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Can increased glacial melting resulting from global change provide attached algae with transient protection against high irradiance?

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SUMMARY

1. Climate change is altering temperatures and precipitation patterns all over the world. Melting glaciers increase surface run-off, thereby increasing the transport of suspended solids through streams. The increased load of suspended solids affects turbidity, which decreases the availability of photosynthetically active radiation for primary producers.

2. We analysed how glacial loading of clays influences the light : nutrient ratio and photosynthetic parameters (measured with a pulse amplitude modulated fluorometer) and the carbon : phosphorus (C : P) elemental ratio of periphytic primary producers. A field study was conducted in two canopy-free streams that receive water from the glaciers of Mount Tronador (Patagonia, Argentina), one with high glacial load and the other with clear water. In addition, we conducted an *in situ* colonisation experiment with three different light treatments.

3. We observed that periphytic biomass (chlorophyll *a* and carbon content) in the streams and in the experiment varied directly with turbidity. Moreover, photosynthetic parameters varied similarly because of an increase in the efficiency of electron transfer per open reactive centre in the more turbid stream and a chronic photoinhibition of photosystem II in the clearer stream.

4. Periphytic C : P also varied with turbidity as we observed a decrease in C : P with an increase in light in both streams and in the experiment.

5. Our main conclusion is that an increase in glacial melting with its associated increase in glacial clay load should protect primary producers against high irradiances (photosynthetic active radiation + ultraviolet radiation) in canopy-free streams.

Keywords: C : P ratio, glacial clay, periphytic algae, photosynthetic parameters, turbidity

Introduction

Increased losses in glacier volume due to ice ablation and negative mass balances have affected the global freshwater cycle (Dyurgerov, 2003), with increased air temperature being the major force in glacier change (Wolfe & Lind, 2008). Because glaciers around the globe are melting rapidly (Masiokas *et al*., 2008), thereby increasing meltwater flux, new questions regarding downstream ecosystems are becoming increasingly important. The meltwater carried by rivers contains large amounts of suspended sediment particles (mainly inorganic clay) that may influence physical, chemical and biological conditions (Lind, Chrzanowski & Dávalos-Lind, 1997; Uehlinger *et al*., 2010). This increase in glacial clay causes an increase in total suspended solids that reduces light availability (Modenutti *et al*., 2000), which in turn affects the distribution of primary producers and herbivores (Hylander *et al*., 2011; Laspoumaderes *et al*., 2013; Modenutti *et al*., 2013). In addition, the amounts of particles vary seasonally and within and among systems (Eisma, 1993; Wood & Armitage, 1997), influencing patterns of photosynthetic fixation of energy by primary producers (Kirk, 1994). In rivers, much of this productivity is associated with stream periphyton (i.e. the attached benthic community that

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consists mainly of algae and bacteria) (Dodds *et al.*, 1996). Thus, periphytic phototrophs can be expected to be affected by the decrease in light availability.

Photosynthesis involves numerous biochemical and developmental responses to light that help to optimise photosynthesis and growth (Falkowski & Raven, 2007), including adjustments in absorption cross sections of the antenna systems and changes to the number of reaction centres (Long, Humphries & Falkowski, 1994). In addition, stream periphyton responds very quickly to pulses or constant fluxes of limiting nutrients (i.e. phosphorus) (Davies & Bothwell, 2012). The links between light and phosphorus as two key resources for primary producers are highlighted in the 'light : nutrient hypothesis' (Sterner *et al.*, 1997). This hypothesis predicts that under high-light intensities and low levels of inorganic phosphorus, nutrient limitation of producers becomes more severe, resulting in a biomass with a disproportionate accumulation of carbon (C) relative to phosphorus (P). Light attenuation by glacial clays is of great ecological importance because reduced light penetration directly affects the photosynthetic process in primary producers (Kirk, 1994). Consequently, changes in light conditions can be expected to be reflected by changes in C fixation and the C : P ratio. Primary producers are characterised by variable nutrient contents that result in a plastic balance in the C : nutrient ratio because their elemental composition may reflect their underlying biochemical allocations and ecological strategies (Cross *et al.*, 2005; Elser *et al.*, 2010; Wang, Sterner & Elser, 2012). Thus, it can be expected that in canopy-free streams under low-clay inputs and high irradiance, the C : nutrient ratio will increase, whereas the ratio should decrease as the clay input increases.

Photosystem II (PSII) is a protein complex in the light-dependent reactions of oxygenic photosynthesis and is susceptible to strong light. The effects of strong light are referred to as photodamage or photoinhibition (Allakhverdiev *et al.*, 2005). In particular, the extent of photoinhibition represents a balance between rates of photodamage and the repair of PSII, wherein the latter electron transport (ETR) and ATP synthesis are two important related processes (Murata *et al.*, 2007; Takahashi & Badger, 2011). It has been proposed that most environmental stresses inactivate PSII by inhibiting the mechanisms for repairing photodamage rather than by directly attacking it (Murata *et al.*, 2007). An excess of light would produce an inhibition of the activity of PSII that, in turn, causes a decrease in the overall number of functional reaction centres, thereby contributing to a decrease in the maximum photochemical efficiency of PSII (Maxwell & Johnson, 2000). Therefore, one of the most important issues for primary producers is the balance between C fixation and photoinhibition. In this sense, migration to deeper levels is a suitable strategy for motile phytoplanktonic cells (Callieri *et al.*, 2007; Ross & Sharples, 2008); however, periphytic primary producers cannot migrate deeper into the water column. In addition, the complexity of the periphytic matrix itself means that light exposure differs at different levels in the substrata (Steinman, 1992). Accordingly, these communities should be dominated by periphytic growth forms that balance the utilisation of light. Consequently, the final stoichiometric composition of periphyton can be expected to result from light and nutrient availability (Hill & Fanta, 2008) and to be related to periphyton diversity and species composition (Dickman, Vanni & Horgan, 2006).

The Patagonian Andes is the largest glaciered area in South America, and in this region, the 0 °C isotherm has moved up 400 m (2300–2700 m) in the last 54 years (Carrasco, Osorio & Casassa, 2008). Thus, glaciers have shown a continuous and maintained recession (Masiokas *et al.*, 2010) towards the altitude where accumulation and melting rates are equal (Schaefer *et al.*, 2013). In particular, glaciers of Tronador Mountain (3554 m.a.s.l.) have receded over the last 30 years (Masiokas *et al.*, 2008), causing an increased input of glacial clay during the warmer months of the year (Iriondo, 1974). Because of the clay load, the streams that drain these glaciers are classified as 'white-waters' (Chillrud *et al.*, 1994). We took advantage of this natural scenario of turbid and clear canopy-free streams, performing a field and experimental study to test the hypothesis that glacial melting would change the light : nutrient ratio for periphytic producers. This outcome would result as a consequence of changes in periphytic photosynthetic parameters due to differences in light, because glacial clay would protect against photoinhibition. Thus, C fixation would be modified as a consequence of climate change that is driving glacier melting that, in turn, would result in differences in the primary producers' stoichiometries.

Methods

Study site

Mount Tronador is the highest mountain in Nahuel Huapi National Park and straddles the border between Chile and Argentina in northern Patagonia (Fig. 1). The dominant bedrock includes volcanic (basalts), granites, and pyritised, silicic metamorphic rocks (Chillrud *et al.*,

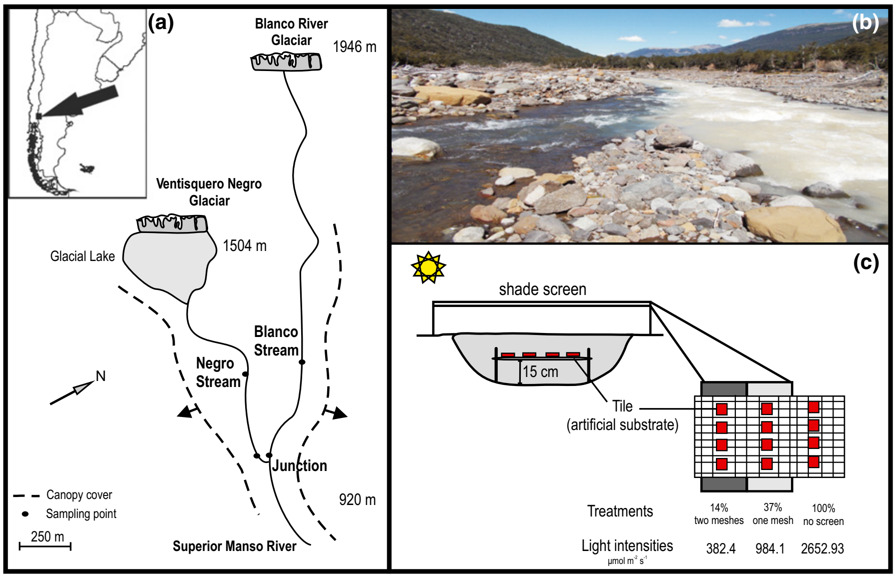

Fig. 1 (a) Location of sampling points in the Negro and Blanco streams. (b) Photograph of the junction of the two streams. (c) Experimental design.

