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RESEARCH PAPERS

Pigments and Photosynthesis of Understory Grasses: Light Irradiance and Soil Moisture Effects[1]

L. J. Selzer[a] and C. A. Busso[b]

[a] *Centro Austral de Investigaciones Científicas (CADIC), Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), and Instituto de Ciencias Polares, Ambiente y Recursos Naturales Universidad Nacional de Tierra del Fuego, Antártida e Islas del Atlántico Sur, Ushuaia, Tierra del Fuego, Argentina*

[b] *Departamento de Agronomía y Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), CONICET, Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina*

e-mail: lselzer@untdf.edu.ar

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Abstract—*Phleum alpinum* L. and *Poa pratensis* L. are major forage species that often grow in various environments in Tierra del Fuego, Argentina. We performed a greenhouse experiment to investigate how these species acclimate to the different irradiance of the microenvironments where they grow. Both grass species were exposed to three levels of incident irradiance (I4: 4%; I26: 26%, or I64: 64% of ambient sunlight) and two levels of soil moisture content (M30: 30–50% or M60: 60–80% of field capacity) under greenhouse conditions. As irradiance levels increased, the contents of chlorophyll per unit surface area and fresh weight basis increased, and the chlorophyll *a/b* and carotenoids/chlorophyll ratio also increased. Maximum photosynthetic rate and the light compensation point increased with increasing light availability. Values for these variables varied with time. However, the relationship of these values was not modified in *P. alpinum* between the irradiance treatments. Contrarily, temporal changes of those variables showed that the maximum photosynthetic rate was similar to that in March in all treatments in *P. pratensis.* Results indicated that *P. alpinum* and *P. pratensis* were able to acclimate to the various experimental environments.

Keywords: Poa pratensis, *Phleum alpinum*, leaf photosynthetic rates, chlorophyll, carotenoids

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INTRODUCTION

The energy obtained through the photosynthesis process provides plants almost all their chemical energy, and it is critical to determine their competitive ability and reproduction capacity. This process is directly and greatly influenced by the amount of light that reaches the plant [1]. In general, the photosynthetic rate increases as light increases during plant growth [2]. These changes are the result of acclimation to a morphological and physiological scale. At a morphological level, leaves increase their thickness because of an increase in the quantity or thickness of the palisade cells, reducing their specific leaf area as a result [3]. However, this increase in thickness implies a reduction of leaf area which translates into a lower light interception. To a physiological level, changes include less chlorophyll per unit nitrogen, a higher chlorophyll *a/b* ratio, a higher electron transport capacity per unit chlorophyll, and a slightly higher ratio of electron transport capacity per unit of ribulose bisphosphate carboxylase/oxygenase (Rubisco) [3].

Pigments are critically important for the leaf physiological function. Chlorophyll absorbs light energy which is transferred to the photosynthetic apparatus. Carotenoids can also contribute light to it [2]. Leaves exposed to direct solar irradiance are significantly different from those grown under shading [2]. The photosynthetic apparatus of light-leaves is adapted to high photosynthetic rates [1].

Variations in the content of pigments can provide information on the physiological stage of leaves because of their importance in leaf functioning. For example, the decline of chlorophyll level is more rapid than that of carotenoids when plants are exposed to stressful conditions or during leaf senescence [4]. Water stress also produces a decline in chlorophyll content by inhibiting its formation or increasing its catabolism [5].

Phleum alpinum and *Poa pratensis* are two perennial grass species, which are palatable (i.e., preferred) to wildlife herbivores like guanaco (*Lama guanicoe* Müller) and also to domestic livestock [6–8]. They

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Abbreviations: AR—aggregate retention; BDR—border of disperse retention with influence of the aggregate retention; Car/Chl—ratio between carotenoids and chlorophyll; DR—disperse retention; PF—primary forest.

Table 1. Climatic variables in the greenhouse where *Phleum alpinum* and *Poa pratensis* plants were exposed to three irradiance levels

Month	Air temperature, °C			Air humidity, %			Soil temperature, °C		
	I64	I26	I4	I64	I26	I4	I64	I26	I4
Oct	8.5 ± 2.7	9.1 ± 2.4	9.5 ± 2.3	66 ± 8	66 ± 7	65 ± 7	9.1 ± 2.1	8.1 ± 1.3	8.0 ± 1.4
Nov	11.4 ± 2.5	12.5 ± 2.4	12.5 ± 2.3	67 ± 8	66 ± 7	66 ± 6	12.2 ± 1.8	11.2 ± 1.3	11.0 ± 1.3
Dec	11.9 ± 3.2	12.9 ± 3.1	13.0 ± 3.0	67 ± 6	66 ± 5	67 ± 5	12.1 ± 2.1	11.4 ± 1.7	11.3 ± 1.8
Jan	16.3 ± 1.2	16.9 ± 1.5	16.9 ± 1.4	65 ± 2	65 ± 2	64 ± 2	16.6 ± 1.0	14.4 ± 0.7	14.3 ± 0.8
Feb	13.1 ± 2.1	13.6 ± 1.9	13.4 ± 1.8	66 ± 10	66 ± 9	68 ± 9	13.6 ± 1.3	12.4 ± 0.7	12.1 ± 0.9
Mar	10.9 ± 2.4	11.2 ± 2.4	10.8 ± 2.3	67 ± 7	68 ± 7	69 ± 7	11.2 ± 1.7	10.4 ± 1.2	10.1 ± 1.3

Climatic variables throughout a growing season at Ushuaia city, Argentina. I64 = 64% of natural incident irradiance; I26 = 26% of natural incident irradiance; I4 = 4% of natural incident irradiance. Values are mean ± SE.

inhabit various environments in Tierra del Fuego, ranging from moist grasslands, open scrub to closed deciduous forests [9]. These environments differ in its openness and therefore in the light that reaches the lowest level of vegetation, which in turn will promote plants' acclimation [2].

