Article

Photosynthetic Pigments in Siberian Pine and Fir under Climate Warming and Shift of the Timberline

Nina Pakharkova[iD]**, Irina Borisova, Ruslan Sharafutdinov and Vladimir Gavrikov ***

Chair Ecology and Nature Management, Siberian Federal University, Svobodny pr. 79, Krasnoyarsk 660041, Russia; nina.pakharkova@yandex.ru (N.P.); irina_borisova77@mail.ru (I.B.); ruslanate@mail.ru (R.S.)
* Correspondence: vgavrikov@sfu-kras.ru; Tel.: +7-913-042-4304

check for updates

Abstract: *Research Highlights:* For the first time, the *Pinus sibirica* Du Tour and *Abies sibirica* L. conifer forest at the West Sayan ridge timberline has been explored to reveal which species is likely to react to climate change and a shift of the timberline. Such a shift may modify the ecological functions of the forests. *Background and Objectives:* Long-term climate change has become obvious in the mountains of southern Siberia. Specifically, a half-century rise in annual mean temperatures has been observed, while precipitation remains unchanged. Trees growing at the timberline are likely to strongly react to climate alterations. The objective was to estimate which of the two species sharing the same habitat would benefit from climate alteration and shifting of the timberline. *Materials and Methods:* At several altitudes (from 1413 to 1724 m a.s.l.), samples of *P. sibirica* and *A. sibirica* needles have been collected and contents of chlorophyll *a* and *b* as well as carotenoids were measured in June 2019. The temperature of needles of the two species was measured in both cloudy and sunny weather conditions. *Results:* The studied species have been shown to have different patterns of pigment variations with the growth of altitude. The decline of chlorophylls and carotenoids was more pronounced in *P. sibirica* (ratio at timberline ca. 2.2) than in *A. sibirica* (ratio ca. 3.1). Accordingly, the electron transport rate decreased more strongly in *P. sibirica* at the timberline (ca. 37.2 µmol of electrons/m^{-2} s^{-1}) than in *A. sibirica* (56.9 µmol of electrons/m^{-2} s^{-1}). The temperatures of needles in both cloudy and sunny weather were higher in *A. sibirica* (10.5 and 43.3 °C, respectively) than in *P. sibirica* (3.8 and 24.2 °C, respectively). *Conclusions:* The considered physiological and ecological traits show that *P. sibirica* is better protected from higher-altitude hazards (excess insolation, rise of temperature etc.) than *A. sibirica*. *P. sibirica* may be therefore a more likely winner than *A. sibirica* in the movement of the mountain timberline under climate warming in the area.

Keywords: timberline; higher altitude; chlorophyll; carotenoids; climate change; *Pinus sibirica*; *Abies sibirica*

1. Introduction

In various regions of the globe, climate warming has become a matter of fact. Forest vegetation may react more weakly or strongly to the change, but those forests occupying the very extreme edges of distribution are expected to be more sensitive to the climatic trend [1]. The shift of the forested zone borderline is an apparent indicator of climate change and of important events shaping the outlook of landscapes. The ability of trees to move up the timberline will to a great extent arise from their ability to acclimate and grow.

Broadly seen, the system of tree photoreceptors ensures the start of acclimation to overwinter freezing temperatures [2]. Habitat warming may, however, distort the work of photoperiod receptors. As has been demonstrated in experiments with a warming of spruce bog communities [3], the trees

extend their growth period but may fail to cue the photoperiod, which leads to premature loss of frost hardiness and tissue damage.

The growth potential of higher altitudes trees is often studied from the viewpoint of how their photosynthetic system functions under extremely harsh conditions. In subalpine *Abies faxoniana* Rehder & E.H. Wilson., the content of chlorophyll in mature trees needles was reported to be lower than that in juvenile trees [4]. The juvenile fir trees were seen as more vulnerable to climate change because altitude exerts a sufficient impact on their leaf traits. The seedlings of another fir species, Abies alba Mill., were subjected to experimental temperature increases [5]. The heating stress led to a decrease of chlorophyll and carotenoid pigments in the seedling leaves.

Poulos et al. [6] explored a stratification pattern in four pine species across the elevation gradient in the mountains of southern California. They found that both the lowest elevation species (*Pinus attenuata Lem*.) and highest elevation one (*Pinus contorta* Doug.) had high carotenoid and anthocyanin indices, which are considered to be a result of their acclimation to high ultraviolet radiation in upper habitats and also to drought stress in foothills. In another mountainous area, the Appalachian, the high-elevation *Abies fraseri* (Pursh) Poir. was compared to the lower-elevation *Picea rubens* Sarg. [7]. It was found that both green (chlorophyll) and yellow (carotenoids) pigments showed a higher content in *A. fraseri*.

Photosynthetic pigments may be of importance in assessing the general health of forest stands. Kopačková et al. [8] reported the carotenoid-to-chlorophyll ratio to be sensitive to soil geochemical conditions, and it could serve as a non-specific indicator of tree stress.

In the northern macro-slope of West Sajan Mountains, the timberline belt is dominated by two conifer species, Siberian pine (*Pinus sibirica* Du Tour) and Siberian fir (*Abies sibirica* L.). The species often grow in a mixture, but at the very elevated edge of the distribution the fir tends to form bush-like spatial clumps, while the pine presents lone-standing trees. Compared to Siberian pine the fir is more seldom an object of research, probably because of its much lower commercial value. However, it plays an important ecological role in covering large areas of mountainous slopes in wet northern Asian climates.

Sobchak and Zotikova [9] measured the content of pigments in differently aged needles of Siberian pine. The sampled 60–80 year-old trees grew at the altitude 2350 m a.s.l. in the vicinity of a glacier in the Altai Mountains. As the needles grow old, the content of both chlorophyll and carotenoid pigments first rises to achieve a maximum at the age of 4 years and then decreases significantly. The authors noted that the decrease of carotenoid content was weaker, which they attributed to the protection function of the pigments. Measurements of photochemical activity of chloroplasts showed that the two- and three-year-old needles were the most photosynthetically active.

Another study in the Altai Mountains [10] considered a 14-km-long transect from a plain site to 2110 m a.s.l. which corresponded to open woodland at the timberline. In two-year-old Siberian pine needles the largest pigment pool was recorded at lower altitudes, with chlorophyll *a* comprising the largest share in the pool. At the highest altitudes, however, green pigments were sufficiently smaller in content whereas carotenoids were at their maximum compared the whole transect. Also, the species exhibits definite variations of the pigment content during the seasons of the year. For Siberian pine that was an introduced species to mountainous sites of the Far East, it has been shown [11] that the green pigments achieve their maximum in August and then steadily decrease in autumn and the early winter months. Inversely, carotenoids were at a minimum in August and at a maximum in November (ca. 0.32 µkg/mkg, green weight). At the same time, the total pool of pigments stayed relatively stable (1.6–1.7 µkg/mkg, green weight) from May to November.

