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Effects of realistically simulated, elevated UV irradiation on photosynthesis and pigment composition of the alpine snow alga *Chlamydomonas nivalis* and the arctic soil alga *Tetracystis* sp. (Chlorophyceae)

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Abstract

An indoor sun simulator was used to provide elevated UV-B radiation (280–315 nm) in combination with realistic ratios to PAR (400–700 nm) and UV-A radiation (315–400 nm) in order to test the physiological response of a soil- and snow microalga during a three-day stress scenario, which may occasionally occur in their respective arctic and alpine habitats.

Chlamydomonas nivalis and *Tetracystis* sp. are initial colonizers of harsh habitats like summer snow fields and bare arctic soils. The two species were chosen because of their role as primary successors in places where life is generally limited by extreme climatic and nutritional conditions.

The influence of the increased UV-B irradiation (1.43 W m^{-2}; control: 0.52 W m^{-2}) on photosynthesis and pigment composition was measured. Both species survived this incubation without any morphological signs of damage, but oxygen production was reduced by 20–56%. Under control conditions, the amount of chlorophylls (Chls) and carotenoids (Cars) per dry mass increased after three days due to optimal light conditions. After the same period, the treated samples of the soil alga *Tetracystis* sp. showed a smaller increase in Chls and primary Cars than the control. However, the production of extraplastidal, secondary Cars was induced. On the contrary, the snow alga *C. nivalis* already had high amounts of secondary Cars before the experiment, and after exposure, all pigment classes increased more compared to control conditions. The results show that these microalgae can tolerate short episodes of enhanced UV-B radiation. Photosynthesis may be temporally impaired, but the cells respond by the production of secondary Cars, which can shield their chloroplasts against excessive irradiation or quench reactive oxygen species.

Additional keywords: microalgae; secondary carotenoids; sun simulator; UV-B exposure.

Introduction

Snow algae and polar soil algae appear as typical members of environments, where low temperatures prevail, regarded as the main limitation for this living matter. The evidently existing climatic adaptations suggest that these species are considered as possible test objects for UV and temperature resistance research. Microalgae that thrive in alpine summer snow or on bare arctic soils are essential primary producers in such ecosystems, where phototrophic life is restricted to a few specialised organisms (Morgan-Kiss *et al.* 2006). These high-altitude snow fields and high-latitude permafrost soils frequently exhibit harsh environmental conditions and the duration of the growing season is restricted to a few weeks per year. During summer, strong variations in temperatures, a limited water availability or excessive irradiation can cause multiple abiotic stresses.

Generally, algae like *C. nivalis* as well as *Tetracystis* sp. survive in such environments regarded to be extreme. However, their habitats differ: the first species is a "true" snow alga, its life cycle is restricted to long-lasting, wet snow beds during alpine and polar summers (Hoham and Duval 2001, Remias *et al*. 2005). These snow algae grow in the melt water between snow grains and can cause, under suitable conditions, monospecific mass accumulations that visually change the colour of snow surfaces to green, yellow, or red. The samples of *C. nivalis* used in

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Abbreviations: A – antheraxanthin; Car(s) – carotenoid(s); Chl(s) – chlorophyll(s); DM – dry mass; F_v/F_m – variable fluorescence/ maximum fluorescence; MAA – mycosporine-like amino acid; PAR – photosynthetic active radiation; PS – photosystem; V – violaxanthin; Z – zeaxanthin.
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this study came from red-coloured snow fields in the European Alps (Austria, Province Tyrol) at 3,000 m a.s.l.

The second species, *Tetracystis* sp., was collected from periodically water filled, small cavities around the high arctic settlement of Ny Ålesund, Svalbard (79° N). This alga causes orange-coloured mats in the bottom of ephemeral waterbodies. During the arctic summer, these sites are ice- and snow-free. Other typical places where such cold-tolerant phototrophic organisms can be found are arctic soil crusts (Breen and Lévesque 2008, Büdel 2005). In polar regions, *Tetracystis* populations were especially reported from tundra surfaces (Patova and Dorokhova 2008), *e.g.* in the proximity of retreated glaciers (Kaštovská *et al.* 2005, Leya *et al.* 2000).

