Phelan, C.A.; Johnson, D.R.; Rood, S.B. Seasonal photosynthetic gas exchange and leaf reflectance characteristics of male and female cottonwoods in a riparian woodland. *Tree Physiol.* **2008**, *28*, 1037–1048. [CrossRef]
47. Cotrozzi, L.; Townsend, P.A.; Pellegrini, E.; Nali, C.; Couture, J.J. Reflectance spectroscopy: A novel approach to better understand and monitor the impact of air pollution on Mediterranean plants. *Environ. Sci. Pollut. Res.* **2018**, *25*, 8249–8267. [CrossRef]
48. Durako, M.J.; Howarth, J.F. Leaf spectral reflectance shows Thalassia testudinum seedlings more sensitive to hypersalinity than hyposalinity. *Front. Plant Sci.* **2017**, *8*, 1127. [CrossRef]
49. Deng, X.; Xiao, W.; Shi, Z.; Zeng, L.; Lei, L. Combined Effects of Drought and Shading on Growth and Non-Structural Carbohydrates in Pinus massoniana Lamb. Seedlings. *Forests* **2020**, *11*, 18. [CrossRef]
50. Climent, J.; e Silva, F.C.; Chambel, M.R.; Pardos, M.; Almeida, M.H. Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. *Ann. For. Sci.* **2009**, *66*, 407. [CrossRef]
51. Kuusk, V.; Niinemets, Ü.; Valladares, F. Structural controls on photosynthetic capacity through juvenile-to-adult transition and needle ageing in Mediterranean pines. *Funct. Ecol.* **2018**, *32*, 1479–1491. [CrossRef]
52. Wang, H.; Wu, F.; Wu, C.; Yu, S.; Zhu, X.; Xie, W. Growth and physiological responses of seedlings with different leaf shapes to drought and re-watering in Pinus massoniana. *J. Northeast For. Univ.* **2018**, *46*, 1–6.
53. Wang, H.; Wu, F.; Zhu, X.; Xie, W. Effects of leaf types on growth and chlorophyll fluorescence characteristics in Pinus massoniana seedlings. *Sci. Silv. Sin.* **2019**, *55*, 183–192.
54. Liu, Z.; Jiang, F.; Li, F.; Jin, G. Coordination of intra and inter-species leaf traits according to leaf phenology and plant age for three temperate broadleaf species with different shade tolerances. *For. Ecol. Manag.* **2019**, *434*, 63–75. [CrossRef]
55. Alsiņa, I.; Dūma, M.; Dubova, L.; Šenberga, A.; Daģis, S. Comparison of different chlorophylls determination methods for leafy vegetables. *Agron. Res.* **2016**, *14*, 309–316.
56. Gitelson, A.A.; Gritz, Y.; Merzlyak, M.N. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *J. Plant Physiol.* **2003**, *160*, 271–282. [CrossRef]
57. Blackburn, G.A. Hyperspectral remote sensing of plant pigments. *J. Exp. Bot.* **2007**, *58*, 855–867. [CrossRef]
58. Gamon, J.; Serrano, L.; Surfus, J. The photochemical reflectance index: An optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia* **1997**, *112*, 492–501. [CrossRef]
59. Guo, Y.-P.; Guo, D.-P.; Zhou, H.-F.; Hu, M.-J.; Shen, Y.-G. Photoinhibition and xanthophyll cycle activity in bayberry (Myrica rubra) leaves induced by high irradiance. *Photosynthetica* **2006**, *44*, 439. [CrossRef]
60. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]
61. Miyake, C.; Miyata, M.; Shinzaki, Y.; Tomizawa, K.-i. CO2 response of cyclic electron flow around PSI (CEF-PSI) in tobacco leaves—Relative electron fluxes through PSI and PSII determine the magnitude of non-photochemical quenching (NPQ) of Chl fluorescence. *Plant Cell Physiol.* **2005**, *46*, 629–637. [CrossRef]

62. Sukhova, E.; Mudrilov, M.; Vodeneev, V.; Sukhov, V. Influence of the variation potential on photosynthetic flows of light energy and electrons in pea. *Photosynth. Res.* **2018**, *136*, 215–228. [CrossRef]
63. Baker, N.R. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **2008**, *59*, 89–113. [CrossRef]
64. Dere, S.; Gunes, T.; Sivaci, R. Spectrophotometric Determination of Chlorophyll-A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents. *Turk. J. Bot.* **1998**, *22*, 13–17.
65. Sánchez, F.J.; Manzanares, M.a.; de Andres, E.F.; Tenorio, J.L.; Ayerbe, L. Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crops Res.* **1998**, *59*, 225–235. [CrossRef]
66. Sedmak, J.J.; Grossberg, S.E. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Anal. Biochem.* **1977**, *79*, 544–552. [CrossRef]
67. Miller, G.; Honig, A.; Stein, H.; Suzuki, N.; Mittler, R.; Zilberstein, A. Unraveling Δ1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. *J. Biol. Chem.* **2009**, *284*, 26482–26492. [CrossRef] [PubMed]
68. Deng, Y.; Shao, Q.; Li, C.; Ye, X.; Tang, R. Differential responses of double petal and multi petal jasmine to shading: II. Morphology, anatomy and physiology. *Sci. Hortic.* **2012**, *144*, 19–28. [CrossRef]
69. Yuan-Bing, L.A.-R.Z.; Deng-Ke, C. Effects of salt stress on the growth and the antioxidant enzyme activity of Thellungiella halophila. *Bull. Bot. Res.* **2006**, *26*, 216–221.