In the northern part of the Siberian pine area (61°–62° northern latitude), the two- and three-year-old needles of the trees exhibit a maximal photosynthetic activity [12]. In the spring–summer season, the share of green pigments decreased while that of yellow pigments increased closer to the dormant period in winter.

The pool of photosynthetic pigments in Siberian fir has been the focus of several kinds of research. Silkina [13] analyzed one- to five-year-old needles of the fir with respect to green pigments. Throughout the growing season, the content of both chlorophyll *a* and *b* was maximal in three–four year-old needles of older trees, ca. 2–4 μkg/mkg dry weight. Meanwhile, the pool of pigments was higher in five-year-old needles of younger fir undergrowth. Across the season, a maximal content of the pigments was observed in May, after which the content gradually decreased until next March. The author has also recommended using three–four year-old needles from the lower part of the crown because the needles of this location showed a maximal pigment content among other locations.

The seasonal dynamics of the pigments was followed in two-year-old fir needles [14]. The authors reported that the green pigments were at a minimum in March and May (ca. 2.4–2.5 μkg/mkg dry weight) and grew until December (3.82 μkg/mkg dry weight). The same was true for the yellow pigments, which were at a minimum in May (0.5 μkg/mkg dry weight) and at a maximum in December (0.83 μkg/mkg dry weight). Measurements of chlorophyll fluorescence have shown that maximal quantum yield of photochemical activity was maximal in July and minimal in March. Also, the fir needles showed higher levels of CO_2 exchange in summer months in comparison with some other conifers, spruce, and juniper [14].

It is widely believed that temperature is the limiting factor that inhibits or speeds up the growth in trees at higher altitudes. Presumably, the timberline shifts upward when the climate is warming. In the case of a monospecies tree community, only one species will occupy higher altitudes. In a multiple species case, however, climate change may not only shift the timberline but also change the species distribution. The aims of the study were, therefore, (1) to evaluate photosynthetic traits in Siberian pine and Siberian fir sharing the same habitats at the mountainous timberline in the West Sayan ridge, and on that basis (2) to make a prognosis of how the climate change would alter the forest communities at the timberline. Specifically, we explored which parts of the trees or the stages of their life cycles led to the formation of the upper timberline in the area.

2. Materials and Methods

2.1. Area of Research

The study area is located in the central part of the Western Sayan Mountains (Figure 1). After the Köppen classification, the climate of the area is continental (Dwb). Several continental air masses, Atlantic, Arctic, and central Asian, overlap over the area. Summertime lasts for ca. two months. Average July temperature varies from 17 °C to 6 °C while the annual mean over the area is −3.9 °C (Olen'ya Rechka weather station). Every 100 m of elevation up the slope brings a decrease of temperature by 0.6 °C. The annual sum of precipitation reaches 1480 mm at higher elevations and 800 mm in a low mountain belt. Half of the precipitation falls from June to August. The depth of snow cover is over 1.5 m and sometimes reaches up to 2 m. The surface relief is rather dissected, with altitudes varying from 400 to 2740 m. The higher mountain belt bears visible signs of Pleistocene glaciations, while dome-shaped peaks not touched by glaciers are common in the middle mountain belt. The river net is dense and branchy, with the rivers receiving water from both glaciers in spring and precipitation and permafrost in summer. The duration of the growing season is from 100 to 120 days.

The background soils of the area are Hyperskeletic Histic Leptosols Humic, Folic Leptic Skeletic Histosols Arenic Hyperorganic, Leptic Skeletic Cambisols Arenic Ferric Humic, and Leptic Skeletic Cambic Phaeozems Arenic Hyperhumic (World Reference Base [15]). Leptosols are formed on the west slope on eluvial facies (altitude 1630–1730 m). The structure and granulometrical composition were not determined due to the absolute dominance of the mineral material in the profile. The depth of the soil profile was no more than 35 cm.

Histosols are formed on the west slope on trans-accumulative facies (altitude 1560–1630 m). No gley materials are found but the profile is water-saturated. This soil has a light granulometrical composition. In terms of pH of the aqueous extract, the soil varies from highly-acid in organic horizons

to slightly acid in mineral horizons. The soil profile has high rock fragment content. The depth of the profile is no more than 40 cm.

Cambisols are formed on the west slope on transeluvial facies (altitude 1475–1560 m). An illuvial-ferruginous diagnostic horizon with iron neoplasms and highly acidic reactions is characteristic of the soil type. The soil profile has also a high content rock fragments. The depth of the profile is no more than 35 cm.

Phaeozems are formed on the west slope on transeluvial facies, but at lower altitudes (1340–1475 m). A hyperhumic diagnostic horizon and highly acidic reactions are characteristic of the soil. The soil profile is strongly differentiated into organic and metamorphic (BM) horizons because of light granulometrical composition. The profile has also a high content rock fragments. The depth of the profile is no more than 45 cm.

Regarding humus abundance, the soils of the research area are described as having very high humus content (10%–17%), and according to the pH value of the aqueous extract, they are strongly acidic (pH 4.3–4.5). All the investigated soils are light in particle size distribution and belong to a cohesive sand group. It can be assumed therefore that all the soils have a low absorption capacity associated with a low content of finely dispersed fractions that constitute the soil-absorbing complex.
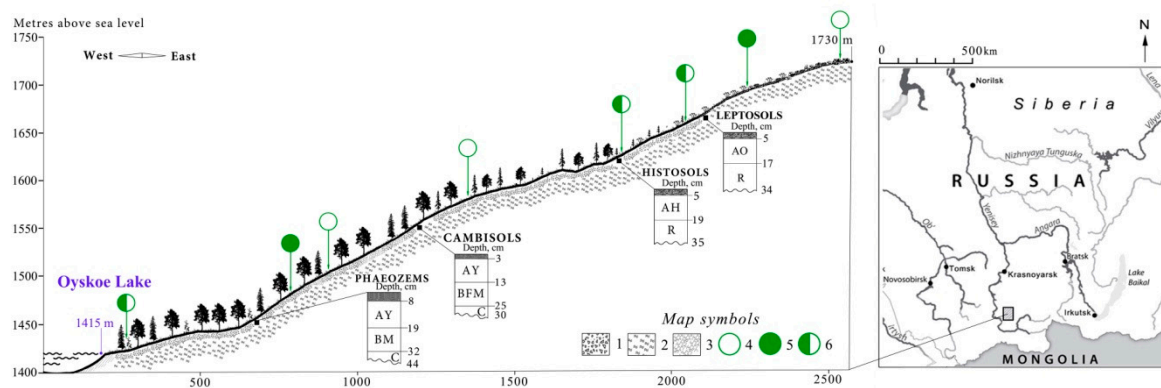

Figure 1. A graphic representation of area of research. The mountain top on the upper right has the coordinates N52.845872, E93.274658. Legend: 1—detritus; 2—metamorphic schist; 3—moraine loam; 4—*Pinus sibirica* sample points; 5—*Abies sibirica* sample points; 6—sample points for both *P. sibirica* and *A. sibirica*; AO = the coarse humic horizon; AY = the gray humic horizon; AH = the hyperhumic horizon; BFM = the ferruginous metamorphic horizon; BM = structural-metamorphic horizon; C = substratum, un-weathered geologic material; R = underlying bedrock.