There are no studies on the capacity of photosynthetic acclimation in *P. alpinum*; and there is only one report on the effects of photoperiod and various temperatures on the growth of two bipolar *P. alpinum* populations [10]. On the other hand, there are several contributions for *P. pratensis*, since this plant is of agronomic importance [11]. Van Huylenbroeck and van Bockstaele [11] also studied the effects of 100 and 65% irradiance levels on growth of these species.

Our objective was to determine the changes in the content of photosynthetic pigments, and the photosynthetic plasticity of *P. alpinum* and *P. pratensis* under contrasting conditions of irradiation and soil moisture levels. The hypotheses were that (1) the contents of chlorophyll decrease, and the ratios of chlorophyll *a/b* and carotenoids/chlorophyll increase, when irradiation increases, (2) chlorophyll content decreases as soil moisture content also decreases, and (3) the maximum net photosynthetic rate is higher in plants acclimated to high than to low irradiances, while the light compensation point follows an inverse pattern.

MATERIALS AND METHODS

Sampling procedures. We collected *Poa pratensis* L. and *Phleum alpinum* L. plants that grew in forest near Ushuaia (54°43'09" S, 68°08'26" W). We divided these plants up to 2–3 tillers per plant. These tillers were placed in 3-L pots, which were filled with a 1 : 1 : 1 mixture of forest soil, sand, and peat [12].

We placed the plants inside a greenhouse covered with 100-μm nylon. Inside, we produced three levels of light intensity by using two, one or no layers of shade cloth. We measured light intensity using a ceptometer (AccuPAR LP-80, "Decagon Devices", United States). The three light levels produced had 64, 26, and 4% (I64, I26 and I4, respectively) of the total solar radiation. Mean solar radiation during summer is about 2085 ± 534 μmol/(m^2 s), with a maximum value of 2702 μmol/(m^2 s) [12]. Within each light level, plants were exposed to either high (60–80% of field capacity; M60) or low (30–50%; M30) moisture levels. We followed the procedure described by Selzer et al. [12] to produce the different moisture levels.

We used a thermostat and forced ventilation to control the temperature. In this way, temperature was set to below 24°C at the plant level. Air and soil temperatures and air humidity within the greenhouse during the study period are shown in Table 1.

Compared to field measurements, the light intensities inside the greenhouse reflected natural conditions: I4 matched light conditions under an undisturbed primary forest with a completely closed canopy; I26 matched either open areas inside an undisturbed forest or closed sectors in harvested stands (up to 30 m^2/ha basal area). Finally, I64 matched post-harvest conditions of managed forests with wide-open areas (10–15 m^2/ha basal area) [13].

Six plants per irradiance and moisture treatments were randomly sampled for pigment and photosynthesis measurements in December 2006, and January and March 2007.

Pigment measurements. Pieces of variable length weighting between 0.1 to 0.3 g were cut from fully

expanded, youngest leaves. Thereafter, pigments were extracted with acetone and Tris-buffer (4 : 1, v/v, pH 7.8). Centrifugation was conducted to remove particles, and the floating portion was used to measure pigments using a spectrophotometer at the 470, 537, 647, and 663 nm following Sims and Gamon [4]. Equations used to determine pigments, obtained from Sims and Gamon, were as follows:

$$\text{Anthocyanins (Ant)} = 0.08173A_{537} - 0.00697A_{647} - 0.002228A_{663}, \quad (1)$$

$$\text{Chl}_a = 0.01373A_{663} - 0.000897A_{537} - 0.003046A_{647}, \quad (2)$$

$$\text{Chl}_b = 0.02405A_{647} - 0.004305A_{537} - 0.005507A_{663}, \quad (3)$$

$$\text{Carotenoids} = [A_{470} - (17.1(\text{Chl}_a + \text{Chl}_b) - 9.479\text{Ant})]/119.26. \quad (4)$$

Units of all equations are μmol/mL.

Despite anthocyanin can be calculated using equation (1), confidence of the results is low because of the high variation in the anthocyanin structure, and the fact that degradation is time-dependent in neutral buffer [4].

Pigment units were transformed to mmol/m^2 and mmol/g fr wt, and total chlorophyll (sum of Chl *a* and *b*), Chl *a/b* ratio, and the ratio between carotenoids and chlorophyll (Car/Chl) were derived.

Photosynthesis measurements. The net CO_2 flux was measured using an infrared gas analyser (Model S151, Qubit Systems, United States) with leaf camera of 9 cm^2 and thermal, electronic analyser of gas fluxes. The equipment is an open system that determines the CO_2 flux by differential measurements. Measurements were taken under controlled laboratory conditions. Temperature within the camera was 18.4 ± 3.3°C, humidity—37.0 ± 3.8%, and the atmospheric concentration of CO_2—366 ± 11 ppm.

Measurements were made using the youngest, completely expanded leaf from each plant studied. Leaves were detached from the plant before measurements. The response of photosynthesis to light was obtained at 0, 25, 50, 100, 250, 500, 750, and 1000 μE/(m^2 s). Previous to the photosynthesis measurements, leaves were allowed to acclimate to new light intensity conditions during 3–5 min. Sampling of CO_2 was conducted 500 times/min, and the measurement was taken when it stabilized at least 150 times with variation ± 1 ppm.

Non-linear mixed model was adjusted to the photosynthesis measurements because data constituted repeated measurements [14]. The used equation to model photosynthetic response was that of [14]. It was as follows:

$$A = A_{\max}\,(1 - e^{(A_{qe}(\text{PFP}-\text{LCP}))}).$$

$A_{\max}$ represents the plateau of photosynthesis at saturating irradiance, A_{qe} is the initial slope at low light intensities, LCP is the interception on the X axis when photosynthesis equals zero, PFP is the incident photosynthetic photon flux, and A is the net photosynthesis (i.e., the response variable). Each of the unknown parameters has a physiological meaning related with plant yield. Those used in the model identify photosynthetic rate at saturating light ($A_{\max}$), apparent quantum yield (A_{qe}), and light compensation point of photosynthesis (LCP).