1994). The upper part of the mountain is covered by a continuous ice cap and has 11 outlet glaciers with a total glacier area of approximately 64 km^2 (Masiokas *et al.*, 2010). The glacier that drains into Argentina's Manso River valley flows down the south-eastern flanks of Mount Tronador and several hundred metres below is separated from the ice cap by a steep cliff. This secondary glacier is fed by snow, ice and debris avalanches from the steep slopes above, and due to the thick debris layer that covers large portions of the ice, it is locally known as Ventisquero Negro (the Black Glacier) (Worni *et al.*, 2012). The rapid thinning and recession of the glacial tongue in recent years have resulted in the formation of a large, rapidly growing proglacial lake between the glacier margin and the main moraine ridges (Masiokas *et al.*, 2010). Negro stream, which is characterised by high turbidity due to large discharges of glacial clay, begins at this glacial lake. Blanco stream runs parallel to Negro stream, both being located in the same canopy-free flood plain at 920 m.a.s.l. (Fig. 1a). Blanco stream originates from the Blanco glacier, which is characterised by a low amount of suspended solids (i.e. glacial clays) and therefore has high transparency. The Negro (turbid) and Blanco (clear) streams join to form the Superior Manso River (Fig. 1b).

Periphyton sampling

We sampled the Negro and Blanco streams during the early autumn, spring and summer of 2012–2013 on six sampling occasions. Each stream was sampled at one sampling point located 500 m upstream from the junction (Fig. 1a). On two occasions, the streams were sampled at another sampling point 2 m upstream from the junction. We measured turbidity (nephelometric turbidity units: NTU) using a portable turbidity meter (Lutron TU-2016, Taipei, Taiwan) and temperature, conductivity and dissolved oxygen with an oxymeter–conductimeter (YSI 85, OH, U.S.A.). In addition, we collected 2 L samples of stream water in acid-washed plastic containers that were transported to the laboratory in thermally insulated containers. For the periphyton study, following Hauer & Lamberti (2007), three stones were randomly selected at each sampling point from a 6-m section of the main channel. On each stone surface, we determined the *in situ* periphytic photosynthetic parameters (qP, Y, NPQ, *Y*/qP and ETR, see below photosynthetic fluorescence parameters) with a WATER-PAM equipped with a Water-EDF fibre optic unit and WinControl software (Heinz Walz GmbH, Effeltrich, Germany). After being measured, each stone was individually stored in a plastic bag and immediately carried to the laboratory under dark conditions in thermally insulated containers.

Photosynthetic fluorescence parameters

The photosynthetic parameters were measured with a PAM fluorometer to provide information about the operating conditions of PSII (Fig. 2). A PAM fluorometer typically consists of four light sources that provide quantitatively different radiation, inducing changes in a redox state of components of the electron transport chain and causing chlorophyll fluorescence. We per-

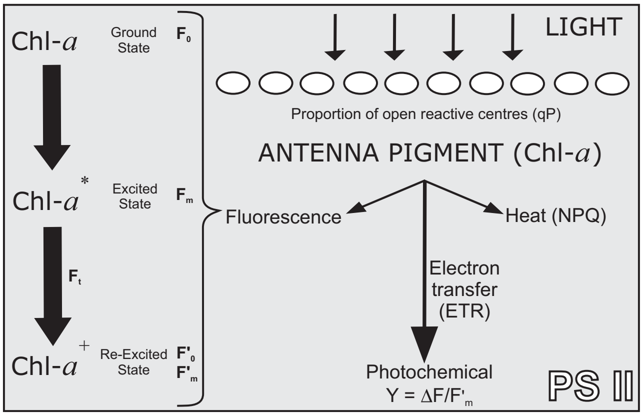

Fig. 2 Simplified three-level scheme of the redistribution of excitation energy of light (heat, photochemical and fluorescence) absorbed by the reaction centre of the light harvesting antenna of PSII. Energy diagram shows the ground state (Chl-*a*), the excited state (Chl–*a**) and the re-excited state (Chl-a^+), and its fluorescence parameters. See text for further explanation.

formed two types of measurements on each stone: saturating actinic light (SAL), three times per stone, and rapid light curve (LC), one time per stone. SAL is a light pulse of 1004 μmol photons m^{-2} s^{-1}, and LC measurements were obtained by exposing the samples to blue radiation at nine incremental steps of irradiance ranging from 0 to 1403 μmol photons m^{-2} s^{-1} in 10-s intervals. The measurement is initiated by switching on the measuring light, obtaining F_0 (ground state, Fig. 2), which is the level of fluorescence of the antenna pigment (Chlorophyll *a*, Chl-*a*) when all reactive centres of PSII are open. A saturating flash of light is then applied, allowing the measurement of F_m. The steady-state value of fluorescence immediately prior to the flash is termed F_t. After a period of time, another saturating light flash allows recording of the initial minimum fluorescence (F'_0) and maximal fluorescence (F'_m) in the light (Maxwell & Johnson, 2000). These fluorescence values were used to determine the photosynthetic parameters: the effective quantum yield ($Y = (F'_m – F_t \backslash F'_\mathrm{m}) = (\Delta F \backslash F'_m)$) of PSII, the electron transport rate (ETR = μmol electrons m^{-2} s^{-1}), the photochemical quenching ($\mathrm{qP} = (F'_m – F_t \backslash F'_m – F'_0)$) and the non-photochemical quenching ($\mathrm{NPQ} = F_m – F'_m \backslash F'_m$) (Rohácek, Soukupová & Bartak, 2008).

Under high-light exposure, it is expected that qP (the proportion of open reaction centres of PSII) will be lower than under shaded conditions (Fig. 2). In addition, under high-light conditions, the effective quantum yield (Y) will reflect a decrease due to photoinhibition/photodamage, as well as a decrease in the electron transport rate (ETR) (Murata *et al.*, 2007). Therefore, the Y/qP ratio (i.e. the photosynthetic efficiency per open reactive centre) will show a low value because a high proportion of energy will not be used by PSII. However, the NPQ response is dependent on the light history of the organism (Müller, Xiao-Ping & Niyogi, 2001) because high light generates reactive oxygen species (ROS) that inhibit the repair of PSII by suppressing the synthesis of proteins (Murata *et al.*, 2007).

Laboratory procedures

In the laboratory, periphyton was obtained by scraping individual stones with a brush and rinsing them carefully with distilled water. The final volume obtained was taken to a constant volume (100 mL) and expressed in relation to 2/3 of the total area of the stone (cm^2) (Biggs & Close, 1989), which was estimated from the lengths of the three main axes (Graham, McCaughan & McKee, 1987). The obtained volume was designated for the estimation of chlorophyll *a* (Chl-*a*), C and P concentrations, and algal composition. For Chl-*a* concentration determination, a 1-mL aliquot of the periphyton suspension was filtered through glass fibre filters (GF/F; WhatmanTM, Maidstone, U.K.). Chl-*a* was extracted in hot ethanol following filtration (Nusch, 1980) and was measured with a 10-AU fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.) with previous calibration against spectrophotometric measurements. Another 5 mL of the suspension was filtered on ashed GF/F filters, dried at 60 °C for 48 h and analysed for organic particulate C using a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific, Milano, Italy). The periphyton P concentration was determined by filtering another 5 mL of the suspension onto acid-washed (10% HCl) and ashed GF/F filters and drying them at 60 °C for 48 h. The filters were combusted at 550 °C for 1 h, ashes were then placed in flasks, and P concentrations were analysed with the ascorbate-reduced molybdenum method (Eaton *et al.*, 2005). Periphyton Chl-*a*, C and P concentrations are reported as mg m^{-2}, with each parameter being estimated in three replicates (three stones). A volume of 20 mL of the periphyton suspension was fixed with acid Lugol's solution for examination of the attached algae.