In our study, a slope with a uniform climb and minimal microrelief was surveyed, which allowed us to consider the factor of increasing altitude to be a dominant one, as well the related changes in temperature, humidity, light, and other derivative parameters. The sampling points were taken at more or less equal distances from one another but taking into account the presence of trees of close age, ca. 40–50 years. The sampling of 50-cm-long shoots was done from a lower part of the crowns, with only vegetative shoots taken (no presence of female or male cones).

2.2. Climatic Data

The data on climate have been taken from station Olen'ya Rechka that operates under index 29974 as a part of the Regional Synoptic Net (Region II) of the World Meteorological Organization. For the goals of the study, mean annual temperatures and total annual precipitation data were taken to check if a climate change was significant for the area of research. The range of the data was from 1967 to 2017.

2.3. Measurements of Pigment Content

To find out the pigment composition in needles, the second-year growth needles of a peripheral shoot at the bottom of the crown was sampled. Because Siberian pine and fir grow often disjunctively

at a higher elevation, the sampling followed sometimes an ad hoc approach—where both pine and fir might have been sampled at the same location/elevation. In a few instances, however, pine and fir trees were sampled at different but still close locations (see Figure 1).

The cut shoots were delivered to the laboratory in dense paper bags within a day, and then the shoots were placed in vessels with water and were kept in the same light and temperature conditions.

The content of chlorophylls and carotenoids was quantified in an alcohol extract on a SPEKOL 1300 spectrophotometer (Analytik Jena AG, Jena, Germany) using wavelengths of 440.5, 649, 654, and 665 nm. The calculations were carried out according to the formulas of Smith and Benitez [16].

Having established the concentration of the pigment in the extract, its content in the test needles was found through the formula:

$$F = (V \times C)/P$$

where F is the pigment content in the plant material, μkg/mkg; V is the volume of the extract, l; C is the pigment concentration, μkg/l; and P is the weight of sample material, mkg. All the data were recalculated to dry weight.

2.4. Measurement of Leaf Fluorescence

The analysis of chlorophyll fluorescence (ChlF) provides a large amount of information on the physiology of conifers, especially concerning the response of plants to various environmental factors. This approach is based on the fact that the light energy absorbed by chlorophyll *a* molecules in the photosystem II (PSII) antenna has three alternative paths: it may be used by the PSII reaction center (RC) in photosynthesis, dissipate as heat, or emit as light with a shifted wavelength (ChlF). The alternatives correlate with the condition of the plants as well as with other ecophysiological factors affecting the plants [17,18]. Chlorophyll fluorescence measurements are very sensitive; various tools and analytical methods have been developed that can be used both on individual leaf and as remote sensing [18–21].

In this study, the induced chlorophyll fluorescence was measured using a JUNIOR-PAM handheld fluorimeter (Heinz Walz GmbH, Effeltrich, Germany). The electron transport rate (ETR) coefficient was taken as the main indicator, in μmol of electrons/m^{-2} s^{-1}, calculated through the WinControl-3 software (Heinz Walz GmbH, Effeltrich, Germany).

Because chlorophyll fluorescence is a measure of re-emitted light (in the red wavebands) from PSII, any ambient light can interfere with the measurement of fluorescence and thus many early systems had to be used in darkness and/or highly controlled light environments. This issue was overcome by the development of modulating systems where the light used to induce fluorescence (the measuring beam) is applied at a known frequency (modulated) and the detector is set to measure at the same frequency [22]. In this way, the detector will only measure fluorescence that results from excitation by the measuring beam and will not permit interference from ambient light. The clear advantage of this is that measurements can be made without darkening the leaf.

The fluorescence parameters of two-year-old needles were measured directly on the sampling day after dark adaptation of shoots under room conditions.

Additionally, the surface temperature of the needles and soil was measured in the field using a Flir E5 thermal imager (Flir Systems, Tallinn, Estonia). The geographical coordinates and the altitudes were found with the help of satellite receivers RTK-GPS system Trimble R8S GSM (Trimble Navigation, Sunnyvale, California, USA). The RTK-GPS system has the accuracy of ca. 50 cm at measurements of altitudes.

3. Results

As follows from Figure 2, there are definite climatic changes in the higher altitude areas of West Sayan ridge. In the period of the last 50 years, mean annual temperatures rose by ca. 2 °C, while large year-to-year variations still took place. In the same period, the total annual precipitation varied greatly but no significant trend was observed.

The highest amount of chlorophyll *a* in the needles of *P. sibirica* was found in the needles of trees on sampling point 1 in the lower part of the slope; in its middle part at heights of 1505–1637 m a.s.l. (sampling points 2 to 4) the amount of chlorophyll *a* in the needles remains constant; then, from 1637 m upward the chlorophyll content is reduced (Table 1).
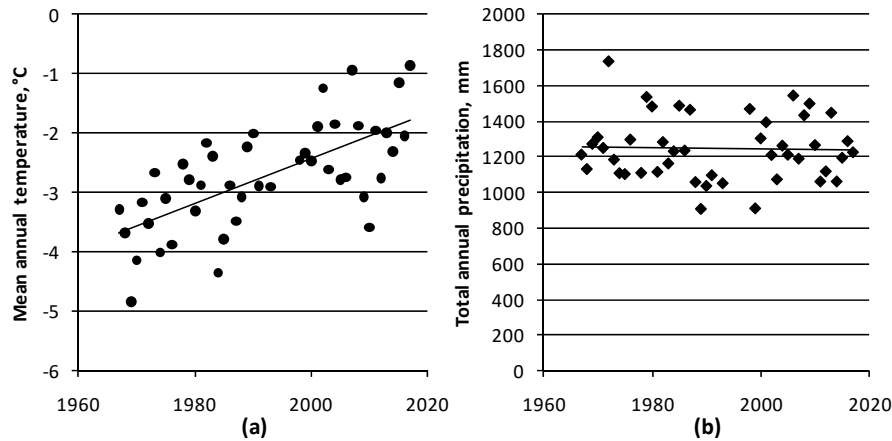

Figure 2. Long-term climatic trends over the area of research: (**a**) Annual mean temperatures; (**b**) Total annual precipitation. Straight lines give an idea of linear components of the trends. The slope of the temperature trend (~0.038) is significant at $p < 0.05$, $R^2 = 0.45$. The slope of precipitation trend (~−0.343) is not significant.