We assumed that the mean of these parameters vary on the study treatments, and that these coefficients follow a multivariate normal distribution because all three parameters vary individually per plant. Use of a multivariate normal distribution allows that $A_{\max}$, A_{qe}, and LCP are correlated. Thereafter, a plant with $A_{\max}$ above the average is likely to have an initial slope (A_{qe}) greater than the average.

Statistical analysis. For analysis of the pigments, data of chlorophyll content and chlorophyll *a/b* ratio were transformed to their cubic root, and those of Car/Chl were transformed to their square, to comply with the assumptions of homoscedasticity and normality. Data were then analyzed using a four-way-ANOVA [15]: (1) date (three levels; December, January, March), (2) species (two levels; *P. alpinum* and *P. pratensis*), (3) light (three levels; I4, I26, I64), and (4) soil moisture content (two levels; M30 and M60). Whenever interactions were found, they were graphically analyzed, and thereafter simpler ANOVAs were made. When significant differences were found, means were compared using the Tukey's test at $p < 0.05$.

To analyze the photosynthesis data, correlation matrix of second order moving window average was used because the residuals were correlated. In addition, heterocedasticity was detected among the residuals. This was modeled with an exponential function of variances, where the residual variances increase exponentially according to the predicted value. A procedure of adjustment of a mixed, non-linear model of the package nlme (i.e., function nlme) from the statistical software R [16] was used to model photosynthetic response curve.

RESULTS

Pigments

Significant differences were found in all studied variables as dependent on incident radiation. However, light effects on the various parameters differed not according to the species, except Car/Chl ratio.

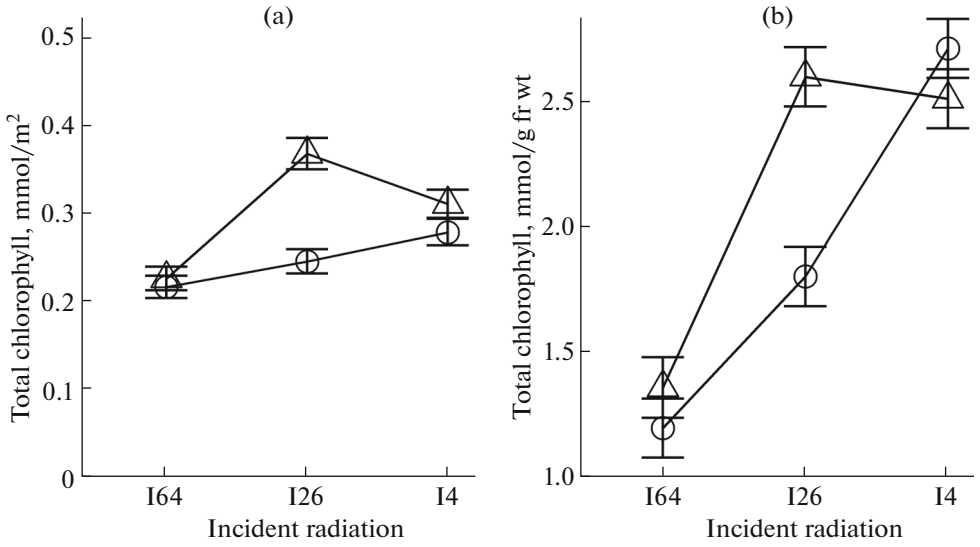

Fig. 1. Total chlorophyll content per unit surface area (a) or fresh weight (b) in *Phleum alpinum* (circles) and *Poa pratensis* (triangles) growing under greenhouse conditions at three irradiance levels: I64 (64% of total sunlight), I26 (26% of total sunlight), I4 (4% of total sunlight). Data are average of measurements made in December, January, and March. Data are mean values ± SE (n = 12).

In general, chlorophyll contents per unit surface area (Fig. 1a) and weight (Fig. 1b) followed a similar pattern. Under both units of measurements, they were higher in *P. pratensis* ($F_{1;178} = 6.58$, $p = 0.0018$) than in *P. alpinum* ($F_{1;178} = 9.59$, $p = 0.0001$) only under I26 (Figs. 1a and 1b).

Content of total chlorophyll per unit surface area was significantly higher ($p < 0.05$) under I26 and I4 than under I64 in *P. pratensis* (Fig. 1a). On the other hand, it increased as light irradiation decreased in *P. alpinum*, and it was significantly higher ($p < 0.05$) under I4 than I64 (Fig. 1a); values under I26 were intermediate and did not differ ($p > 0.05$) from those under I4 and I64. However, when values were expressed on per unit fresh weight basis, total chlorophyll was similar ($p > 0.05$) under I26 and I4, and values under these irradiances were higher ($p < 0.05$) than those under I64 in *P. pratensis* (Fig. 1b). When total chlorophyll was expressed in this unit, it increased ($p < 0.05$) as light irradiance decreased in *P. alpinum* (Fig. 1b). Chlorophyll content per unit surface area and fresh weight was significantly higher ($p < 0.05$) in December than in January and March in both species ($F_{2;178} = 19.55$, $p < 0.0001$; $F_{2;178} = 20.77$; $p < 0.0001$) (Table 2).

A three-way-interaction was found among species, irradiance and soil moisture content ($F_{2;178} = 3.93$; $p = 0.021$). However, no distinguishable pattern was found, and there were no significant differences between soil moisture contents within each combination of species and irradiance after the Tukey's test.