A volume of 250 mL of stream water from each river for each date was filtered through ashed GF/F filters and analysed for particulate C and N. Total dissolved phosphorus (TDP) and dissolved organic carbon (DOC) were determined for the filtered stream water. DOC was measured with a carbon analyser (Shimadzu TOC VCSH; Shimadzu, Kyoto, Japan). The samples for TDP measurements were digested with potassium persulphate at 125 °C at 1.5 atm for 1 h, and the concentrations were analysed using the ascorbate-reduced molybdenum method (Eaton *et al.*, 2005). Total nitrogen was estimated following Valderrama (1981). Total suspended solids (TSS) were quantified by filtering 250 mL of stream water through pre-weighed GF/F filters, which were dried for at least 48 h at 60 °C and then reweighed.

Light measurements

Light absorption by suspended clay was measured in two ways. First, we measured light absorption directly with a PUV500B submersible radiometer (Biospherical Instruments, San Diego, CA, U.S.A.) at selected points in the Negro and Blanco streams. We also measured ambient light beside the streams. In addition, unfiltered stream water was scanned with a double beam spectrophotometer (Shimadzu UV 2450, Kyoto, Japan) from 250 to 700 nm; (Merck Millipore, Darmstadt, Alemania) water was used as the blank. The mean absorbance, *A*, was transformed into *a*, expressed as m^{-1}, using

$$a = \ln(10_A)/L,$$

where *L* is cuvette length (Morris *et al.*, 1995).

The light attenuation coefficient (K_d) was estimated from the direct light measurements and spectrophotometric scans. The incident light in each sample (I_z), adjusted for turbidity level (spectrophotometric scans of unfiltered water), was calculated from the light attenuation coefficient following Lambert–Beer equation, $I_z = 1\ e^{(-Kz)}$ (Kirk, 1994), where z is the depth at which the periphyton occurred. Light measurements were expressed as available photosynthetic active radiation (PAR) at stream bottom in μmol photons $m^{-2}\ s^{-1}$.

Field experiment

The effects of a light gradient on primary producers were studied in an *in situ* experiment in the Blanco stream over 2 weeks in the summer (January 2012). We established three different light conditions (full: 100%, medium: 37% and low: 14% of solar radiation). Light quantity was manipulated by reducing irradiance with increasing layers of shade screens to achieve the three levels of incident irradiance: 100% no screen, 37% one mesh, 14% two meshes (Fig. 1c). These meshes were placed 15 cm above the stream surface and were fixed with metal pegs 1 m from the stream margin. Artificial substrata (12 ceramic tiles, 6 × 6 cm) were placed under each treatment 15 cm from the stream bed (to avoid herbivores) and 15 cm below the stream surface. The experiment was run in four replicates per treatment, and during the experiment, temperatures and stream water levels were monitored with a data logger (U20 HOBO; Onset, Bourne, MA, U.S.A.).

After 2 weeks of exposure to different light conditions, photosynthetic parameters were estimated *in situ* (qP, Y, NPQ, Y/qP and ETR) for each tile with a WATER-PAM equipped with a Water-EDF fibre optic unit. Next, we sampled each tile individually in a plastic container and immediately transported it to the laboratory under dark conditions in thermally insulated containers. In the laboratory, substrata were scraped as previously explained for the natural stones, and the same parameters were estimated.

During this period, the sky was generally cloudless, with maximum daily incident irradiance for PAR of 2784.9 μmol photons $m^{-2}\ s^{-1}$. Maximum incident intensities for ultraviolet radiation (UVR) at 320 and 340 nm were 44.8 and 78.1 μW $cm^{-2}\ nm^{-1}$, respectively. All light intensities were measured with a PUV500B radiometer.

Algal analysis

The attached algae were identified, counted and measured with direct microscopy using 0.0088-mL chambers. Cell dimensions were measured and processed with an image analysis system (Image ProPlus; Media Cybernetics, Warrendale, PA, U.S.A.), and cell volume was estimated by applying average dimensions of a minimum of 20 cells per species per sample to the geometric shape best approximating the cell shape of each species (Sun & Liu, 2003). Filamentous chrysophyte (i.e. *Hydrurus foetidus*) biovolume was based on measurements of individual cells and did not include the mucilaginous envelope. Diatom identification was performed on permanent slides with clean diatom valves mounted on glass slides using Naphrax™ resin.

Statistical analysis and calculations

All data were analysed with Sigma Plot.12 (Systat Software Inc., San Jose, CA, U.S.A.). Depending on sample distribution and variances, we conducted paired-sample *t*-tests (normality of differences and equality of variances) or Wilcoxon's tests (no normality and/or no equality of variances) to detect effects of turbidity on various physicochemical and photosynthetic parameters. To compare the experimental results, we performed one-way ANOVA or Kruskal–Wallis one-way analysis. The relationships among TSS (mg L^{-1}), turbidity (NTU), irradiance at stream bottom (μmol m^{-2} s^{-1}), Chl-*a* (mg m^{-2}), C : Chl-*a* (g g^{-1}), C : P (atomic) and photosynthetic parameters (qP, Y, NPQ, Y/qP and ETR) were explored using Pearson's correlations and Spearman's rank correlations; when we found significant relationships, we performed linear regressions. When data followed a nonlinear regression, we used dynamic fitting.

Results

The streams

We found significant differences between Negro (turbid waters) and Blanco (clear waters) streams, especially in TSS content (Paired *t*-test; d.f. = 7, $P = 0.004$). The TSS concentration in the Negro stream was sixteen-fold that of the Blanco stream (50.2 ± 35.1 mg L^{-1} and 2.8 ± 4.0 mg L^{-1}, respectively). We found no difference in TSS concentration between upper and lower sampling points in either stream. Therefore, we pooled all of the data to analyse the turbidity (NTU)–TSS concentration relationship, obtaining a significant positive regression (Fig. 3a). Spectrophotometric scans of unfiltered water showed that clay did not increase the absorption of any specific wavelength and the whole 250–700 nm spectrum was equally reduced by the presence of clay particles. Consequently, UVR varied according to changes in PAR (linear regression $r^2 = 0.94$, d.f. = 7, $P < 0.001$). Accordingly, light availability for periphyton growth was significantly lower in Negro Stream due to the high concentration of suspended clay (Table 1). In addition, we observed changes in turbidity with sampling dates, especially in January (mid-summer) in Negro Stream, while Blanco Stream exhibited a more narrow range (Fig. 3). In contrast to the observed light conditions, TDP concentrations were similar with no significant differences between streams (Table 1). Accordingly, the TN : TP atomic ratio showed no differences between streams (Negro: 38.4 ± 20.5; Blanco: 34.5 ± 12.4). DOC concentrations were also very low, with no significant differences between the streams (Negro: 0.7 ± 0.1 mg L^{-1}; Blanco: 0.4 ± 0.2 mg L^{-1}).

Periphyton biomass and elemental ratios

Periphyton biomass (as periphytic C and Chl-*a* mg m^{-2}) varied with sampling period (Fig. 4a); however, there were significant differences between the Negro and Blanco streams. We found a higher Chl-*a* concentration in Negro Stream than in Blanco Stream (12.1 ± 9.3 and 0.6 ± 0.5 mg m^{-2}, respectively, paired *t*-test, d.f. = 7, $P = 0.005$), showing a direct relationship with turbidity (Fig. 4b). Periphyton carbon concentration was twofold, significantly higher in Negro than in Blanco Stream (655.3 ± 381.2 and 281.4 ± 197.4 mg m^{-2}, respectively, paired *t*-test, d.f. = 7, $P = 0.028$). The C : Chl-*a* ratio (g g^{-1}) also responded to light conditions, showing an exponential increment as light increased (Fig. 5a). However, differences in periphyton P concentrations were not significant between streams (Table 1). The C : P ratio was significantly higher in Negro Stream (Table 1, Wilcoxon, $n = 8$, $P = 0.008$). Furthermore, we observed that the C : P ratio–light relationship fitted an exponential decay curve (Fig. 5b).