Table 1. A summary of the measured values of the pool of photosynthetic pigments at various altitudes in the vicinity of the timberline of West Sayan ridge. Sp = sample point, Chl = chlorophyll.

	Sp [1], Altitude a.s.l.,	Sp1	Sp2	Sp3	Sp4	Sp5	Sp6
P. sibirica	m	1418	1505	1567	1637	1660	1724
	Chl *a* (μkg/mkg)	1941 ± 0.069 [2]	1741 ± 0.063	1786 ± 0.065	1773 ± 0.065	1489 ± 0.066	1329 ± 0.047
	Chl *b* (μkg/mkg)	0.790 ± 0.026	0.744 ± 0.031	0.772 ± 0.028	0.782 ± 0.028	0.798 ± 0.019	0.799 ± 0.018
	Carotenoids (μkg/mkg)	0.681 ± 0.024	0.624 ± 0.022	0.575 ± 0.020	0.638 ± 0.023	0.695 ± 0.025	0.952 ± 0.034
A. sibirica	Sp, Altitude a.s.l.,	Sp1	Sp2	Sp3	Sp4	Sp5	
	m	1413	1475	1640	1660	1704	
	Chl *a* (μkg/mkg)	0.894 ± 0.031	1605 ± 0.056	2022 ± 0.128	2150 ± 0.052	1508 ± 0.047	
	Chl *b* (μkg/mkg)	0.348 ± 0.012	0.643 ± 0.020	0.897 ± 0.032	0.954 ± 0.032	0.635 ± 0.023	
	Carotenoids (μkg/mkg)	0.375 ± 0.013	0.555 ± 0.020	0.508 ± 0.018	0.811 ± 0.029	0.685 ± 0.024	

[1] Sample point index. [2] Standard deviation.

For other species of the *Pinus* genus, in particular, for *P. canariensis*, a decrease in the chlorophyll content in the treeline zone in winter was also found, which reduced the risk of overexcitation photoinhibition and photodamage, making rebuilding of photosynthetic activity possible in spring [23].

The amount of chlorophyll *b* remains constant at all points of sampling. The content of carotenoids in the needles of *P. sibirica* begins to increase from sampling point 3 (1567 m a.s.l.) upward and reaches a maximum at the point 6 (1724 m a.s.l.), where the trees transform into a shrub form.

In the needles of *A. sibirica*, an increase in the entire pigment pool from sampling point 1 (1413 m a.s.l.) to point 4 (1660 m a.s.l.) is observed; by sampling point 5 (1704 m a.s.l.) the pigment content is decreasing. Above this elevation, fir is not found.

The data obtained show that at the beginning of the growing season the higher the altitude the smaller the amount of chlorophylls in the needles of *P. sibirica*, while the amounts of carotenoids increase (Figure 3). In *A. sibirica* however, the ratio has a pronounced peak in middle elevations.

The chlorophyll *b* content in fir remains almost constant. Accordingly, the ratio of chlorophyll *a* to chlorophyll *b* in the needles decreases with altitude (Figure 4). In *P. sibirica*, the decrease is obviously more expressed.

　　Up the slope, the electron transport rate (ETR) varies in both *P. sibirica* and *A. sibirica* (Figure 5). However, after peaking at ca. 1567 m a.s.l. the *P. sibirica* ETR decreases up to the timberline. The ETR value in *A. sibirica* peaks higher, at ca. 1660 m a.s.l., and remains bigger at the timberline (ca. 1704 m a.s.l.) than that of *P. sibirica*. These data are related to chlorophyll *a* content (Table 1) and give evidence that *A. sibirica* needles have higher photosynthetic activity at altitudes of 1640 and 1660 m a.s.l.
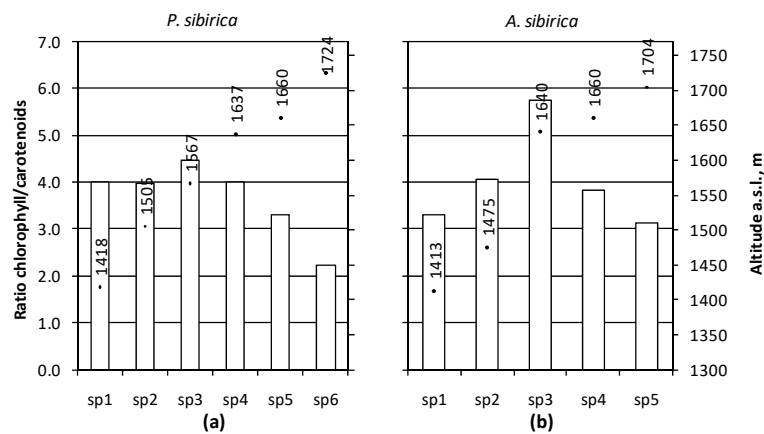
Figure 3. Change of ratio chlorophyll/carotenoids (white bars) along the study slope: (**a**) *P. sibirica*; (**b**) *A. sibirica*. sp1–sp6 denote the numbers of sample points. Vertically oriented numbers are altitudes of the sample points.
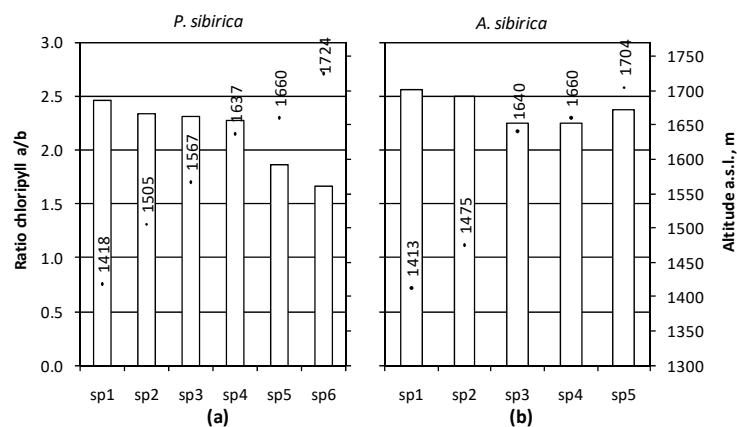

Figure 4. Change of ratio chlorophyll *a*/chlorophyll *b* (white bars) along the study slope: (**a**) *P. sibirica*; (**b**) *A. sibirica*. sp1–sp6 denote the numbers of sample points. Vertically oriented numbers are altitudes of the sample points.