The chlorophyll *a/b* ratio increased as irradiance increased in both species ($F_{2;178} = 39.53$, $p < 0.0001$). However, it was found an interaction between irradiance and species ($F_{2;178} = 4.63$; $p = 0.0109$). This was because such ratio was equal under I26 and I4 in *P. alpinum* (Fig. 2). There were no effects ($p > 0.10$), neither of month, nor of soil moisture, on chlorophyll *a/b* ratio ($p > 0.10$) (data not shown).

Table 2. Monthly mean of total chlorophyll content per surface area and weight on plants of *Phleum alpinum* and *Poa pratensis* growing under greenhouse conditions

Total chlorophyll content	December	January	March
Phleum alpinum, mmol/m^2	0.31 ± 0.10^a	0.22 ± 0.07^b	0.23 ± 0.06^b
Poa pratensis, mmol/m^2	0.36 ± 0.11^a	0.28 ± 0.12^b	0.30 ± 0.12^b
Phleum alpinum, mmol/kg	2.45 ± 1.01^a	1.69 ± 0.89^b	1.63 ± 0.76^b
Poa pratensis, mmol/kg	2.51 ± 0.94^a	1.92 ± 0.78^b	2.11 ± 0.90^b

Each value represents the average of the three irradiance levels within each month. Values are the mean ± 1 standard error of $n = 36$. Different letters within a same row indicate significant differences ($p < 0.05$) among months using the Tukey's test.

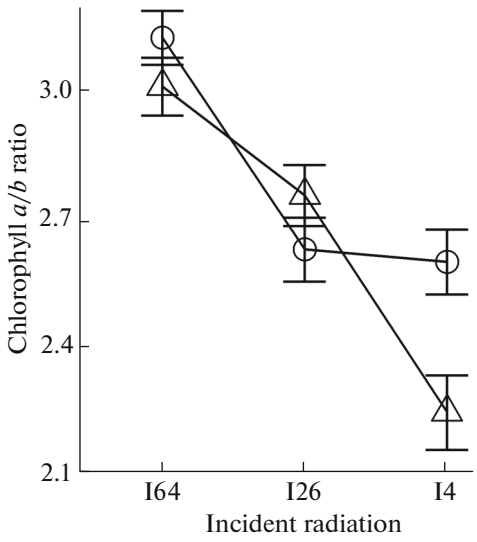

Fig. 2. Chlorophyll *a/b* ratio in *Phleum alpinum* (circles) and *Poa pratensis* (triangles) growing under three irradiance conditions in the greenhouse: I64 (64% of total sunlight), I26 (26% of total sunlight), I4 (4% of total sunlight). Data are mean values ± SE ($n = 36$).

An interaction among month, species and light was found when the Car/Chl ratio was studied. This was because differences between species were found only in March under I26 ($F_{4;178} = 3.41$; $p = 0.01$): the ratio was higher in *P. alpinum* than in *P. pratensis* (Fig. 3). In December, the Car/Chl ratio was significantly higher under I64 than I26 and under both irradiances than under I4 (Fig. 2). In January and March, changes in Car/Chl ratio with irradiance levels were similar to those in December, although there were no significant differences between I26 and I4.

Photosynthesis

The plateau of photosynthesis at saturating irradiance varied with the irradiance level, species and date ($F_{4;1404} = 3.20$; $p = 0.0125$). In all sampling dates, A_{max} was significantly greater under I64 than under I26, and under I4 in *P. alpinum* (Fig. 4). A_{max} varied with sampling date in both species depending on the irradiance level: under I64 and I26 A_{max} was higher in January than in December and March; no significant differences were detected among dates under I4 (Table 3).

On the other hand, A_{max} in *P. pratensis* was similar in I64 and I26 in December and January, although there was a tendency to be higher under I64. A_{max} under both irradiance levels were significantly higher than under I4 in these months (Table 3). No significant differences were found in A_{max} in March among irradiance levels (Table 3).

Maximum photosynthesis (i.e., A_{max}) was significantly higher in *P. alpinum* than in *P. pratensis* under I64 in all sampling dates (Table 3). In December, it was greater only in *P. pratensis* under I26. However, differences were not significant between species under this irradiance level in January and March (Table 3). There were no significant differences between species under I4.

There were no significant differences in A_{qe} among light, species and sampling dates. Apparent quantum yield (i.e., A_{qe}) was significantly higher under I4 than I26, and under these irradiance levels than under I64 in both species ($F_{2;1404} = 10.97$; $p < 0.0001$) (Table 3). Within each species, A_{qe} was significantly higher in December than in the remaining sampling dates ($F_{2;1404} = 23.86$; $p < 0.0001$) (Table 3). Apparent quan-