Photosynthetic parameters

Photosynthetic parameters (Fig. 6a) were estimated using the mean of the PAM measurements of the actinic

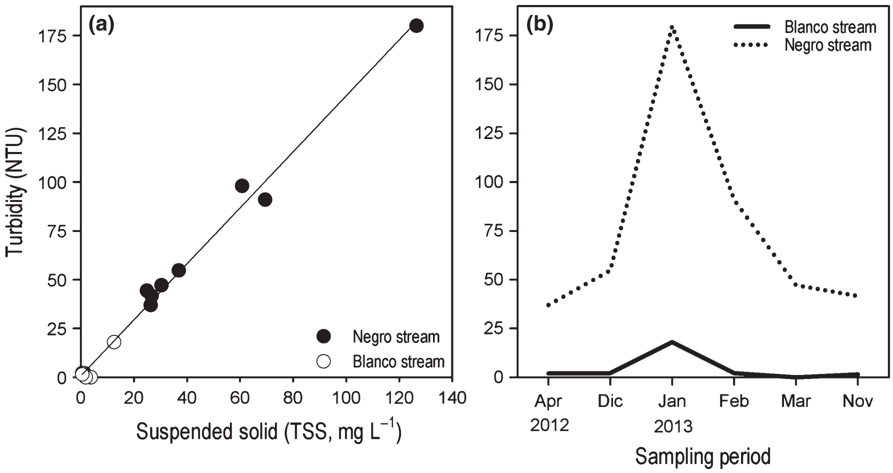

Fig. 3 (a) Relationship between total suspended solid (TSS) and turbidity ($r^2 = 0.99$, d.f. = 15, $P < 0.001$). (b) TSS concentration during the sampling period.

Table 1 Light and nutrient parameters of the Blanco and Negro streams measured on eight dates in the sampling period; additionally, in February and March, we sampled at the junction. The daily dose irradiances were calculated by integrating for the whole day. Light refers to available PAR at stream bottom

	Daily dose irradiance		Light (μmol m^{-2} s^{-1})		TDP (μg L^{-1})		Periphytic P (mg m^{-2})		C : P (Atomic)	
	PAR (mol m^{-2})	UV-320 (kJ m^{-2} nm^{-1})	Blanco	Negro	Blanco	Negro	Blanco	Negro	Blanco	Negro
12-April	37.6	3.9	2115	1220	19.1	23.9	4.2	8.9	95.6	155.1
12-December	66.5	8.8	2006	885	21.5	29.7	2.0	4.8	134.4	162.4
13-January	60.2	7.0	1420	131	26.0	24.0	5.7	2.6	101.7	778.9
13-February	57.5	6.3	1859	518	6.4	11.8	5.1	9.3	125.6	228.1
			1982	392	5.4	6.4	2.4	6.0	113.9	315.9
13-March	42.9	4.5	1808	969	16.5	18.4	4.8	3.2	157.3	217.4
			2147	959	16.1	16.1	3.6	2.9	165.7	218.3
13-November	51.8	5.8	1894	1152	21.0	22.6	4.2	11.0	146.9	202.3
Mean	51.5	5.9	1903	778	16.5	19.1	4.0	6.1	130.1	284.8
Test			Paired *t*-test		Paired *t*-test		Paired *t*-test		Wilcoxon	
P-value			$P < 0.001$		$P = 0.061$		$P = 0.141$		$P = 0.008$	

TDP, Total dissolved phosphorus.

light pulse for each sampling date in the two streams. Photosynthetic parameters were related to light condition (turbidity) because photosynthetic efficiency per open reactive centre of the PSII (Y/qP) increased as light availability decreased (Fig. 7a). Therefore, photosynthesis was affected by turbidity. The efficiency of light used in the photochemical system ($Y = \Delta F/F'_m$) was significantly higher in Negro Stream than in Blanco Stream (Paired *t*-test, d.f. = 6, $P = 0.008$) as well as the efficiency per open reactive centre (Y/qP, Paired *t*-test, d.f. = 6, $P < 0.001$).

We observed a decrease in NPQ during times of increased light, as shown by an exponential decay curve with increasing light (Fig. 7b). The NPQ was 10.5-fold higher in Negro Stream (Paired *t*-test, d.f. = 6, $P < 0.001$) although Blanco Stream had saturated light conditions. In the obtained light curves, we also observed differences between the streams in the ETR and the NPQ parameters (Fig. 8). In particular, in Blanco Stream, we found a lower thermal dissipation response (Fig. 8a) and a lower electronic transfer (ETR, Fig. 8b).

Experimental study

During the 2 weeks of experimentation, temperature varied over a narrow range between 9.3 and 12.1 °C (HOBO data logger), TN : TP was 32.4 ± 10.2 and P concentration remained almost invariable (TDP = 14.7 ± 1.5 μg L^{-1}). Thus, the main variable in the experiment was light treatment.

In the experimental tiles, periphyton Chl-*a* and C concentrations as well as photosynthetic parameters were related to the experimental light conditions (Fig. 6b). At the end of the experiment, there were differences

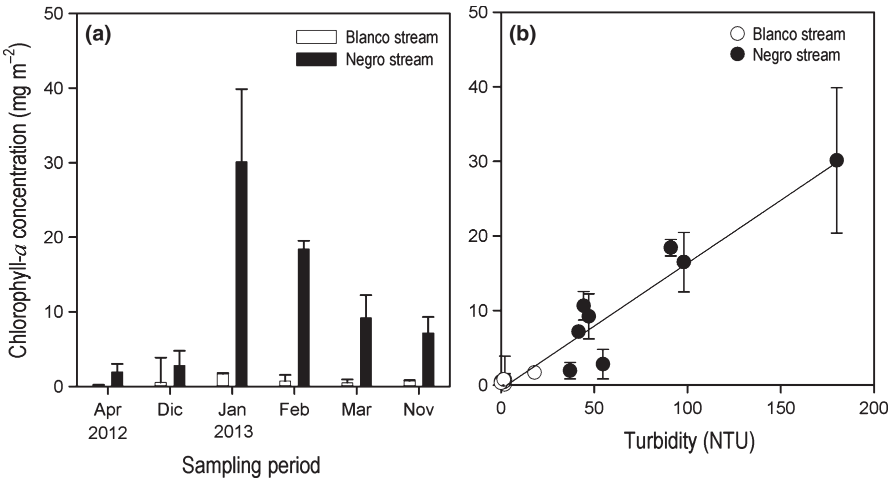

Fig. 4 (a) Periphytic chlorophyll *a* during the sampling period and (b) relationship between turbidity and chlorophyll *a* ($r^2 = 0.91$, d.f. = 15, $P < 0.001$).