70. Favaretto, V.F.; Martinez, C.A.; Soriani, H.H.; Furriel, R.P. Differential responses of antioxidant enzymes in pioneer and late-successional tropical tree species grown under sun and shade conditions. *Environ. Exp. Bot.* **2011**, *70*, 20–28. [CrossRef]
71. Riikonen, J. Pre-cultivation of Scots pine and Norway spruce transplant seedlings under four different light spectra did not affect their field performance. *New For.* **2016**, *47*, 607–619. [CrossRef]
72. Yao, X.-y.; Liu, X.-y.; Xu, Z.-g.; Jiao, X.-l. Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *J. Integr. Agric.* **2017**, *16*, 97–105. [CrossRef]
73. Lin, J.; Zhang, R.; Hu, Y.; Song, Y.; Hänninen, H.; Wu, J. Interactive effects of drought and shading on Torreya grandis seedlings: Physiological and growth responses. *Trees* **2019**, *33*, 951–961. [CrossRef]
74. Reich, P.B.; Wright, I.J.; Cavender-Bares, J.; Craine, J.; Oleksyn, J.; Westoby, M.; Walters, M. The evolution of plant functional variation: Traits, spectra, and strategies. *Int. J. Plant Sci.* **2003**, *164*, S143–S164. [CrossRef]
75. Cheng, X.; Yu, M.; Wang, G.; Wu, T.; Zhang, C. Growth, morphology and biomass allocation in response to light gradient in five subtropical evergreen broadleaved tree seedlings. *J. Trop. For. Sci.* **2013**, 537–546.
76. Zhang, Y.; Liu, A.; Huang, S. Effects of shading on some morphological and physiological characteristics of Begonia semperflorens. *Pak. J. Bot.* **2018**, *50*, 2173–2179.
77. Fernandes, A.M.; Fortini, E.A.; de Carvalho Müller, L.A.; Batista, D.S.; Vieira, L.M.; Silva, P.O.; do Amaral, C.H.; Poethig, R.S.; Otoni, W.C. Leaf development stages and ontogenetic changes in passionfruit (*Passiflora edulis* Sims.) are detected by narrowband spectral signal. *J. Photochem. Photobiol. B Biol.* **2020**, *209*, 111931. [CrossRef] [PubMed]
78. Viera Silva, D.; Dos Anjos, L.; Brito-Rocha, E.; Dalmolin, A.C.; Mielke, M.S. Calibration of a multi-species model for chlorophyll estimation in seedlings of Neotropical tree species using hand-held leaf absorbance meters and spectral reflectance. *iFor. Biogeosci. For.* **2016**, *9*, 829. [CrossRef]
79. Kelly, J.; Landhäusser, S.; Chow, P. The impact of light quality and quantity on root-to-shoot ratio and root carbon reserves in aspen seedling stock. *New For.* **2015**, *46*, 527–545. [CrossRef]
80. Gao, Z.; Khalid, M.; Jan, F.; Jiang, X.; Yu, X. Effects of light-regulation and intensity on the growth, physiological and biochemical properties of Aralia elata (miq.) seedlings. *S. Afr. J. Bot.* **2019**, *121*, 456–462. [CrossRef]
81. Schmitt, F.-J.; Renger, G.; Friedrich, T.; Kreslavski, V.D.; Zharmukhamedov, S.K.; Los, D.A.; Kuznetsov, V.V.; Allakhverdiev, S.I. Reactive oxygen species: Re-evaluation of generation, monitoring and role in stress-signaling in phototrophic organisms. *Biochim. Biophys. Acta Bioenerg.* **2014**, *1837*, 835–848. [CrossRef]
82. Liu, B.; Liu, Q.; Daryanto, S.; Guo, S.; Huang, Z.; Wang, Z.; Wang, L.; Ma, X. Responses of Chinese fir and Schima superba seedlings to light gradients: Implications for the restoration of mixed broadleaf-conifer forests from Chinese fir monocultures. *For. Ecol. Manag.* **2018**, *419*, 51–57. [CrossRef]
83. Ballaré, C.L.; Scopel, A.L.; Sanchez, R.A. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. *Science* **1990**, *247*, 329–332. [CrossRef]
84. Ogburn, R.M.; Edwards, E.J. Quantifying succulence: A rapid, physiologically meaningful metric of plant water storage. *Plant Cell Environ.* **2012**, *35*, 1533–1542. [CrossRef]
85. Xie, H.; Yu, M.; Cheng, X. Leaf non-structural carbohydrate allocation and C: N: P stoichiometry in response to light acclimation in seedlings of two subtropical shade-tolerant tree species. *Plant Physiol. Biochem.* **2018**, *124*, 146–154. [CrossRef] [PubMed]