Both species share several morphological characteristics (Ettl *et al.* 1983, Ettl *et al.* 1988). They are mostly single-celled and of spherical shape. Moreover, they often store high amounts of secondary Cars in cytoplasmic lipid globules, which colour the cells orange or red, causing the green chloroplast to be visually covered by these pigments. Field samples consist almost completely of nonmoving cyst stages enclosed with thick cell walls. Motile, flagellated stages with frequent cell cycles are rarely observed because they are limited to short periods of favourable conditions like at the beginning of summer (snowmelt).

To elucidate the range of UV adaptation, especially in the biologically important wavelength region of UV-B radiation (280–315 nm), considering a "global change" scenario (and the assumption of long-term variations of the ozone layer in high latitudes; *see* Rozema *et al.* 2005 and Blumthaler 2007), we applied realistic elevated UV-B radiation over a three-day simulation under cool temperatures, whilst keeping UV-A radiation (315–400 nm: 15 W m^{-2}) and photosynthetically active radiation (PAR, 400–700 nm: 730 μmol m^{-2} s^{-1}) at a realistic level similar to their growth environment. In general, ecologically relevant studies require the use of realistic global radiation conditions, which not only refer to the intensity but also to the spectral composition of the irradiation. Our experiment was performed in a sun simulator of the Helmholtz Zentrum München (Germany), where these requirements are fulfilled (Döhring *et al.* 1996, Thiel *et al.* 1996).

The physiological responses of the two selected green algal species were observed in order to answer whether these organisms are able to cope with periodically severe high UV-B radiation. The physiological parameters that were measured during this survey include photosynthesis (light-dependent oxygen production, chlorophyll fluorescence) and HPLC quantification of primary and secondary pigments. These data were compared between samples that received ambient UV-B radiation (0.5 W m^{-2}, control) and an approximately threefold higher load of UV-B radiation (exposure). Great attention was especially paid to any alterations in the secondary pigments because of their capability in shielding algae against harmful irradiation. Moreover, the light adaptation of the cells as observed in the field should be partially explained by these simulation experiments.

Materials and methods

Patches of red-coloured snow, which were caused by *Chlamydomonas* cf. *nivalis* in the proximity of the Tiefenbach glacier (European Alps, Austria, province of Tyrol, N46°55.04 E10°55.89; 2980 m a.s.l.) were sampled on September 11th, 2008. The field material was slowly melted and the cells kept vital at 5°C and approx. 50 μmol m^{-2} s^{-1} PAR (16-h day) prior to the beginning of the experiments. The meltwater cell suspension was mixed 1:1 with Bolds Basal Medium (BBM; according to Leya 2004, however without vitamin B solution), but the algae did not grow or divide and remained in spherical cyst stages.

Tetracystis sp. (morphological genus determination according to Ettl and Gärtner 1988) caused orange-coloured mats in occasionally water filled cavities in and around the arctic settlement of Ny Ålesund (Svalbard, N78°55.09 E11°57.02; 6 m a.s.l.). This alga has been deposited at the Culture Collection of Cryophilic Algae (CCCryo) as strain 340b-08 at IBMT Potsdam-Golm, Germany (for collection information, *see* Leya *et al.* 2009). Field samples were collected from a small basin in July 2004 and kept permanently frozen at –20°C for four years. After rethawing at 5°C and 50 μmol m^{-2} s^{-1} PAR (16-h day), the cells that had survived the four years freezing were isolated and transferred into sterile BBM media. Before measurements, the population developed to actively growing, mostly green stages and was kept at 10°C and 100 μmol m^{-2} s^{-1} PAR (16-h day) prior to use.

For the UV-B treatment, the algae were placed in the sun simulator at the Helmholtz Center Munich to study the algae's response under a natural photobiological environment. In the sun simulator, a combination of four lamp types (metal halide lamps: *Osram Powerstar HQI-TS 400W/D*, quartz halogen lamps: *Osram Haloline 500W*, blue fluorescent tubes: *Philips TL-D 36W/BLUE*, and UV-B fluorescent tubes: *Philips TL 40W/12*) was used to obtain a natural balance of simulated global radiation throughout the UV to infrared spectrum. The lamp types were arranged in several groups to get the natural diurnal variations of solar irradiance by switching appropriate group of lamps on and off. The short-wave cut-off was achieved by selected soda-lime and acrylic glass filters. A detailed description of the sun simulator facility is given by Döhring *et al.* 1996 and Thiel *et al.* (1996).