　　Thus, right after the disappearance of the snow cover, there is an increase in altitude and a decrease in photosynthetic activity and chlorophyll content in the needles of *P. sibirica*, as well as an increase in the content of carotenoids. This feature contributes to the preservation of the photosynthetic apparatus at low temperatures and high insolation conditions. Adaptations of the kind allow *P. sibirica* to climb up the slopes under conditions of climate change, increasing the elevation of the timberline. For *A. sibirica*, such a clear adaptive picture is not observed throughout the entire slope, but when approaching the timberline, similar tendencies can be seen in the needles of fir trees.
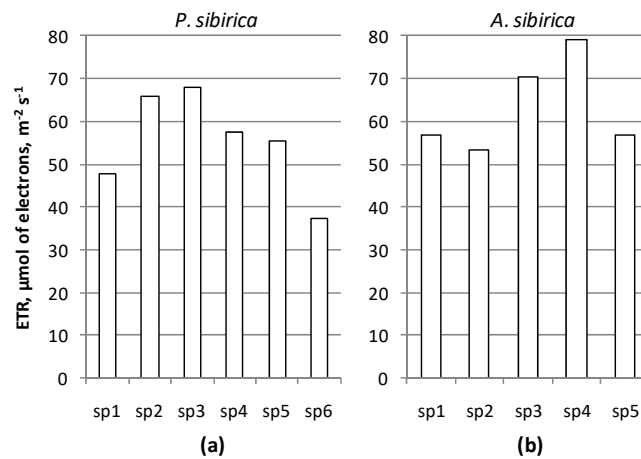
Figure 5. Change of electron transport rate (ETR) (white bars) along the study slope: (**a**) *P. sibirica*; (**b**) *A. sibirica*. sp1–sp6 denote the numbers of sample points.

At the time of measurements, the temperature of the litter was slightly positive, while the temperature of the soil under the litter had a temperature of about −5 °C (Figure 6).
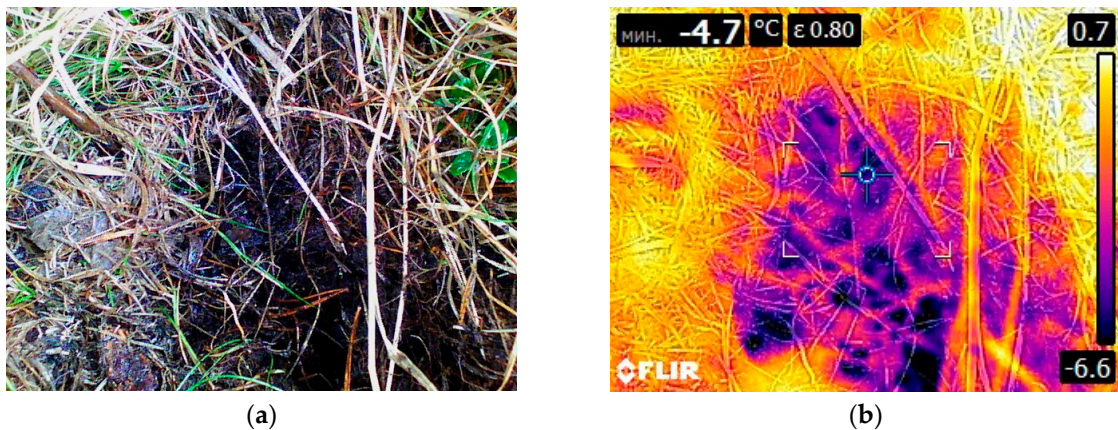

Figure 6. Photograph (**a**) and thermal image (**b**) of the soil under litter. The both images were received as single-step with the help of thermal imager Flir E5 under mean daily ambient temperature of +9.4 °C (data from the Olen'ya Rechka weather station).

At daytime, mean air temperatures rose to about +12.5 °C (according to data of Olen'ya Rechka weather station located at 1404 m a.s.l.). As per the thermal image data (Figure 7), under identical weather conditions of a cloudy morning the temperature of *A. sibirica* needles is much higher than that of *P. sibirica* (+10.5 °C and +3.8 °C, correspondingly).

On sunny days, when maximum air temperatures rose to about +18.9 °C, these differences become even more pronounced; temperatures of +43.3 °C for *A. sibirica* needles and +24.2 °C for *P. sibirica* needles were recorded. The lower needle temperatures in *P. sibirica* can be explained by the higher transpiration rate.
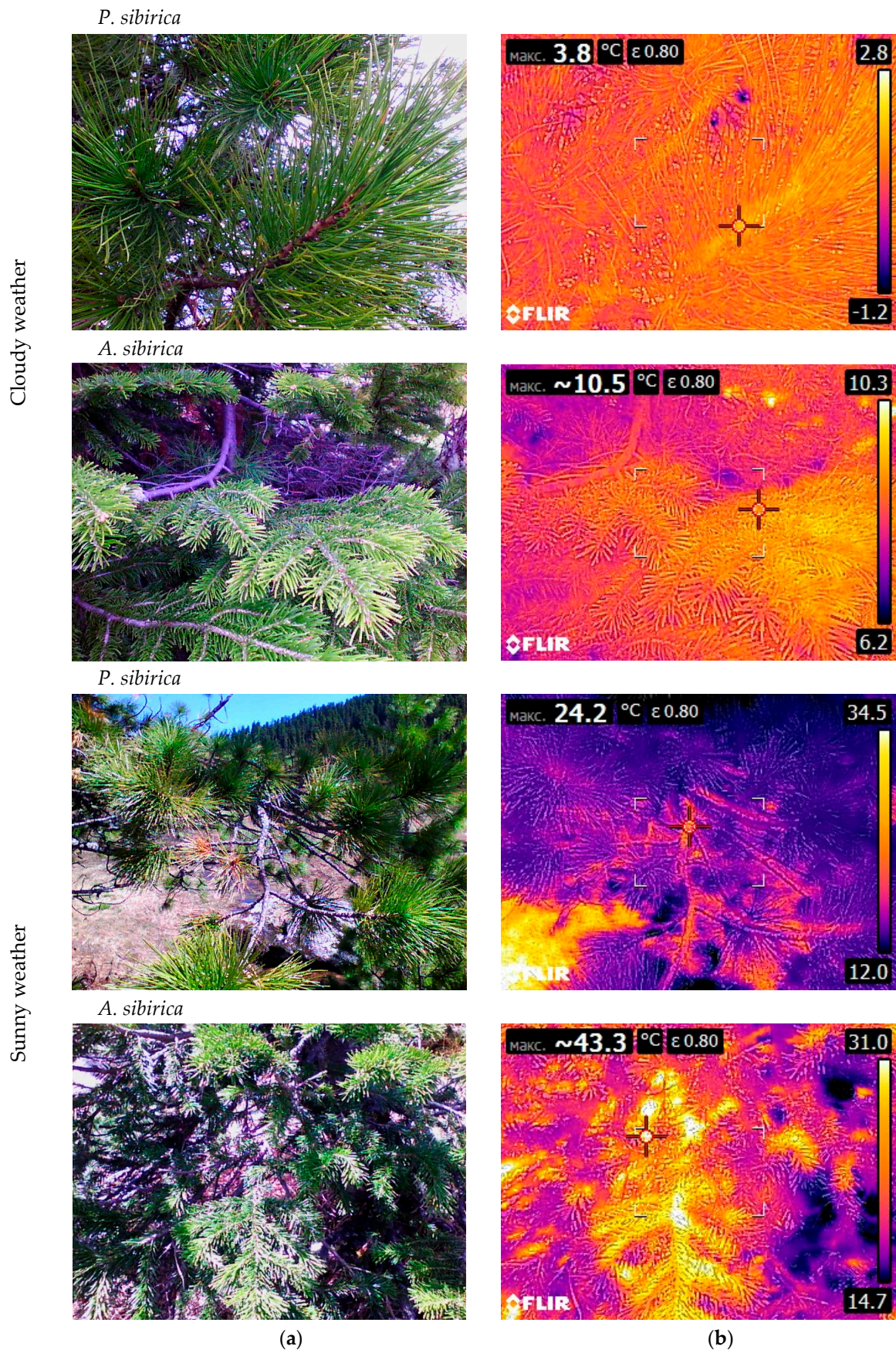
Figure 7. Photographs (**a**) and thermal images (**b**) of the young needles in *P. sibirica* and *A. sibirica* under cloudy (upper four images) and sunny (lower four images) weather.