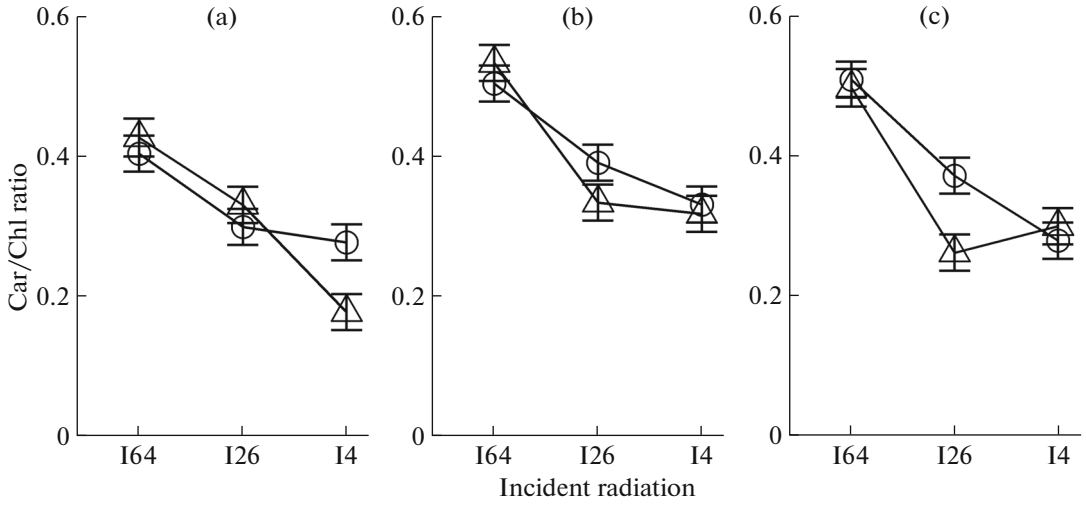

Fig. 3. Carotenoids/chlorophyll ratio in (a) December, (b) January, and (c) March in *Phleum alpinum* (circles) and *Poa pratensis* (triangles) growing under three irradiance levels: I64 (64% of total sunlight), I26 (26% of total sunlight), I4 (4% of total sunlight). Data are mean values ± SE ($n = 12$).

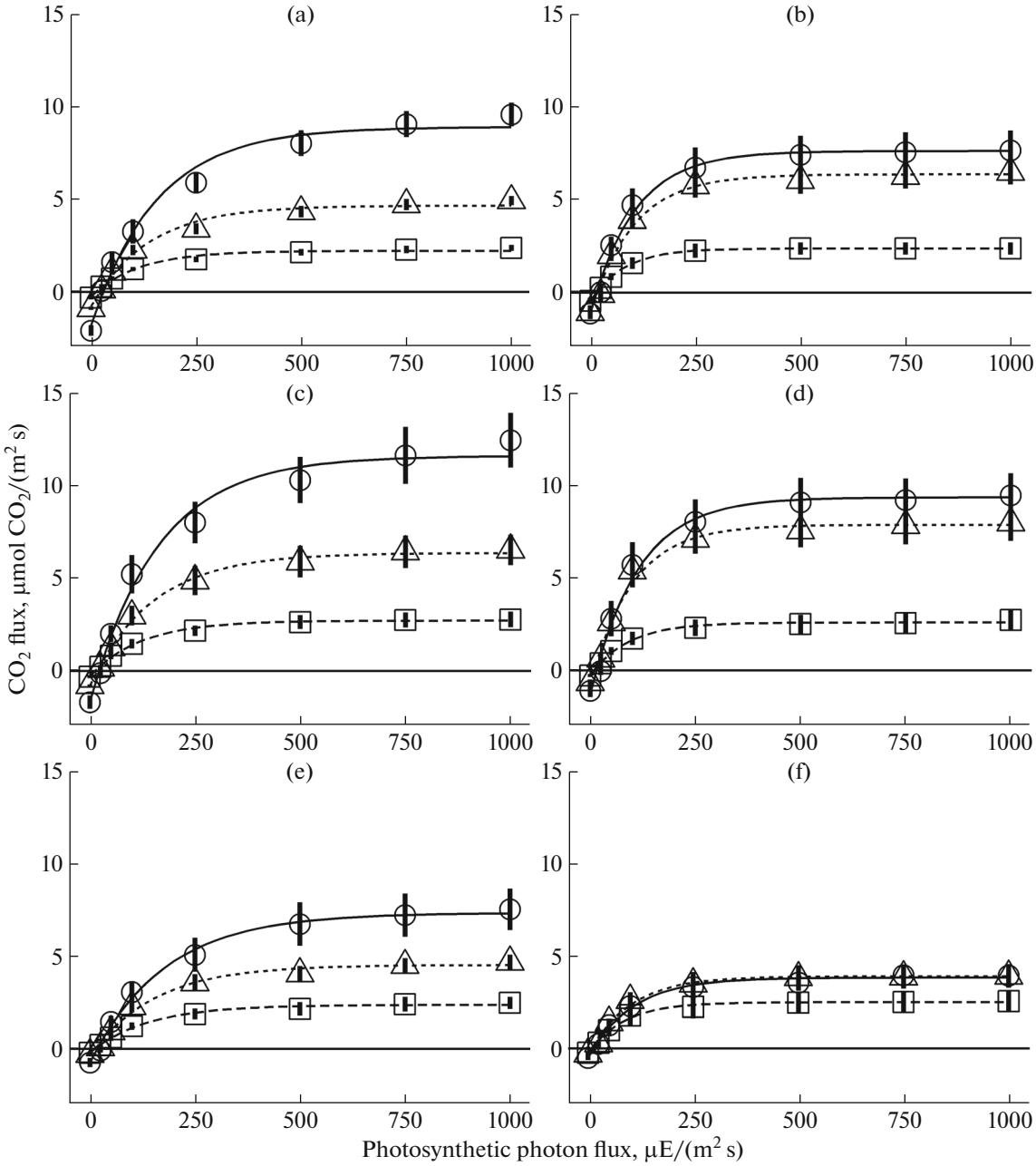

Fig. 4. Variation of net photosynthesis in December (a, b), January (c, d), and March (e, f) in *Phleum alpinum* (a, c, e) and *Poa pratensis* (b, d, f) that grew in the greenhouse under three light intensities. Open circles and solid lines: I64—64% of solar irradiance; open triangles and dotted lines: I26—26% of solar irradiance; open squares and dashed lines: I4—4% of solar irradiance. Data are mean values ± SE (n = 12). Lines were obtained from the parameters estimated by the model (see MATERIALS AND METHODS section).

tum yield was significantly higher in *P. pratensis* than in *P. alpinum* ($F_{1;1404} = 186.97$; $p < 0.0001$) (Table 3).