The algae were exposed to UV-A radiation and PAR for 16 h per day and to UV-B radiation for 12 h, including a diurnal variation as follows: two hours of

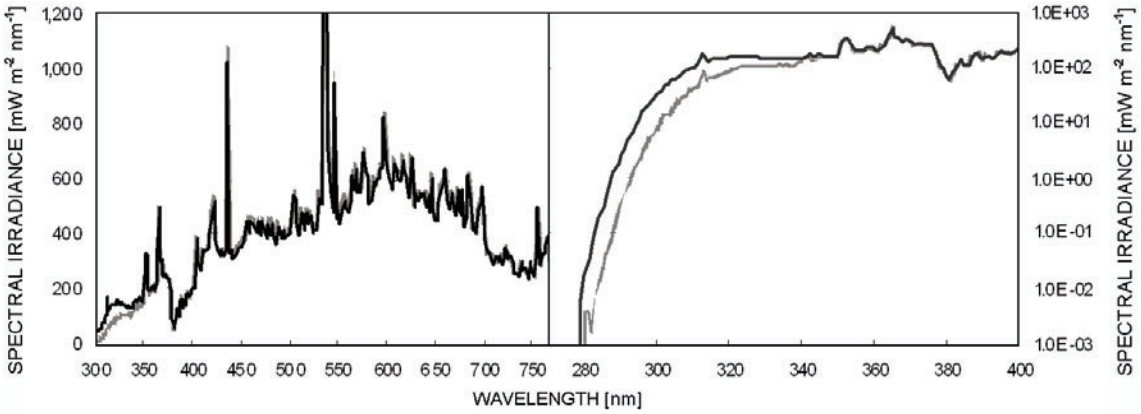

Fig. 1. Sun simulator spectra for ambient radiation (control, *grey line*) and elevated radiation (exposure, *black line*) used in the experiment. *See* Table 1 for irradiance and exposure values.

Table 1. Irradiance and exposure values of control and exposure during the treatment in the sun simulator. The biological effective UV-B radiation UV-B_{be} is calculated after Caldwell (1971), normalized at 300 nm.

Treatment	control	exposure
PAR [µmol m^{-2} s^{-1}]	737	724
UV-A [W m^{-2}]	14.0	15.9
UV-B [W m^{-2}]	0.52	1.43
UV-B_{be} [mW m^{-2}]	146	540
Daily PAR [mol m^{-2} d^{-1}]	29.9	29.4
Daily UV-A [kJ m^{-2} d^{-1}]	526	595
Daily UV-B [kJ m^{-2} d^{-1}]	13.8	38.2
Daily UV-B_{be} [kJ m^{-2} d^{-1}]	3.9	14.4

dawn and dusk each with 18% of PAR and 15% of UV-A. Before and after the maximum time range from 10 to 18 h, two hours of 67% PAR, 56% UV-A and 16% of UV-B were applied. The maximum values during the experiment are listed in Table 1 and the respective spectra are plotted in Fig. 1. The day before the measurements, both species were allowed to acclimate to the light conditions for 6 h at 130 µmol m^{-2} s^{-1} PAR, 2.1 W m^{-2} UV-A and 0.08 W m^{-2} UV-B radiation. The irradiance and exposure values were calculated from spectroradiometric measurements performed with a double monochromator system *DTM-300* (*Bentham*, Reading, UK).

During the experiments, the algae were exposed in open glass Petri dishes filled with BBM : Millipore water (1:1). Evaporation was reduced by setting the relative humidity in the exposure chambers to 70%. To avoid undesired heating of the samples due to the high irradiation, the Petri dishes were placed in a water bath cooled with ice during the light phases. The air temperature in the chamber was kept at 10°C during night and increased at daytime due to solarisation to 15°C maximum.

Before and after the treatment, samples were harvested onto preweighed 45-mm glass fibre filters (*Whatman GF/C*) for further analysis by a vacuum hand pump and immediately frozen at –80°C. The frozen filters were lyophilized for 24 h and afterwards the dry mass (DM) of the algae was determined. Algae used for photosynthesis were harvested similarly and the freeze-dried cells were resuspended into dimethylformamide (Sigma Aldrich) for measuring the chlorophyll (Chl) content according to Porra *et al.* 1989.