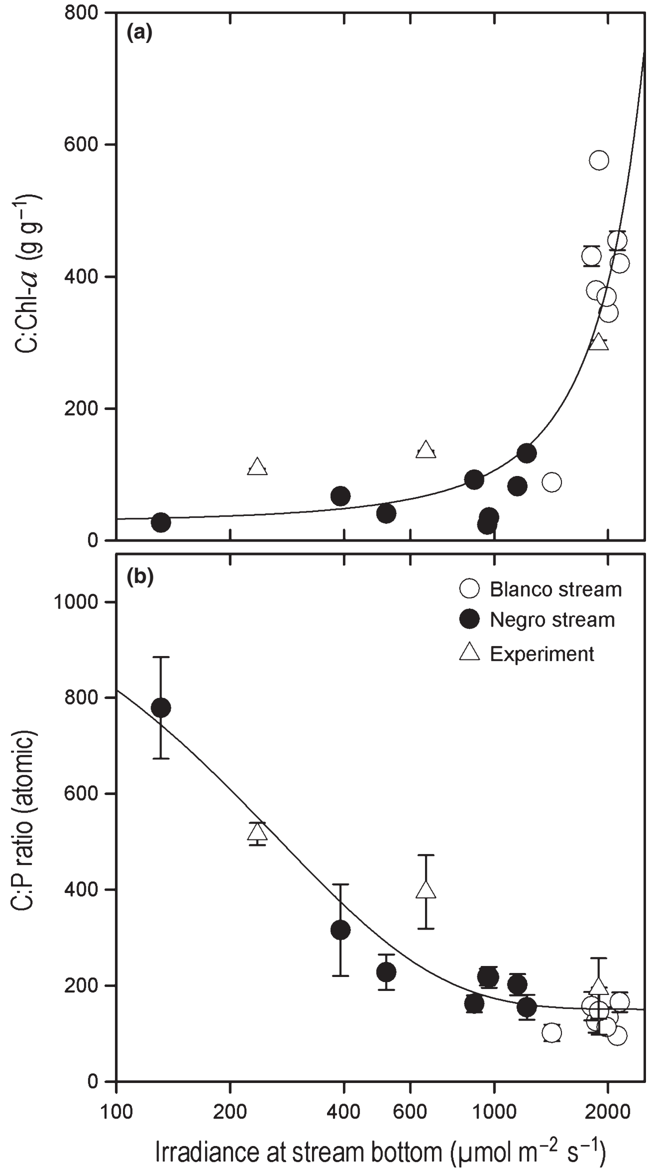

Fig. 5 Relationships between available light and (a) periphytic C : Chl-*a* ratio ($r^2 = 0.83$, d.f. = 18, $P < 0.0001$) and (b) periphytic C : P ratio ($r^2 = 0.90$, d.f. = 18, $P < 0.0001$).

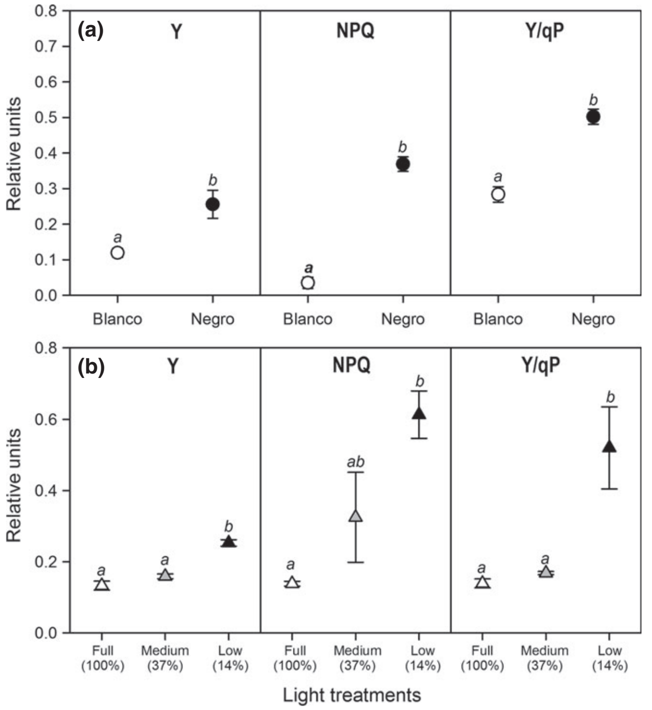

Fig. 6 (a) Photosynthetic parameters (effective quantum yield: $Y = \Delta F/F'_m$, non-photochemical quenching: NPQ, and efficiency of open reactive centre: Y/qP) in the two streams (open circles: Blanco, filled circles: Negro). (b) Photosynthetic parameters in the experimental treatments (open triangles 100% of incident light, grey triangles 37% of incident light and black triangles 14% of incident light). Data are given as means and standard errors.

(one-way ANOVA, $F_{2,9} = 16.4$, $P < 0.001$) in Chl-*a* concentration (14% light treatment = 2.8 ± 0.3, 37% = 1.6 ± 0.3, and 100% = 1.6 ± 0.3 mg m^{-2}) that were significant between 14% and the others two treatment (100% and 37%, *a posteriori* Tukey's test, $P = 0.002$). We also found significant differences in C concentration among treatments (one-way ANOVA, $F_{2,8} = 33.8$, $P < 0.001$): 14% = 303.4 ± 34.2 mg C m^{-2}, 37% = 206.3 ± 18.2 mg C m^{-2} and 100% of incident light = 140.3 ± 25.6 mg C m^{-2} (*a posteriori* Tukey's test, between all pairs $P < 0.05$). The C : Chl-*a* ratio exhibited significant differences (one-way ANOVA, $F_{2,8} = 31.1$, $P < 0.001$) between the 14% versus the 37% and between the 14% versus the 100% treatments (*a posteriori* Tukey's test, between all pairs $P < 0.001$), with the ratio being higher in the treatment of 100% of incident light, fitting the same exponential curve as the field data (Fig. 5a, see triangles). The periphyton P concentration did not vary among treatments (one-way ANOVA, $F_{2,9} = 1.1$, $P = 0.4$). However, because of the change in periphyton C concentration, we observed significant differences in the C : P ratio (one-way ANOVA, $F_{2,8} = 24.6$, $P < 0.001$, see triangles in Fig. 5b). The full light treatment (100%) differed from the two reduced light treatments (100% versus 14% *a posteriori* Tukey's test, $P < 0.001$; and 100% versus 37% *a posteriori* Tukey's test, $P = 0.006$), whereas there was no significant difference between the 14% and 37% treatments (*a posteriori* Tukey's test, $P = 0.06$).

We observed higher photosynthetic efficiency ($Y = \Delta F/F'_m$) in the 14% treatment (Fig. 6B, one-way ANOVA, $F_{2,9} = 41.5$, $P < 0.001$) and significant differences between the 14% versus the 100% and the 14% versus the 37% treatments (*a posteriori* Tukey's test, $P < 0.001$). The 100%

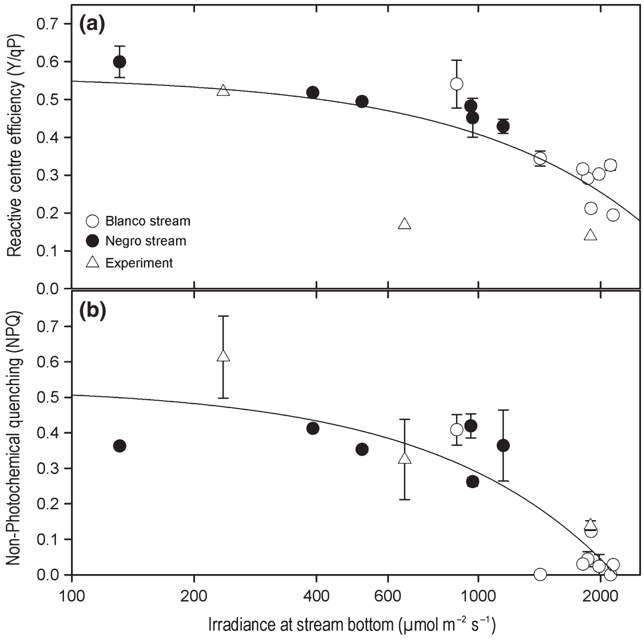

Fig. 7 Relationships between available light and (a) the efficiency per open reactive centre (*Y*/qP) (r^2 = 0.54, d.f. = 16, *P* = 0.004) and (b) heat dissipation (NPQ) (r^2 = 0.79, d.f. = 13, *P* = 0.0002).