The light compensation point was significantly higher under I64 than under I26 and I4, and there were no significant differences in LCP between these two later irradiation levels ($F_{2;1404} = 21.99$; $p < 0.0001$) (Table 3). *P. pratensis* showed lower LCP than *P. alpinum* ($F_{1;1404} = 57.73$; $p < 0.0001$) (Table 2). Within each species, this parameter was significantly higher in December than in January and March ($F_{2;1404} = 17.69$; $p < 0.0001$) (Table 3).

Similarly, leaf dark respiration rate was higher under I64 than under I26 and both than under I4 ($F_{2;180} = 154.58$, $p < 0.0001$) (Fig. 5). Both species showed a similar pattern, but *P. pratensis* respiration under I64 was lower than that in *P. alpinum* in Decem-

Table 3. Means of the photosynthetic parameters (± SE) in *Phleum alpinum* and *Poa pratensis* that grew in the greenhouse under three light intensities

Species	Date	Irradiance*	A_{max},[1]	A_{qe},[2]	LCP,[2]
			μmol $CO_2/(m^2\ s)$	A_{max}/LCP	μmol PPF/(m^2 s)
P. alpinum	Dec	I64	8.91 ± 0.49^{g}	0.0064 ± 0.0003^{Aa}	28.86 ± 2.04^{Aa}
		I26	4.65 ± 0.48^{d}	0.0074 ± 0.0006^{Ab}	20.99 ± 2.70^{Ab}
		I4	2.22 ± 0.47^{f}	0.0088 ± 0.0030^{Ac}	11.95 ± 4.79^{Ab}
	Jan	I64	11.61 ± 0.50^{b}	0.0062 ± 0.0002^{Ba}	21.67 ± 1.96^{Ba}
		I26	6.36 ± 0.48^{a}	0.0068 ± 0.0004^{Bb}	17.39 ± 2.48^{Bb}
		I4	2.72 ± 0.48^{ef}	0.0082 ± 0.0010^{Bc}	11.33 ± 4.34^{Bb}
	March	I64	7.35 ± 0.49^{a}	0.0056 ± 0.0004^{Ba}	16.97 ± 2.56^{Ba}
		I26	4.52 ± 0.48^{d}	0.0066 ± 0.0007^{Bb}	12.35 ± 3.36^{Bb}
		I4	2.38 ± 0.48^{ef}	0.0072 ± 0.0010^{Bc}	10.72 ± 5.44^{Bb}
P. pratensis	Dec	I64	7.61 ± 0.48^{a}	0.0096 ± 0.0005^{Aa}	14.59 ± 2.02^{Aa}
		I26	6.35 ± 0.48^{a}	0.0098 ± 0.0006^{Ab}	16.29 ± 2.10^{Ab}
		I4	2.37 ± 0.47^{ef}	0.0127 ± 0.0020^{Ac}	14.25 ± 3.18^{Ab}
	Jan	I64	9.35 ± 0.48^{c}	0.0089 ± 0.0004^{Ba}	14.52 ± 1.95^{Ba}
		I26	7.87 ± 0.48^{ac}	0.0101 ± 0.0005^{Bb}	9.10 ± 2.06^{Bb}
		I4	2.59 ± 0.47^{ef}	0.0111 ± 0.0020^{Bc}	8.16 ± 3.77^{Bb}
	March	I64	3.79 ± 0.48^{de}	0.0095 ± 0.0010^{Ba}	12.94 ± 2.88^{Ba}
		I26	3.86 ± 0.47^{de}	0.0101 ± 0.0010^{Bb}	8.33 ± 2.99^{Bb}
		I4	2.47 ± 0.47^{ef}	0.0118 ± 0.0020^{Bc}	7.82 ± 3.76^{Bb}

A_{max}—maximum photosynthesis, A_{qe}—apparent quantum yield, LCP—light compensation point. Values are the mean ± SE, $n = 6$.
* I64—64% of solar irradiance, I26—26% of solar irradiance, I4—4% of solar irradiance.
[1] A_{max}—different letters indicate significant differences between each combination of irradiance, data and species after the Tukey's test.
[2] A_{qe} and LCP—different capital letters indicate significant differences between data within each species. Different lowercase letters indicate significant differences among irradiance treatments within each date and species, both after the Tukey's test.

ber and January ($F_{4;180} = 4.31$, $p = 0.0023$). The interaction between month, light and species was due to the decreasing differences in the response variable in March. In December and January, we detected significant differences among all light levels (Fig. 5). However, only in March we detected significant differences between I64 and the other light levels in *P. alpinum*.

DISCUSSION

Pigments

Values found for total chlorophyll content in both species, between 0.21 and 0.36 mmol/m^2, are within the range reported in other studies [4, 10]. However, values for *P. alpinum* (0.21 and 0.27 mmol/m^2) were lower than those reported for this species by Heide and Solhaug [10] (from 0.41 to 1.10 mmol/m^2). Values shown by *P. pratensis* (between 0.22 and 0.36 mmol/m^2) were also relatively lower than those found in this species (0.51 mmol/m^2) by Westbeek et al. [17]. These differences in values found by different studies could be due to differential growing conditions. In both cited studies, plants grew under controlled temperature and light conditions. Also, plants grew in hydroponics in the study of Westbeek et al. [17]. In our study, conducted under greenhouse conditions, we could only partially control light and temperature levels. In addition, differences on results between studies could also be due to differences on plant nutritional conditions [18].