Photosynthesis was measured on the third day of exposure with a *Presens Fibox 3* oxygen optode *PSt3* (*Presens*, Nürnberg, Germany), using a 3-ml thermostatic acrylic chamber *DW1* (*Hansatech Instruments*, King's Lynn, UK) combined with a magnetic stirrer. 2 ml of algal suspension was mixed with 1 ml of a 0.1 M HCO_3^- source prior to use. Four light levels (PAR of 42, 69, 261, and 460 µmol m^{-2} s^{-1}) were calibrated with a *Hansatech QRT1 PAR* sensor and set with *Hansatech A5* neutral filters. The values of two respiratory dark phases (before and after the light cycle) were averaged. The O_2 production per time was normalised to the amount of total Chl per sample.

The pigments were analysed with a *ChemStation 1100 HPLC* gradient system (*Agilent Technologies*, Weilheim, Germany). Cars and Chls were quantified with calibration standards and separated with a *Lichrospher RP C18 250* × 4.6 mm column according to Remias and Lütz (2007), using a diode array detector. The results are expressed as the relative (sum of all pigment classes by mass = 100%) and absolute amounts [µg g^{-1} (DM)] of Chls, primary Cars and secondary Cars. Light microscopy and cell vitality staining with fluorescein diacetate (FDA, *Sigma*) were performed according to Müller *et al.* (2001) and Remias *et al.* (2009). Fast Chl fluorescence (Kautsky effect) was measured with a pulse-modulated *Handy PEA* (*Hansatech Instruments*, King's Lynn, UK). One drop of suspended algae was placed into a leaf clip, equipped with a moistened paper filter, each sample was kept dark for 30 min. Five measurements were averaged and the standard deviation calculated.

Results

Light microscopical (LM) observations: The overall morphology and colour of the samples was monitored by LM. Fig. 2 shows typical red cysts of *C. nivalis* and greenish vegetative cells of *Tetracystis* sp. During the treatment, no changes in cell morphology were observed (which could have been signs of stress or destruction). When staining the cells with the vitality marker FDA, all cells of both species exhibited a green fluorescence signal and hence were regarded as vital, also directly after the elevated UV-B treatment (data not shown).

Changes in pigment composition: During the three-day exposure, a shift in the relative pigment composition in *Tetracystis* sp. was observed. Before the experiment, no secondary pigment was present in this species, grown under laboratory conditions (100 µmol m^{-2} s^{-1} PAR, no UV-B). In the control (PAR 730 µmol m^{-2} s^{-1} plus ambient UV-B), its portion rose from 0 to 3% and in the UV-treatment to 6%, respectively. The Chls were slightly reduced (87 to 82%, respectively 80%), while the primary Cars remained about the same relative level (13 to 15%, respectively 14%). *C. nivalis* had a very high content of secondary carotenoids already before the experiments (90%). Likewise, the relative amount of Chls in this species slightly decreased from 9 to 7% (respectively 5% after exposure) in favour of the secondary Cars while the primary pigments remained at 1%.

The shift of absolute pigment contents, based on DM basis, showed different patterns in both species concerning the three harvests, namely at start, the control and the exposure (Fig. 3). The transfer from the regime of 100 µmol m^{-2} s^{-1} PAR to 730 µmol m^{-2} s^{-1} PAR plus ambient UV-B for three days induced an increase of about 2.7 fold total Chls and about 3.3 fold in thylakoid Cars in *Tetracystis* sp. The PAR plus UV-B treatment reduced total Chls by about 1.8 fold (but remained still higher compared to the start conditions), and thylakoid Cars were reduced similarly (by about 1.9 fold). Secondary pigments showed an increase from 203 µg g^{-1}(DM) to 235 µg g^{-1}(DM) under additional UV. *C. nivalis* reacted differently, because Chls (1.5 fold) and primary Cars (1.6 fold) showed only a slighter increase after three days under control conditions. The additional UV-exposure did not change these values considerably: all plastid pigments increased by less than 10% compared to the high PAR/ambient UV-B exposure, whereas the DM content of secondary Cars increased: from the start to the control harvest the amount was nearly doubled, and in the exposure it rose nearly threefold to a value of 14.3 mg g^{-1} (1.4% of DM, respectively).