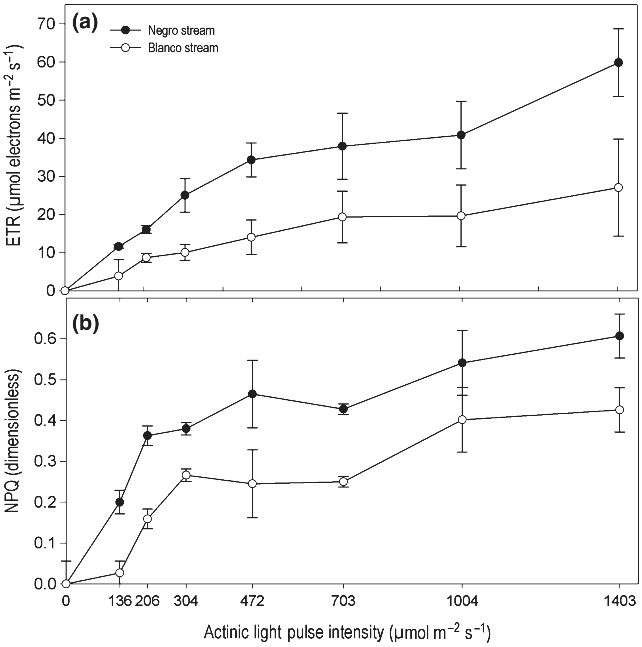

Fig. 8 Rapid light curves in the two streams (all sampling dates were pooled, and values are given as means and standard errors). (a) Non-photochemical quenching (NPQ). (b) Electron transfer rate (ETR) in the PSII.

and the 37% light treatments did not show significant differences (*a posteriori* Tukey's test, *P* = 0.2). A higher efficiency per reactive centre (Y/qP) was also found in the 14% treatment (Fig. 6B, one-way ANOVA, $F_{2,8}$ = 14.4, *P* = 0.002), the 14% versus the 100% and the 14% versus the 37% treatments (*a posteriori* Tukey's test, *P* < 0.01). Heat dissipation (NPQ) was higher in the 14% light treatment (one-way ANOVA, $F_{2,8}$ = 7.07, *P* = 0.02, *a posteriori* Tukey's test, 14% versus 100% *P* = 0.01).

Attached algal assemblages

Diatoms, chlorophytes and cyanobacteria were the primary constituents of the periphyton in both streams. In the natural substrata of Blanco Stream, the community was a cohesive thin layer dominated by prostrate diatoms, with *Gomphonema intricatum* and *Hannaea* sp. accounting for more than 80% of the biomass throughout the study. On the contrary, filamentous green algae (*Ulothrix* sp.) occurred on the stones of Negro Stream and in early autumn the chrysophyte *Hydrurus foetidus* (Villars) was most prevalent, forming an algal mat (90% of the total biomass). Within this matrix, diatoms such as *Diatoma* sp. and *G. intricatum* were observed. Cyanobacteria were present in both streams but with low contributions to total biomass. Periphyton abundance averaged $6.4 \times 10^4 \pm 5.68 \times 10^4$ cell cm^{-2} in Blanco Stream, whereas periphyton in Negro Stream were more abundant ($1.4 \times 10^5 \pm 7.3 \times 10^4$ cell cm^{-2}).

Ulothrix increased with turbidity (Spearman's ρ = 0.8, d.f. = 10, *P* = 0.009) while *Gomphonema* decreased (Spearman's ρ = −0.8, d.f. = 10, *P* = 0.005). This change in periphyton species abundance was evident during the summer (January) chlorophyll increase in Blanco Stream, when turbidity increased from 2 to 18 NTU. Over the same period, Negro Stream turbidity increased from 54 to 180 NTU, but no significant changes in periphyton species were registered. There were also differences in species composition between experimental treatments. While *Ulothrix* dominated in the 14% and 37% light treatments, in the full light treatment, we found a thin layer dominated by *Hannaea* sp. and *G. intricatum*.

Discussion

The periphytic primary producers were affected by changes in light conditions due to glacial clay inputs, with a light increase associated with a decrease in Chl-*a*. Variation in glacial flour was the primary regulator of variation in both PAR and UVR attenuation in these glacially fed rivers, as we found that the relative contributions of DOC were negligible because of their very low concentrations. The chlorophyll-light patterns were observed in both streams (clear and turbid), as well as in the experiment in which only light conditions were manipulated. An increase in Chl-*a* content in the primary producers leads to an increase in photosynthetic efficiency due to low photon availability (Hill, Fanta & Roberts, 2009). As a consequence of low-light availability, autotrophs usually increase chlorophyll (increased antenna size) and increase the PSII reaction centres to enhance light capture and energy transfer (Schansker *et al.*, 2011) or increase the synthesis of membrane-rich chloroplasts (Horton *et al.*, 2008). Indeed, we found less Chl-*a* in Blanco Stream than in Negro Stream, but more important was the increase in the C : Chl-*a* ratio, which reflects a decrease in Chl-*a* per biomass unit in the high-light exposed stream (19.5 times higher Chl-*a* content in Negro than in Blanco Stream). Frost, Hillebrand & Kahlert (2005) reported that more than 60% of samples of periphyton had a C : Chl-*a* ratio above 200 and only 25% had a ratio in excess of 500. Our observed values lay between these limits, in particular those from Blanco Stream and from the full light treatment of the experiment, while Negro Stream values remained below 200.

Our results showed an increase in the efficiency per open reactive centre of PSII under high turbidity, demonstrating that periphyton in Negro Stream had a higher efficiency per open PSII reactive centre than the periphyton in Blanco Stream (Fig. 5b). When light intensity increases above 1000 μmol photons m^{-2} s^{-1} (Murata, Allakhverdiev & Nishiyama, 2012), the rate of repair is reduced, disrupting the photodamage/repair balance, and thus the activity of PSII decreases (Nishiyama, Allakhverdiev & Murata, 2011), as seen in our data. To prevent this, non-photochemical quenching (NPQ) dissipates the excess of captured light as heat (Horton *et al.*, 2008), particularly under moderate excess of light when the accumulated damage in PSII reaction centres is low. Long exposure to high irradiance may cause chronic photoinhibition (Häder *et al.*, 2002) that is characterised by a decrease in photosynthetic electron transport (Critchley & Russell, 1994), due to the promotion of non-assimilatory electron transport to dissipate excess harvested light energy (Schansker *et al.*, 2011; Takahashi & Badger, 2011). This effect of long exposure to high irradiance was evident in Blanco Stream (clear water), where there was almost no dissipation of excess energy through the NPQ and a significant decrease in ETR in the rapid light curves. Therefore, NPQ protects PSII against short-term high-light conditions (Müller *et al.*, 2001; Nishiyama *et al.*, 2011). In this sense, NPQ is a photoprotective process that can prevent damage before it occurs (Murchie & Niyogi, 2011).

Therefore, we were able to identify two different types of photoinhibition: (i) preconditions of low-light availability (Negro Stream) in which excessive excitation energy is dissipated thermally (NPQ) with high-light harvesting by the antenna, and (ii) preconditions of high-light availability (Blanco Stream) caused a decrease in the photosynthetic electron transport (ETR) but with low NPQ due to a low-light harvesting by the antenna. Under high-light conditions, electronic transfer (ETR) decreased significantly to avoid overloading the system with excess photons, which could not be thermally dissipated (NPQ) (Häder *et al.*, 2002; Schansker *et al.*, 2011). In both cases, the PAM pulse consisted of 1004 μmol photons m^{-2} s^{-1}, which represents a normal photon flux for Blanco Stream (maximum irradiance at noon was 2000 μmol m^{-2} s^{-1}), but represents 50% more than maximum at noon of a sunny day for Negro Stream (750 μmol m^{-2} s^{-1}). This means that the photosynthetic machinery of Blanco Stream was prepared for high light and the antenna harvested a low proportion of light, with a low requirement for dissipation and therefore a low NPQ. In contrast, with low-light adaptation in Negro Stream, the antennae were ready to capture a greater proportion of incident light and the pulse resulted in a high dissipation with high NPQ.

Photosynthetic parameters (particularly: $Y = \Delta F/F'_m$ and NPQ) differed between the streams and among experimental treatments in response to turbidity and light intensities. Water turbidity not only reduces light intensity in the PAR spectrum (400–700 nm) but also in the UV range, and it has been shown that both the quantum yield of PSII and C fixation of autotrophs decline with an increase in UV radiation (Villafañe *et al.*, 1999; Modenutti *et al.*, 2004; Leu *et al.*, 2006). We also found under high PAR + UVR intensities in Blanco Stream, and in the full sunlight treatment, a reduction in effective quantum yield (Y) followed by a decrease in efficiency per reactive open centre in the PSII (Y/qP), which decreased the number of absorbed photons.