Total chlorophyll content in *P. alpinum* was 11 and 22% lower under I26 and I64, respectively, than under I4. It diminished by 38% from I64 to I26, and by 15% between I4 and I26 in *P. pratensis*. However, no significant differences were found between I26 and I4 in any of these two species (Figs. 1a,b). These results are in agreement with our first hypothesis that chlorophyll content increases as radiation levels increase. Our results agree with those reported for several dicotyledons [19] and monocots [20]. However, van Huylen-

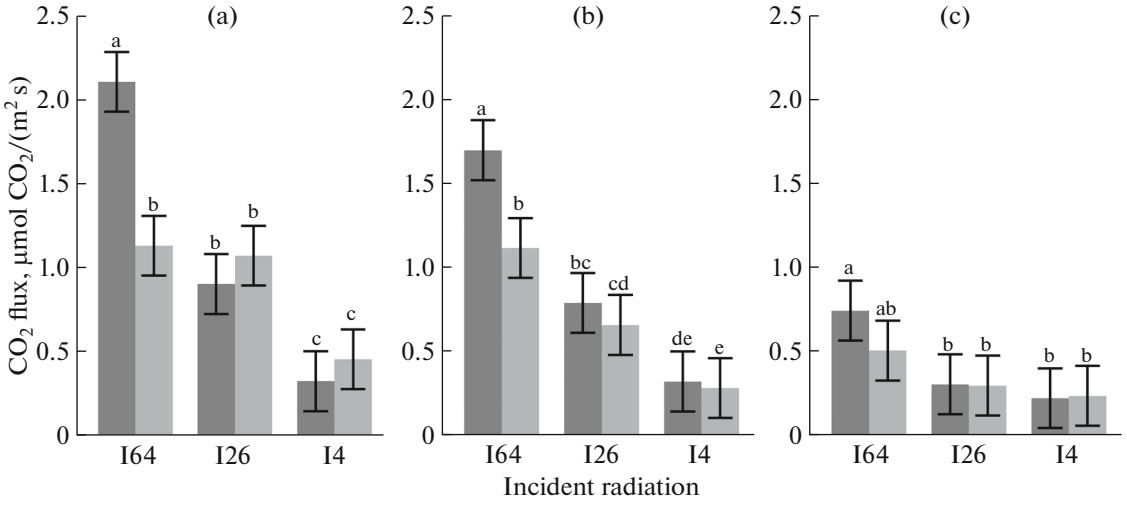

Fig. 5. Leaf respiration rate in December (a), January (b), and March (c) in *Phleum alpinum* (dark grey) and *Poa pratensis* (light grey) that grew in the greenhouse under three light intensities (I64—64% of solar irradiance, I26—26% of solar irradiance, I4—4% of solar irradiance). Data are mean values ± SE (n = 12). Vertical bars represent the 95% confidence limit. Different letters indicate significant differences between species and irradiance combinations.

broeck and van Bockstaele [11] found that chlorophyll content can decrease as irradiance also decrease.

Changes in chlorophyll content might optimize nitrogen use according to growth irradiances [3], because this nutrient is one of the major constraints in plant growth [18]. Light availability becomes a constraint for plant growth at lower levels of irradiance. As a result, chlorophyll and protein contents change during acclimation (for example, proteins of photosystems), therefore, increasing the interception of light quanta [21]. This allows the plant to maintain an appropriate balance among the various compartments of photosynthesis, light capture, electron transport chain and proteins of the dark phase of photosynthesis. On the other hand, the number of proteins of the dark phase of photosynthesis increases during acclimation to high irradiances. These proteins may limit photosynthesis under conditions of high photosynthetic flux density [21].

Decreases in the proportion of chlorophyll *a/b* are common, and increases in the relation Car/Chl are widespread, in all plant groups [20]. These differences are due to the leaf responses during acclimation to high irradiances, which have much lower proportion of "light harvesting" proteins (LHCII), and much greater number of reaction centers in relation to the total amount of chlorophyll [22]. Similarly, the higher values of Car/Chl ratio at higher than at lower irradiances (Fig. 3), also agree with our first hypothesis, which might be explained by lower content of LHCII. This is because proteins of LCHII have lower values of Car/Chl ratio (between 0.07 and 0.14) in comparison to proteins of the reaction centers CPa and CPI (0.14 to 0.25) [23].

Increases in the Car/Chl ratio contribute to protect the photosynthetic apparatus [24]. Plant xanthophylls, a group of compounds within the carotenoids, interconvert themselves within their various forms via epoxidations according to the excess light to which leaves are exposed. When light is excessive, zeaxanthin, the more epoxidated form, is accumulated [2]. The zeaxanthin protects the photosynthetic apparatus because it allows an increase in the release of excess energy; it also increases the content of carotenoids in leaves exposed to high irradiance, response that is independent on the plant species [2].

The 33% decrease in chlorophyll content in January and March as compared to December could be related to leaf age. Woledge [25] reported that chlorophyll content decreased with leaf age from the time leaves reached their maximum length, and that this decrease was faster in leaves exposed to low irradiance levels. We used leaves in the same position from the plant at each sampling date; however, it is possible that the sampled leaves had been of different age, and that leaves sampled in December had been relatively younger. Another possibility is that total irradiance had been lower in December than in January and March, and that chlorophyll content was greater as a result.

Similarly, the 20% increase in the Car/Chl ratio could indicate leaf aging since chlorophylls degrade faster than carotenoids [4].

There was not a clear effect of soil moisture, which does not agree with our second hypothesis. This might be partially due to the fact that air relative moisture humidity was high in the greenhouse (Table 1), which might have compensated effects of low soil moisture in the experiment. For example, Sánches-Díaz et al. [26] determined that net photosynthetic rate of the flag leaf

in barley plants was higher at low than at high vapor pressure deficit either under well-watered or water-deficit conditions. This is, gas exchange was influenced by atmospheric humidity independently of soil water content [26]. However, Sarker et al. [27] obtained the same results to ours on wheat plants, except that the chlorophyll *a/b* ratio was higher in irrigated plants. Other studies have reported a reduction in the chlorophyll formation under water stress conditions [5, 27]. It is likely that plants were not exposed to water stress in the M30 treatment since soil moisture content was well above the permanent wilting point [28]. Irrigation frequency was equal in the M30 and M60 treatments, although water addition was lower in M30 than in M60 (data not shown). Then, it may be that lower soil moisture content in M30 could have been masked by the irrigation frequency.