Both species contain the primary pigments common for green algae (details not shown). The main difference was found in the three xanthophyll cycle Cars: while each sample of *C. nivalis* exclusively contained violaxanthin, *Tetracystis* sp. also had antheraxanthin and zeaxanthin (the samples were harvested at the same time and immediately frozen at –80°C, consequently preventing

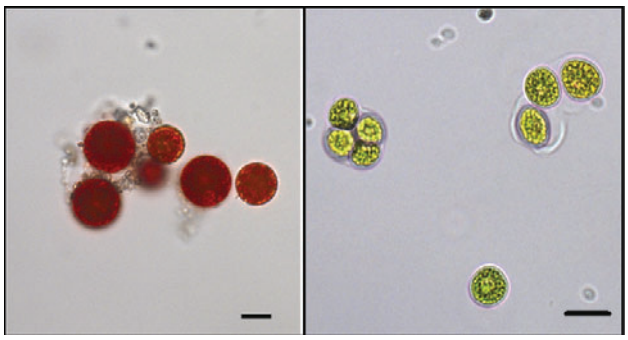

Fig. 2. Light micrographs of *C. nivalis* cysts (*left*) and cells of *Tetracystis* sp. (*right*), taken before the exposure experiment. Scale: 10 µm.

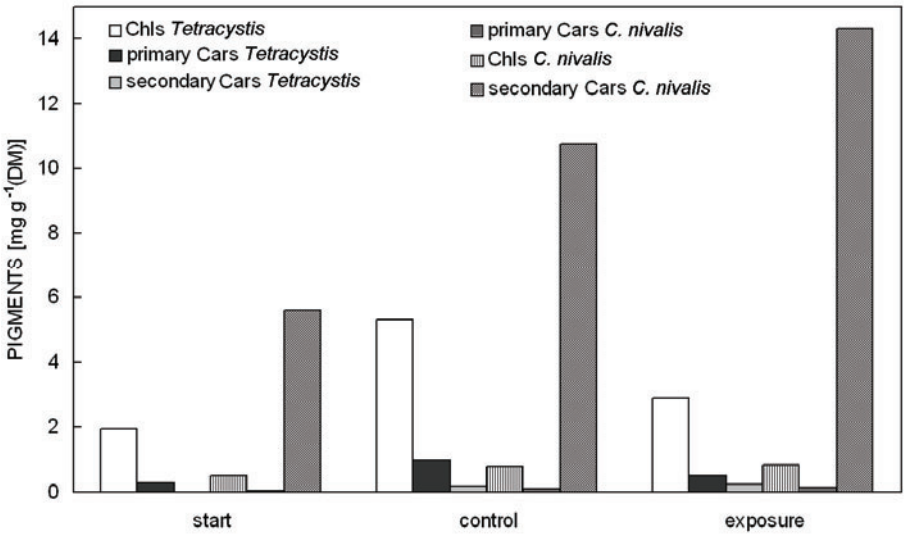

Fig. 3. Content of algal pigment classes of *Tetracystis* sp. and *C. nivalis* per dry mass (DM) before the experiments (start) and after three days of high light plus normal UV (control) and high light plus elevated UV-B (exposure).

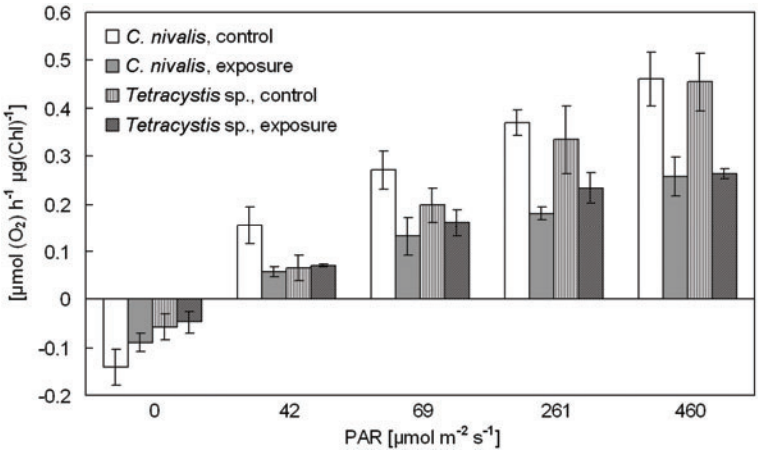

Fig. 4. Net photosynthetic rates [oxygen evolution per chlorophyll (*a*+*b*), Chl] of *C. nivalis* and *Tetracystis* sp. of each the control and the exposure at darkness and under four different light levels. Data points are means of three replicates ± SD.