It might be expected that clay load would affect periphyton negatively (Davies-Colley *et al.*, 1992). However, we observed that increases in clay input were related to a rise in Chl-*a* content in both streams. Thus, if any negative effect of clay abrasion exists, it can be considered negligible compared with the light effect of turbidity. According to the light : nutrient hypothesis (Sterner *et al.*, 1997) and the studies that have reported that the balance between light and nutrients determines algal nutrient content (Fanta *et al.*, 2010), we predicted that glacial clay inputs would decrease the C : P ratioof periphyton. However, we observed the opposite situation, a decrease in C : P with an increase in light in both streams and in the experiment. This result may arise from the influence of solar ultraviolet radiation (UVR) and the higher PAR that reaches the periphyton. In canopy-free streams, such as those in our study, full sunlight (PAR + UVR) reaches the stream surface. On the contrary, in the indoor experiment carried out by Fanta *et al.* (2010), periphyton was exposed to artificial light (without UVR) in the lowest part of the PAR light intensity range of our study. The UVR component of sunlight negatively affects quantum yield and C fixation and, consequently, reduces the C : P ratio of the primary producers (Xenopoulos, Frost & Elser, 2002; Leu *et al.*, 2006). Although it has also been suggested that dissolved organic matter affects periphyton stoichiometry more than UVR (Frost *et al.*, 2007), the studied streams have very low DOC concentrations ($\leq$0.6 mg L^{-1}), an order of magnitude less than that in the Frost *et al.* (2007) study. Patagonian systems receive high UVR due to the Antarctic ozone hole (Häder *et al.*, 2007), and canopy-free streams are particularly exposed because of their shallow depth. In addition, in clear low-order streams with a very low DOC concentration algae are more sensitive to UV stress at low temperature and high PAR (Uehlinger *et al.*, 2010). Suspended clay in a turbid stream would protect algae from photoinhibition as well as from UVR damage to the PSII, as occurred in Negro Stream and in our experiment with a neutral light screen (no UV differential filter). Indeed, when clay concentration increased in Blanco Stream in January, a consequent increase in periphyton Chl-*a* and biomass was observed. The hazardous effect of UVR has also been reported to depend on the biomass of attached algae (McNamara & Hill, 2000) and the vertical structure of periphyton (Hodoki & Ohbayashi, 2005). In this sense, a positive feedback might have occurred in the turbid Negro Stream. The clay load initially would have protected algae from sunlight (including UV); therefore, algae developed into an algal mat that, in turn, allowed new algae to grow, protected by the change in periphyton vertical structure.

Glaciers respond rapidly to climate change, and glacier dynamics directly affect the ecosystems that they feed (Moore *et al.*, 2009). Streams that receive meltwater are particularly vulnerable to global change and associated stressors because they are relatively isolated and fragmented within the terrestrial landscape (Perkins *et al.*, 2010). In a scenario of global warming, with glacial recession taking place all around the world, the transparency of streams receiving melting water will change due to the change in glacial clay load. Depending upon whether clay load increases or decreases, the periphyton of these streams will be modified in many respects, in terms of biomass, Chl-*a* content and also in food quality for herbivores, because the elemental composition of the biofilm depends on light availability and also on UVR incidence. In the short term, an increase in melting results in an increase in clay load and therefore an increase in algal biomass but of lower food quality. However, in the long term, the clay load in streams will be reduced and, consequently, the effect of a light increase will reduce algal biomass, but high-light incidence, including UVR, will result in higher food quality. The final consequence is that glacial melting may shift periphyton from high C and low quality to low C and higher quality food for higher trophic levels.

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References

Allakhverdiev S.I., Tsvetkova N., Mohanty P., Szalontai B., Byoung Y.M., Debreczeny M. *et al.* (2005) Irreversible photoinhibition of photosystem II is caused by exposure of *Synechocystis* cells to strong light for a prolonged period. *Biochimica et Biophysica Acta - Bioenergetics*, **1708**, 342–351.

Biggs B.J.F. & Close M.E. (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology*, **22**, 209–231.

Callieri C., Modenutti B., Queimaliños C., Bertoni R. & Balseiro E. (2007) Production and biomass of picophytoplankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers. *Aquatic Ecology*, **41**, 511–523.

Carrasco J.F., Osorio R. & Casassa G. (2008) Secular trend of the equilibrium-line altitude on the western side of the southern Andes, derived from radiosonde and surface observations. *Journal of Glaciology*, **54**, 538–550.

Chillrud S.N., Pedrozo F.L., Temporetti P.F., Planas H.F. & Froelich P.N. (1994) Chemical weathering of phosphate and germanium in glacial meltwater streams: effects of subglacial pyrite oxidation. *Limnology and Oceanography*, **39**, 1130–1140.

Critchley C. & Russell A.W. (1994) Photoinhibition of photosynthesis in vivo: the role of protein turnover in photosystem II. *Physiologia Plantarum*, **92**, 188–196.

Cross W.F., Benstead J.P., Frost P.C. & Thomas S.A. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**, 1895–1912.

Davies J.-M. & Bothwell M.L. (2012) Responses of lotic periphyton to pulses of phosphorus: P-flux controlled growth rate. *Freshwater Biology*, **57**, 2602–2612.

Davies-Colley R.J., Hickey C.W., Quinn J.M. & Ryan P.A. (1992) Effects of clay discharges on streams. 1. Optical properties and epilithon. *Hydrobiologia*, **248**, 215–234.

Dickman E.M., Vanni M.J. & Horgan M.J. (2006) Interactive effects of light and nutrients on phytoplankton stoichiometry. *Oecologia*, **149**, 676–689.

Dodds W.K., Hutson R.E., Eichem A.C., Evans M.A., Gudder D.A., Fritz K.M. *et al.* (1996) The relationship of floods, drying, flow and light to primary production and producer biomass in a prairie stream. *Hydrobiologia*, **333**, 151–159.

Dyurgerov M. (2003) Mountain and subpolar glaciers show an increase in sensitivity to climate warming and intensification of the water cycle. *Journal of Hydrology*, **282**, 164–176.

Eaton A.D., Clesceri L.S., Rice E.W., Greenberg A.E. & Franson M.A.H. (2005) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, American Water Works Association, Washington DC, USA, 1368.

Eisma D. (1993) *Suspended Matter in the Aquatic Environment*, Springer-Verlag, Michigan, USA, 315

Elser J.J., Fagan W.F., Kerkhoff A.J., Swenson N.G. & Enquist B.J. (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist*, **186**, 593–608.

Falkowski P.G. & Raven J.A. (2007) *Aquatic Photosynthesis*, Princeton University Press, New Jersey, USA, 512.

Fanta S.E., Hill W.R., Smith T.B. & Roberts B.J. (2010) Applying the light: nutrient hypothesis to stream periphyton. *Freshwater Biology*, **55**, 931–940.

Frost P.C., Cherrier C.T., Larson J.H., Bridgham S. & Lamberti G.A. (2007) Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton. *Freshwater Biology*, **52**, 319–330.

Frost P.C., Hillebrand H. & Kahlert M. (2005) Low algal carbon content and its effect on the C: P stoichiometry of periphyton. *Freshwater Biology*, **50**, 1800–1807.

Graham A.A., McCaughan D.J. & McKee F.S. (1987) Measurement of surface area of stones. *Hydrobiologia*, **157**, 85–87.

Häder D.P., Kumar H.D., Smith R.C. & Worrest R.C. (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences*, **6**, 267–285.

Häder D.-P., Lebert M., Sinha R.P., Barbieri E.S. & Helbling E.W. (2002) Role of protective and repair mechanisms in the inhibition of photosynthesis in marine macroalgae. *Photochemical & Photobiological Sciences*, **1**, 809–814.

Hauer F.R., Lamberti G.A. (2007) *Methods in Stream Ecology*, Elsevier Inc., China, 877.