4. Discussion

Altogether, the regional climate is becoming warmer and probably slightly drier because of rising evapotranspiration. These climatic changes are likely to influence the trees' functioning, especially those occupying the very edge of their distribution in the mountains, known as the timberline.

Since timberlines not always be straight but are often fragmented [24,25], the question is whether this fragmentation is the result of the climatic features of a given area or is related to the steepness of the slope, the type of soil, and other heterogeneities of a particular landscape. Harsch and Bader [26] distinguish four primary treeline forms: diffuse, abrupt, island, and krummholz. Growth limitation is dominant only at the diffuse treeline, which is the form that has most frequently responded as expected to growing-season warming, whereas the other forms are controlled by dieback and seedling mortality and are relatively unresponsive.

In our study, a slope with a uniform climb and minimal microrelief was surveyed, which allowed us to consider the factor of increasing altitude to be a dominant one, as well the related changes in temperature, humidity, light, and other derivative parameters.

Mountain plants generally acclimatize to high insolation [27–29] and extreme temperatures [30,31]. Temperature-related phenomena are highly likely to be determinative factors of the position of the forest boundary on a global scale [32,33].

As a rule, even extremely low winter temperatures are not a survival problem for taxa adapted to temperate zones [34–37]. Some authors reported that the treeline advance was positively associated with winter warming and not significantly associated with summer warming [38–40]. During winter dormancy, the vital activity of trees decreases sharply. Therefore, physiological processes under a gradual temperature effect are practically not affected during this period. However, in spring during the transition to the seasonal growth, plants become most vulnerable to temperature fluctuations. Negative effects of winter stress and damage to recruitment, tree survival, and growth appear to contradict the dominance of summer growth control [41–43].

In the spring on the upper edge of the forest, there are two types of threats that are not directly related to the low negative temperatures typical of winter: (1) photoinhibition caused by a combined effect of increasing solar radiation and low temperature, and (2) the so-called "winter drying" (Figure 8).

Figure 8. Altitude 1728 m a.s.l., *P. sibirica* sapling growing above the timberline. Brown dead needles are likely a result of winter desiccation.

Night frosts followed by a combination of strong solar radiation at negative or slightly positive temperatures in the daytime are typical situations in which breaking or even destruction of the photosystem by excess energy can occur. The UV radiation is not a tree-specific stress factor, given the well-designed protective measures developed by plants, but its effect can be seen by a decreased ratio

of chlorophylls to carotenoids [44]. Carotenoids perform many important functions in the process of photosynthesis: they provide additional pigments at the absorbing of solar energy and they protect the photosystem. The protective function includes a quenching of triplet chlorophyll and singlet oxygen, protection of the reaction center of the photosystem from powerful energy flows at high light intensities, and protection of the lipid phase of thylakoid membranes from overoxidation.

In spring, an increase in the carotenoid pool with a lower chlorophyll content may allow *P. sibirica* saplings from higher altitudes to avoid photoinhibition. Also, by reducing the speed of electron transport (Figure 5), the protection of the needles from water deficiency and physiological desiccation is ensured. The desiccation is known to result from the inaccessibility of soil moisture during the period of low temperatures. In the needles of middle altitude *A. sibirica*, a peak of the concentration of pigments is found. In the upward direction, their content decreases and the electron transport speed decreases as well (Figure 5), which indicates a slowing down in general photosynthetic activity. Moreover, the share of *A. sibirica* carotenoids in the total pool of its pigments grows with altitude, similarly to *P. sibirica*, but the growth is weaker (Figure 3).

Some authors believe that acclimation to high insolation at low temperatures reduces the ability for primary photosynthetic reactions, that is, the ability to assimilate carbon and synthesize sugar. Through this acclimation, the plant can use high-intensity light more efficiently while limiting photosynthesis at low light flux without changing the quantum yield [29,31]. Thus, the decrease in the electron transport rate (ETR) in the needles with altitude (Figure 5) is, apparently, a transitional reaction of acclimation at the beginning of the growing season in both *P. sibirica* and *A. sibirica*.

Trees, like any plants, have a variable tissue temperature compared to the air temperature. Under direct insolation, alpine plants can warm up 10–20 °C above air temperature and sometimes even more [45].

For temperate zone plants, the temperature optimum for photosynthesis processes is known to be in the range of 25–30 °C, while higher temperatures lead to a decrease in the rate of photosynthesis. Therefore, heating of needles up to 40 °C and above is unfavorable and is associated most likely with a lack of water for cooling by transpiration. The root system in *P. sibirica* is located superficially, while in *A. sibirica* it lies in deeper layers of the soil, where there is still no accessible (in the liquid phase) water at this time.

The exceptionally low snow cover is a norm in the upper part of the slope and on the peak during cold weather. This leads to deep freezing of the soils which are also as usual shallow ones. The prerequisites are thus created under which the drying out of needles at the end of winter becomes very likely. This is the result of low positive temperatures at daytime (<10 °C) and a light frost at night (>−5 °C), which are often considered to be critical for plant activity [46,47].

In the middle and lower parts of the slope, the soils can be separated from the atmosphere by a layer of snow that also causes trees to experience the warm temperatures of the canopy, while the soils are still cool or even frozen [48]. Furthermore, if thick layers of moss isolate the ground during the summer, the soil does not thaw deep enough or thaw too slowly [49,50].