any light transformation artefacts). The deepoxidation states (AZ/VAZ) of the latter species were of 0.514 (start), 0.606 (control), and 0.524 (exposure). The pattern of secondary Cars differed: *C. nivalis* produces astaxanthin (mainly esterified as mono- and diesters with fatty acids) and trace amounts of adonirubin. *Tetracystis* sp. accumulates adonixanthin and canthaxanthin (field samples also contained echinenone).

Photosynthesis and Chl fluorescence: Net photosynthesis and respiration were measured after a recovery period of 8 h of darkness and approx. 4 h of PAR only (730 µmol m^{-2} s^{-1}). The oxygen turnover was normalized to the total Chl content per sample (Fig. 4). *Tetracystis* sp. exhibited a decrease of approx. 20 to 40% of photosynthesis after treated with elevated UV–B radiation, however at the lowest light level (42 µmol m^{-2} s^{-1} PAR) and during the dark phase, no significant differences between control and exposure were measured. Calculation of gross photosynthesis resulted in the same trends.

C. nivalis had an even more impaired net photosynthesis of exposed samples with declines down to 56%. The respiration decreased under exposure [–0.141 to –0.090 µmol(O_2) h^{-1} µg(Chl)$^{-1}$]. Again, gross photosynthesis showed the same light dependent trends of photosynthetic activity with high oxygen values.

Chl fluorescence assays as a parameter of PSII function were conducted on the second day (data not shown). In the morning at 8 h (before UV irradiation was turned on), the mean active photosynthesis factor F_v/F_m was 0.608 ± 0.035 for *Tetracystis* sp. and 0.580 ± 0.021 for *C. nivalis*. At 12.30 h (4.5 h later), the exposed cells of both species had a decreased mean F_v/F_m of 0.354 ± 0.030 for *Tetracystis* sp. and 0.500 ± 0.034 for *C. nivalis* respectively, thus showing a smaller decrease for the red snow algae. Moreover, the F_v/F_m of both species evolved an overnight recovery to values of 0.609 ± 0.016 respectively 0.514 ± 0.012 in the morning of the next day (8 h).

Discussion

Three days of incubation under ambient *vs*. elevated UV-B radiation is expected to be sufficient in order to study typical UV-stress effects, as they may episodically occur from a few minutes up to several hours in nature (Holzinger *et al.* 2006, Lütz *et al.* 1997, Meindl and Lütz 1996). The applied temperature regime of about 10°C matched the field conditions for *Tetracystis* sp. (own observations). For *C. nivalis*, it was also in the range of temperatures which cysts are subject to after snowmelt (Remias *et al.* 2005).

The maximal rates of photosynthetic oxygen evolution either as net or as gross photosynthesis did not differ strongly between these two unrelated organisms; this holds for control as well as for treated cultures. Independent of different cell and plastid structures, photosynthesis per Chl is surprisingly similar. However, the adaptive reactions occur differently.

C. nivalis evolved reduced respiration and photosynthetic rates after a three-day exposure to elevated UV-B radiation. The physiological origin of this decline remains open, but could be explained by the fact that this species is in the cyst stage where photosynthesis is generally reduced. The F_v/F_m values, which indicate the activity of photosystem II, showed only a slight decline during exposure, but are generally lower compared to green cells like *Tetracystis* sp. The impaired PS efficiency in connection with cyst maturation of *C. nivalis*, where certain physiological processes may prepare for dormancy (the latter is needed in the field *e.g.* after the snow melt), was also found by Stibal *et al.* (2007). In comparison, *Tetracystis* sp. showed less photosynthetic and respiratory reduction after exposure; furthermore the F_v/F_m values showed a greater recovery overnight. The generally lower F_v/F_m levels of *C. nivalis* before the experiment can be explained by the strong light attenuation of the cytoplasmic secondary Cars, typical for these cells in a cyst stage, whereas the *Tetracystis* sp. population is still in the vegetative growing phase and

much less pigmented.