Photosynthesis

The net photosynthetic rates found in this study were within the range found in other studies for *P. alpinum* [10] and *P. pratensis* [29]. Even though Heide et al. [29] conducted studies under different irradiance levels, the focus of their study was not concentrated on this aspect.

P. alpinum and *P. pratensis* acclimated to the various irradiance levels. In both species, the net photosynthetic rate was higher under I64 than under I26, and in both irradiance levels higher than under I4. These results agree with our third hypothesis. In *P. alpinum*, this parameter was 62 to 92% higher under I64 than under I26, and 90 to 134% higher under I26 than under I4. Net photosynthetic rates on *P. pratensis* were 19 to 20% higher in December and January, and 2% lower in March under I64 than under I26, although differences were not significant. However, they were 56 to 204% higher under I26 than under I4.

In general, increases in A_{max} are due to increases in leaf thickness because of the elongation of the mesophyll cells [2]. These results indicate an increase in amount of chloroplasts and enzymes. As a consequence, there is an increase in the photosynthetic capacity per unit surface area [3]. Plants of both species modified their leaf thickness in the different treatments, and showed a greater biomass allocation to leaves as irradiance levels were reduced from I64 to I4 [30]. It is advantageous that plants increase their leaf thickness under higher irradiance conditions. This allows them to increase their photosynthetic capacity per unit surface area, reducing at the same time their transpiration per unit biomass, because transpiration is higher in these environments [30]. In our study, *P. alpinum* plants showed their leaves partially rolled when exposed to I64. This is an acclimation mechanism to high irradiances because the exposure of leaves to light was reduced [21].

Apparent quantum yield was 10 to 30% higher under I4 than under I26, and between 24 to 38% higher at the lowest than at the highest irradiance level. This indicates that light utilization was better under the lowest irradiance levels, reaching A_{max} at lower rather than at higher light levels. Our values are low compared to those in other studies [20, 31]. However, quantum yield was estimated using a different methodology than ours in these research works. In our study, the reported values are similar to those of Aleric and Kirkman [32]. The quantum yield reduction could be indicating that there was photoinhibition [31]; however, this is not evident from our data in Fig. 4.

Another alternative is that the reduction in the apparent quantum yield is indicating changes in the concavity of photosynthetic response curve. Concavity is greater as apparent quantum yield increases. It appeared to be higher under I4 than under I26 and I64, and it was higher in *P. pratensis* than in *P. alpinum* (Fig. 4; Table 3). Differences in apparent quantum yield might be because plants reached saturating irradiance earlier under I4 than under I26 and I64 (Fig. 4). These differences would allow plants to utilize the low light available in environments of low irradiance. This change could be due to changes in the relative concentration of Rubisco in relation to electron transport chain [31].

The light compensation point was 13 to 75% higher under I64 than under I26 and I4. These findings agree with our third hypothesis, and indicate that plants can have positive carbon balance at lower (i.e., I4 and I26) than at higher (i.e., I64) irradiances. This is advantageous for plants that grow in shaded environments where there was lower light availability. In agreement with this finding, plants also reduced their dark respiration rate. On average, respiration was 43 and 70% lower under I26 than under I64 and under I4 than under I64, respectively. This group of plant responses was similar to that found in other species, and is typical of the photosynthetic apparatus acclimation to shaded conditions [3].

Maximum photosynthesis showed a similar temporal pattern in both species under I64 and I26: it increased from December to January, and decreased from January to March. The photosynthesis increase in January could be due to a temperature increase, as in this month the temperature was on average 4 and 6°C higher than in December and March, respectively [12]; a similar response was found in *P. alpinum* exposed to long days [10]. Growth is controlled by photoperiod and temperature: low temperatures and short days inhibit leaf growth [33]. The reduction of A_{max} in March could be due to reduction in growth. This could be due to a shortening of days, as autumn begins in this month, and a temperature reduction. Growth reduction would determine lower need of photosynthates, because photosynthesis is influenced by the carbon need for growth [2].

Light compensation point was 41% lower in January and March than in December in both species. This could be due to the increased age of leaves at the later sampling dates, since younger leaves have faster metabolism [2].

Both species inhabit almost the same environments [9]. In agreement with this, both species showed similar responses in most of the studied variables. For example, total chlorophyll was very similar under I64 and I4; we found differences only under I26 (Fig. 1). There weren't any differences under I4 between the species (Table 3), which coincides with their poor performance in low light [34]. However, *P. pratensis* outperformed *P. alpinum* under I26 but this was reversed under I64. *P. pratensis* forms dense mats of grass where self-shading can greatly reduce incoming light [7], so higher net photosynthesis at intermediate light levels would be beneficiary. On the other hand, *P. alpinum* doesn't form dense mats and, as its name implies, is usually found in alpine environments which have higher irradiances than lowland areas [17]. Furthermore, Westbeek et al. [17] found that alpine species have higher net photosynthesis rate than lowland species such as *P. pratensis*.

In conclusion, as irradiance increased, the contents of chlorophyll per unit surface area and weight decreased, and the ratios of chlorophyll *a/b* and Car/Chl increased in *P. alpinum* and *P. pratensis*. There was a small effect of leaf age in both species. There was not net effect of soil moisture contents. The rate of maximum photosynthesis and the light compensation point increased as irradiance also increased in both grass species. Temporal variations affected these values during the study period. However, the relationship of these values between the irradiance treatments was not modified in *P. alpinum*. On the other hand, temporal changes of those variables determined that the maximum rate of photosynthesis was similar to that in March in all treatments in *P. pratensis*. Our results indicated that *P. alpinum* and *P. pratensis* were able to acclimate to various environmental conditions studied.

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