On the slope studied, trees and undergrowth of *P. sibirica* were located more or less singly, while the undergrowth of *A. sibirica* was distributed as clumps confined to more gentle parts of the microrelief. Apparently, this distribution of *A. sibirica* is due to the species tending to show a loci where more moisture accumulates during the growing season because the *A. sibirica* is less drought-resistant than *P. sibirica*.

However, some authors note that colder soil temperatures under woody vegetation, compared e.g., with grass or shrubs, are a common phenomenon for any forest type [51,52]. This observation would indicate a positive effect of more sparsely located trees on the growth near the timberline, while clumping of forest in densely standing trees would be unfavorable for root temperatures. After the classification by Harsch and Bader [26] *P. sibirica* trees form a diffuse treeline form, whereas an abrupt treeline form or island treeline form are rather characteristic of *A. sibirica*.

5. Conclusions

To conclude, under conditions of possible climate warming in the mountains of Southern Siberia, *P. sibirica* will have an advantage in colonizing the zone above the timberline. Some features of the species, both physiological and biochemical, will ensure its wider distribution; these features include a higher content of carotenoids, characteristic of light-coniferous photophilous plants, and a lower rate of photosynthesis at low temperatures. Morphologically, the surface root system is located in the upper, more warmed up soil layers, which allows the species to have more water available during this period and, despite the high level of insolation, to maintain the optimal temperature of the needles to avoid desiccation. As a result, *P. sibirica* may be a more likely winner than *A. sibirica* in driving up the mountain timberline under climate warming in the area.

References

1. Takahashi, K. Virtual issue: Alpine and subalpine plant communities: Importance of plant growth, reproduction and community assemblage processes for changing environments. *J. Plant Res.* **2018**, *131*, 891–894. [CrossRef]
2. Welling, A.; Palva, E.T. Molecular control of cold acclimation in trees. *Physiol. Plant* **2006**, *127*, 167–181. [CrossRef]
3. Richardson, A.D.; Hufkens, K.; Milliman, T.; Aubrecht, D.M.; Furze, M.E.; Seyednasrollah, B.; Krassovski, M.B.; Latimer, J.M.; Nettles, W.R.; Heiderman, R.R.; et al. Ecosystem warming extends vegetation activity but heightens vulnerability to cold temperatures. *Nature* **2018**, *560*, 368. [CrossRef]
4. Peng, G.; Wu, C.; Xu, X.; Yang, D. The age-related changes of leaf structure and biochemistry in juvenile and mature subalpine fir trees (*Abies faxoniana* Rehder & EH Wilson.) along an altitudinal gradient. *Pol. J. Ecol.* **2012**, *60*, 311–321.
5. Miron, M.S.; Cristea, V.; Sumalan, R.L. Physiological responses of European silver sir (*Abies alba* Mill.) seedlings to drought and overheating induced stress conditions. *J. Hortic.* **2018**, *22*, 115–120.
6. Poulos, H.M.; Berlyn, G.P.; Mills, S.A. Differential stress tolerance of four pines (*Pinaceae*) across the elevation gradient of the San Bernardino Mountains, Southern California, USA. *J. Torrey Bot. Soc.* **2012**, *139*, 96–109. [CrossRef]
7. Jordan, R.K. Comparative Ecophysiology of Two High-Elevation Southern Appalachian Conifers: The Importance of the Winter Season. Ph.D. Thesis, Appalachian State University, Boone, NC, USA, 2018.
8. Kopačková, V.; Lhotáková, Z.; Oulehle, F.; Albrechtová, J. Assessing forest health via linking the geochemical properties of a soil profile with the biochemical parameters of vegetation. *Int. J. Environ.* **2015**, *12*, 1987–2002. [CrossRef]
9. Sobchak, R.O.; Zotikova, A.P. Impact of high altitude conditions on anatomical and physiological characteristics of Siberian pine needles. *Vestnik Tomskogo Gosudarstvennogo Universiteta* **2009**, *326*, 200–202. (In Russian)

10. Bender, O.G.; Zotikova, A.P.; Velisevich, S.N. Water relation features and pigment complex state of Pinus sibirica Du Tour needles in the north-eastern Altai Mountains. *Vestnik Tomskogo Gosudarstvennogo Universiteta Biologia* **2009**, *3*, 63–72. (In Russian)

11. Titova, M.S. Seasonal dynamics of the pigments availability in needles of Siberian pine (Pinus Sibirica) and Korean pine (Pinus Koraiensis). *Vestnik Krasnoyarskogo Gosudarstvennogo Agrarnogo Universiteta* **2010**, *8*, 77–81. (In Russian)

12. Varlam, I.I.; Rusak, S.N.; Kazartseva, K.V. Seasonal changes of the Pinus sibirica pigment structure in the conditions of urboecosystems of northern territories (on the example of Surgut). *Ekologia Urbanisirovannyh Territorij* **2019**, *1*, 82–86. (In Russian)

13. Silkina, O.V. Complex Evaluation of Ecological and Physiological Parameters of Needles in *Abies sibirica* and *Picea abies* in the Process of Vegetation and Its Phytoproduction Activity. Ph.D. Thesis, University of Kazan, Kazan, Russia, 2006. (In Russian).

14. Golovko, T.K.; Yatsko, Y.N.; Dymova, O.V. Seasonal changes in the state of the photosynthetic apparatus in three boreal conifers in middle taiga of European North-East. *Khvoinye Borealnoy Zony* **2013**, *30*, 73–78. (In Russian)

15. IUSS Working Group WRB. *World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015; Available online: http://www.fao.org/3/i3794en/I3794en.pdf (accessed on 12 November 2019).

16. Wintermans, J.E.G.; De Mots, A. Spectrophotometric Characteristics of chlorophyll a and b and their phaeophytins in ethanol. *Biochimica et Biophysica Acta* **1965**, *109*, 448–453. [CrossRef]

17. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]

18. Kalaji, H.M.; Schansker, G.; Ladle, R.J.; Goltsev, V.; Bosa, K.; Allakhverdiev, S.I.; Brestic, M.; Bussotti, F.; Calatayud, A.; Dąbrowski, P.; et al. Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. *Photosynth. Res.* **2014**, *122*, 121–158. [CrossRef]

19. Damm, A.; Guanter, L.; Paul-Limoges, E.; van der Tol, C.; Hueni, A.; Buchmann, N.; Eugster, W.; Ammann, C.; Schaepman, M.E. Far-red sun-induced chlorophyll fluorescence shows ecosystem-specific relationships to gross primary production: An assessment based on observational and modeling approaches. *Remote. Sens. Environ.* **2015**, *166*, 91–105. [CrossRef]

20. Meroni, M.; Rossini, M.; Guanter, L.; Alonso, L.; Rascher, U.; Colombo, R.; Moreno, J. Remote sensing of solar-induced chlorophyll fluorescence: Review of methods and applications. *Remote Sens. Environ.* **2009**, *113*, 2037–2051. [CrossRef]

21. Qiu, N.; Zhou, F.; Gu, Z.; Jia, S.; Wang, X. Photosynthetic functions and chlorophyll fast fluorescence characteristics of five Pinus species. *Chin. J. Appl. Ecol.* **2012**, *23*, 1181–1187.