It was unexpected that the snow algae showed higher short-time impairment than the arctic soil algae, because *in situ* UV-B radiation values are considered to be higher in the mid-latitude Alps than in high-latitude coastal soil ecosystems. In general, both species can be regarded to be UV-tolerant, at least for a three-day period of elevated UV-B radiation, accompanied with realistic UV-A radiation and PAR levels. Xiong *et al*. (1999) reported that many algal strains are less tolerant and that they may respond quite differently to elevated UV-B radiation depending on their adaptation abilities. However, there are reports of freshwater and marine algae, which show quite good short-wave light adaptation if the experiments were performed close to field conditions, especially including sufficient PAR irradiation for photorepair (Holzinger and Lütz 2006). Alternatively, Thomas and Duval (1995) performed *in situ* measurements of photosynthesis of red and green snow. Compared to samples with exclusion of the ambient UV, the photosynthesis field samples decreased about 25% (red snow) and 85% (green snow).

To our knowledge, this is the first light-simulation experiment with microalgae from polar respectively alpine snow origin using realistic ambient light, where the spectral regions of PAR, UV-A and UV-B irradiance were accurately adapted to realistic outdoor conditions. The applied light intensity of 730 µmol m^{-2} s^{-1} PAR was in the range of normal high-light conditions at 79°N (own observations). Other authors exposed similar species also to additional UV irradiance, however not in a ratio to PAR which would approach native conditions. For example, Reisser and Houben (2001) exposed a *Tetracystis* isolated from aerophytic habitats to an elevated UV-B radiation of 3 mmol m^{-2} s^{-1}, which is approx. 1,200 W m^{-2}, for two hours. If their specification is correct, this corresponds to an unrealistic daily UV-B dose of 32 MJ m^{-2} d^{-1} (thousand-times our exposure value). The UV-A dose was not given and their value of PAR was 60 mmol m^{-2} s^{-1} (60,000 µmol m^{-2} s^{-1}). We assume that Reisser and Houben (2001) never worked in such conditions, instead with UV-B radiation of 3 µmol m^{-2} s^{-1} (approx. 1.2 W m^{-2}) and in low PAR of 60 µmol m^{-2} s^{-1} (approx. 13 W m^{-2}). Nevertheless, regarding this error, they performed their experiments in a very artificial environment: the ratio UV-B:PAR in energetic units (W m^{-2}) was about 1:11, contrasting our conditions of 1:300 (control) and 1:100 (exposure). The ratio of natural terrestrial solar radiation during a mid-latitude summer under clear sky yields an approximate noon value of 1:290 and exceeds greater values up to 1:100 in high mountain regions (Götz *et al.* 2010, own observations). By means of Chl fluorescence (maximum quantum yield of photosystem II, F_v/F_m), they did not measure any recovery of this alga after exposure, consequently a lethal dose was applied.

Similar to this study, Duval *et al*. (1999) collected red spores of *C. nivalis* from snow fields (Rocky Mountains, USA) for irradiation experiments. However, they used a 4-W Spectroline UV lamp providing either UV-A radiation (365 nm) or even harsh UV-C radiation (254 nm, UV-C radiation in general means radiation below 280 nm), each of 300 to 310 µW cm^{-2} effectively reaching the sample. The portion of UV-B radiation was not indicated, and PAR light was only used for control, not for exposed samples. UV-A irradiance was applied for the first 8 days and then they changed exclusively to UV-C radiation for another 7 days to measure any changes in the amount of soluble cellular compounds. By photometric assays, they reported the increase of a putative UV-screening pigment due to UV-C irradiation and suggested it to be a phenolic compound, but gave no further analytical evidence. Moreover, they reported that the use of UV-C radiation caused the cells to be completely bleached after 5 to 7 days of continuous exposure. The unnatural dose of UV-C radiation may have provoked intracellular artefacts due to degeneration of molecules. UV-C irradiance at 254 nm is a "killing wavelength" for any organism, resulting in unspecific cleavages of biomolecules; in nature, this wavelength region is absorbed by stratospheric ozone. In such an artificial light regime, nucleotides are changed and essential DNA-repair enzymes like photolyases cannot be activated, since they need blue light or UV-A radiation for their operation (Teranishi *et al.* 2008).