Hill W.R. & Fanta S.E. (2008) Phosphorus and light colimit periphyton growth at subsaturating irradiances. *Freshwater Biology*, **53**, 215–225.

Hill W.R., Fanta S.E. & Roberts B.J. (2009) Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography*, **54**, 368–380.

Hodoki Y. & Ohbayashi K.H. (2005) Species-specific responses of freshwater diatoms to solar ultraviolet radiation. *Archiv fur Hydrobiologie*, **162**, 431–443.

Horton P., Johnson M.P., Perez-Bueno M.L., Kiss A.Z. & Ruban A.V. (2008) Photosynthetic acclimation: does the dynamic structure and macro-organisation of photosystem II in higher plant grana membranes regulate light harvesting states? *FEBS Journal*, **275**, 1069–1079.

Hylander S., Jephson T., Lebret K., Von Einem J., Fagerberg T., Balseiro E. *et al.* (2011) Climate-induced input of turbid glacial meltwater affects vertical distribution and community composition of phyto- and zooplankton. *Journal of Plankton Research*, **33**, 1239–1248.

Iriondo M. (1974) Modelo de sedimentación en el Lago Mascardi Provincia de Río Negro. *Revista Asociación Geológica Argentina*, **29**, 349–358.

Kirk J.T.O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*, Cambridge University Press, New York, USA, 509.

Laspoumaderes C., Modenutti B., Souza M.S., Bastidas Navarro M., Cuassolo F. & Balseiro E. (2013) Glacier melting and stoichiometric implications for lake community structure: zooplankton species distributions across a natural light gradient. *Global Change Biology*, **19**, 316–326.

Leu E., Wängberg S.-Å., Wulff A., Falk-Petersen S., Børre Ørbæk J. & Hessen D.O. (2006) Effects of changes in ambient PAR and UV radiation on the nutritional quality of an Arctic diatom (*Thalassiosira antarctica* var. borealis). *Journal of Experimental Marine Biology and Ecology*, **337**, 65–81.

Lind O.T., Chrzanowski T.H. & Dávalos-Lind L. (1997) Clay turbidity and the relative production of bacterioplankton and phytoplankton. *Hydrobiologia*, **353**, 1–18.

Long S.P., Humphries S. & Falkowski P.G. (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology*, **45**, 633–662.

Masiokas M.H., Luckman B.H., Villalba R., Ripalta A. & Rabassa J. (2010) Little Ice Age fluctuations of Glaciar Río Manso in the north Patagonian Andes of Argentina. *Quaternary Research*, **73**, 96–106.

Masiokas M.H., Villalba R., Luckman B.H., Lascano M.E., Delgado S. & Stepanek P. (2008) 20th-century glacier recession and regional hydroclimatic changes in northwestern Patagonia. *Global and Planetary Change*, **60**, 85–100.

Maxwell K. & Johnson G.N. (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany*, **51**, 659–668.

McNamara A.E. & Hill W.R. (2000) UV-B irradiance gradient affects photosynthesis and pigments but not food quality of periphyton. *Freshwater Biology*, **43**, 649–662.

Modenutti B., Balseiro E., Bastidas Navarro M., Laspoumaderes C., Souza M.S. & Cuassolo F. (2013) Environmental changes affecting light climate in oligotrophic mountain lakes: the deep chlorophyll maxima as a sensitive variable. *Aquatic Sciences*, **75**, 361–371.

Modenutti B., Balseiro E., Callieri C., Queimaliños C. & Bertoni R. (2004) Increase in photosynthetic efficiency as a strategy of planktonic organisms exploiting deep lake layers. *Freshwater Biology*, **49**, 160–169.

Modenutti B.E., Pérez G.L., Balseiro E.G. & Queimaliños C.P. (2000) The relationship between light attenuation, chlorophyll a and total suspended solids in a Southern Andes glacial lake. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **27**, 1–4.

Moore R.D., Fleming S.W., Menounos B., Wheate R., Fountain A., Stahl K. *et al.* (2009) Glacier change in western North America: influences on hydrology, geomorphic hazards and water quality. *Hydrological Processes*, **1**, 42–61.

Morris D.P., Zagarese H., Williamson C.E., Balseiro E., Hargreaves B.R., Modenutti B. *et al.* (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology & Oceanography*, **40**, 1381–1391.

Müller P., Xiao-Ping L. & Niyogi K.K. (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiology*, **125**, 1558–1566.

Murata N., Allakhverdiev S.I. & Nishiyama Y. (2012) The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. *Biochimica et Biophysica Acta - Bioenergetics*, **1817**, 1127–1133.

Murata N., Takahashi S., Nishiyama Y. & Allakhverdiev S. (2007) Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **1767**, 414–421.

Murchie E.H. & Niyogi K.K. (2011) Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiology*, **155**, 86–92.

Nishiyama Y., Allakhverdiev S.I. & Murata N. (2011) Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. *Physiologia Plantarum*, **142**, 35–46.

Nusch E.A. (1980) Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv für Hydrobiologie-Beiheft Ergebnisse der Limnologie*, **14**, 14–36.

Perkins D.M., Reiss J., Yvon-Durocher G. & Woodward G. (2010) Global change and food webs in running waters. *Hydrobiologia*, **657**, 181–198.

Rohácek K., Soukupová J. & Bartak M. (2008) Chlorophyll fluorescence: a wonderful tool to study plant physiology and plant stress. *Plant Cell Compartments - Selected Topics* **2**, 41–104.

Ross O.N. & Sharples J. (2008) Swimming for survival: a role of phytoplankton motility in a stratified turbulent environment. *Journal of Marine Systems*, **70**, 248–262.

Schaefer M., Machguth H., Falvey M. & Casassa G. (2013) Modeling past and future surface mass balance of the Northern Patagonia Icefield. *Journal of Geophysical Research F: Earth Surface*, **118**, 571–588.

Schansker G., Tóth S.Z., Kovács L., Holzwarth A.R. & Garab G. (2011) Evidence for a fluorescence yield change driven by a light-induced conformational change within photosystem II during the fast chlorophyll a fluorescence rise. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **1807**, 1032–1043.

Steinman A.D. (1992) Does an increase in irradiance influence periphyton in a heavily-grazed woodland stream? *Oecologia*, **91**, 163–170.

Sterner R.W., Elser J.J., Fee E.J., Guildford S.J. & Chrzanowski T.H. (1997) The light: nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *The American Naturalist*, **150**, 663–684.

Sun J. & Liu D.Y. (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, **25**, 1331–1346.

Takahashi S. & Badger M.R. (2011) Photoprotection in plants: a new light on photosystem II damage. *Trends in Plant Science*, **16**, 53–60.

Uehlinger U., Robinson C.T., Hieber M. & Zah R. (2010) The physico-chemical habitat template for periphyton in alpine glacial streams under a changing climate. *Hydrobiologia*, **657**, 107–121.

Valderrama G. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry*, **10**, 109–122.

Villafañe V.E., Andrade M., Lairana V., Zaratti F. & Helbling E.W. (1999) Inhibition of phytoplankton photosynthesis by solar ultraviolet radiation: studies in Lake Titicaca, Bolivia. *Freshwater Biology*, **42**, 215–224.

Wang H., Sterner R.W. & Elser J.J. (2012) On the "strict homeostasis" assumption in ecological stoichiometry. *Ecological Modelling*, **243**, 81–88.

Wolfe J.E. & Lind O.T. (2008) Influence of suspended clay on phosphorus uptake by periphyton. *Hydrobiologia*, **610**, 211–222.

Wood P.J. & Armitage P.D. (1997) Biological effects of fine sediment in the lotic environment. *Environmental Management*, **21**, 203–217.

Worni R., Stoffel M., Huggel C., Volz C., Casteller A. & Luckman B. (2012) Analysis and dynamic modeling of a moraine failure and glacier lake outburst flood at Ventisquero Negro, Patagonian Andes (Argentina). *Journal of Hydrology*, **444–445**, 134–145.

Xenopoulos M.A., Frost P.C. & Elser J.J. (2002) Joint effects of UV radiation and phosphorus supply on algal growth rate and elemental composition. *Ecology*, **83**, 423–435.

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