22. Schreiber, U.; Schliwa, U.; Bilger, W. Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* **1986**, *10*, 51–62. [CrossRef]

23. González-Rodríguez, A.; Brito, P.; Lorenzo, J.; Jiménez, M. Photosynthetic Performance in Pinus canariensis at Semiarid Treeline: Phenotype Variability to Cope with Stressful Environment. *Forests* **2019**, *10*, 845.

24. Holtmeier, F.-K. Die Höhengrenze der Gebirgswälder. In *Arbeiten Aus Dem Institut für Landschaftsökologie 8*; Westfälische Wilhelms-Universität: Münster, Germany, 2000.

25. Holtmeier, F.-K. *Mountain Timberlines: Ecology, Patchiness, and Dynamics (Vol. 36)*; Springer Science & Business Media: New York, NY, USA, 2009; p. 436.

26. Harsch, M.; Bader, M. Treeline form—A potential key to understanding treeline dynamics. *Glob. Ecol. Biogeogr.* **2011**, *20*, 582–596. [CrossRef]

27. Anderson, J.M.; Osmond, C.B. Shade-sun responses: Compromises between acclimation and photoinhibition. In *Photoinhibition*; Kyle, D.J., Osmond, C.B., Arntzen, C.J., Eds.; Elsevier: New York, NY, USA, 1987; pp. 1–37.

28. Bailey, S.; Walters, R.G.; Jansson, S.; Horton, P. Acclimation of Arabidopsis thaliana to the light environment: The existence of separate low light and high light responses. *Planta* **2001**, *213*, 794–801. [CrossRef]

29. Walters, R.G. Towards an understanding of photosynthetic acclimation. *J. Exp. Bot.* **2005**, *56*, 435–447. [CrossRef]

30. Huner, N.P.A.; Öquist, G.; Hurry, V.M.; Krol, M.; Falk, S.; Griffith, M. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynth. Res.* **1993**, *37*, 19–39. [CrossRef]

31. Huner, N.P.A.; Öquist, G.; Sarhan, F. Energy balance and acclimation to light and cold. *Trends Plant Sci.* **1998**, *3*, 224–230. [CrossRef]

32. Rossi, S.; Deslauriers, A.; Anfodillo, T.; Carraro, V. Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. *Oecologia* **2007**, *152*, 1–12. [CrossRef]

33. Slatyer, R.O. Altitudinal variation in the photosynthetic characteristics of snow gum, Eucalyptus pauciflora Sieb. ex Spreng. VII. Relationship between gradients of field temperature and photosynthetic temperature optima in the Snowy Mountains area. *Aust. J. Bot.* **1978**, *26*, 111–121. [CrossRef]

34. Larcher, W. Frostresistenz. In *Handbuch der Pflanzenkrankheiten*; Rademacher, B., Ed.; Parey-Verlag: Berlin/Heidelberg, Germany, 1985; Volume 1, pp. 177–259.

35. Larcher, W. Winter stress in high mountains. In *Establishment and Tending of Subalpine Forest: Research and Management*; Turner, H., Tranquillini, W., Eds.; Swiss Federal Institute of Forestry Research: Birmensdorf, Switzerland, 1985; Volume 270, pp. 11–19.

36. Larcher, W. *Physiological Plant Ecology*, 4th ed.; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2003; p. 513.

37. Sakai, A.; Larcher, W. *Frost Survival of Plants: Responses and Adaptation. Ecological Studies 62*; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 1987; p. 321.

38. Kullman, L. Tree line population monitoring of Pinus sylvestris in the Swedish Scandes, 1973–2005: Implications for treelinetheory and climatechangeecology. *J. Ecol.* **2007**, *95*, 41–52. [CrossRef]

39. Rössler, O.; Bräuning, A.; Löffler, J. Dynamics and driving forces of treeline fluctuation and regeneration in central Norway during the past decades. *Erdkunde* **2008**, *62*, 117–128. [CrossRef]

40. Harsch, M.A.; Hulme, P.E.; McGlone, M.S.; Duncan, R.P. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecol. Lett.* **2009**, *12*, 1040–1049. [CrossRef]

41. Tranquillini, W. *Physiological Ecology of the Alpine Timberline*; Springer: New York, NY, USA, 1979; p. 137.

42. Pereg, D.; Payette, S. Development of black spruce growth forms at treeline. *Plant Ecol.* **1998**, *138*, 137–147. [CrossRef]

43. Rickebusch, S.; Lischke, H.; Bugmann, H.; Guisan, A.; Zimmermann, N.E. Understanding the low-temperature limitations to forest growth through calibration of a forest dynamics model with tree-ring data. *Forest Ecol. Manag.* **2007**, *246*, 251–263. [CrossRef]

44. Germino, M.J.; Smith, W.K. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell Environ.* **1999**, *22*, 407–415. [CrossRef]

45. Körner, C. *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems; with 47 Tables*; Springer Science & Business Media: Berlin/Heidelberg, Germany; New York, NY, USA, 2003; p. 359.

46. Körner, C. Winter crop growth at low temperature may hold the answer for alpine treeline formation. *Plant Ecol. Divers* **2008**, *1*, 3–11. [CrossRef]

47. Sutinen, M.L.; Ritari, A.; Holappa, T.; Kujala, K. Seasonal changes in soil temperature and in the frost hardiness of Scots pine (*Pinus sylvestris*) roots under subarctic conditions. *Can. J. For. Res.* **1998**, *28*, 946–950. [CrossRef]

48. Körner, C. Treelines will be understood once the functional difference between a tree and a shrub is. *Ambio* **2012**, *41*, 197–206. [CrossRef]

49. Ballard, T.M. Subalpine soil temperature regimes in southwestern Brithish Columbia. *Arct. Alp. Res.* **1972**, *4*, 139–146. [CrossRef]

50. Scott, P.A.; Bentley, C.V.; Fayle, D.C.F.; Hansell, R.I.C. Crown forms and shoot elongation of white spruce at the treeline, Churchill, Manitoba, Canada. *Arct. Alp. Res.* **1987**, *19*, 175–186. [CrossRef]

51. Körner, C.; Paulsen, J.; Pelaez-Riedl, S. A bioclimatic characterisation of Europe's alpine areas. In *Alpine Biodiversity in Europe*; Nagy, L., Grabherr, G., Körner, C., Thompson, D.B.A., Eds.; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2003; Volume 167, pp. 13–28.

52. Körner, C.; Paulsen, J. A world-wide study of high altitude treeline temperatures. *J. Biogeogr.* **2004**, *31*, 713–732. [CrossRef]