Many authors stress the importance of MAAs in UV protection of certain algae, and their abundance in phototrophic organisms is still under investigation (Karsten *et al.* 2007). Sommaruga and Garcia-Pichel (1999) found no MAA compounds in red spores from snow in the Austrian Alps (this is in accordance with own investigations of *C. nivalis* and *Tetracystis* sp., data not shown). Reisser and Houben (2001) detected neither MAAs nor sporopollenin (the latter as cell wall constituent) in *Tetracystis*.

The UV-shielding potential of *C. nivalis* was investigated by Gorton and Vogelmann (2003). They stated that the secondary carotenoid astaxanthin and a further, so far unknown pigment with an absorption maximum at 335 nm are responsible for UV-B protection in the cytoplasm, but indicated that also integral parts of the cell walls may play a significant role in protection. Accordingly, a spectral survey of the native Car isomers of astaxanthin by Remias and Lütz (2007) supported that they play a significant role particularly in UV-A and PAR reduction. At 320 nm, 13*Z cis*-astaxanthin still has 26% relative absorption compared to λ_{max} in the PAR region. Due to the very high abundance in *C. nivalis*, a shielding capacity of this cytoplasmic pigment also in the UV-B region can be assumed. Our attempts to find flavonoids or phenylpropane precursors, as they are common in higher plants from polar sites (Lütz *et al.* 2008), were negative.

C. nivalis as well as *Tetracystis* sp. showed an increase of secondary Cars during the three-day exposure

in this study. Probably these species, among many other members of the Chlorophyceae (Shick and Dunlap 2002), are not able to synthesize MAAs, instead they may compensate irradiation stress with a massive production of secondary Cars, which have a broad absorption wavelength range. Increased levels of Cars under elevated UV have been reported also for higher plants (Yang and Yao 2008). Our experiments indicate further that secondary Car biosynthesis is not reduced under elevated UV-B radiation. As a consequence, they can protect cells both against excessive PAR harmful for photosynthesis and against UV radiation harmful for many physiological processes. Nonetheless, Cars fulfil further physiological functions like quenching of reactive oxygen species (ROS), which may occur more frequently during irradiation stress. The comparable low percentage of secondary Cars of *Tetracystis* sp. during this survey compared to *C. nivalis* is caused by the fact that this species was almost in a "green state" due to culture conditions before the experiments. The relative content of secondary Cars (mainly canthaxanthin) in arctic field samples of this *Tetracystis* sp. from Spitsbergen was 65.1%, compared to other pigments, resulting in a dark orange colouration of this species *in situ*. Obviously, the three-day exposure was not long enough to exceed a ratio of 6%, thus secondary Car production may be quite slow and cannot be regarded as a short-time response, since pigment synthesis is a longer lasting process. Leya *et al*. (2009) showed for a variety of green algae, isolated from artic habitats, that high light and low nutrients induce an increase of secondary Cars, but again, their massive production required several days. It can be assumed, that microalgae of exposed habitats have to synthesize certain amounts of shielding pigments in advance of a potential harmful irradiation event, also described for snow algae by Bidigare *et al.* (1993). Similarly, Heo and Jeon (2009) described that the xanthophyll fucoxanthin, isolated from marine *Sargassum siliquastrum*, could efficiently protect human cells from UV-B induced oxidative stress.

In *Tetracystis* sp., the amount of plastid-bound pigments changed more drastically compared to *C. nivalis*: the control conditions caused a higher increase in primary Cars than in Chls (for protection of thylakoids), while additional UV-B irradiance resulted in a reduction of all plastid pigments. This indicates a structural reduction in the number of total thylakoid membranes of *Tetracystis* sp. In *C. nivalis*, the plastid pigments changed much less during the treatments, because the high amounts of secondary Cars attenuated the changes in the exposure light conditions. This attenuation explains why violaxanthin could be found as the only xanthophyll-cycle pigment, typical for low-light-adapted thylakoids (antheraxanthin and zeaxanthin were below detection limits).

However, our results are in contrast to MacIntyre *et al.* (2002), who reviewed that algae generally decrease their pigment content on a per-DM basis when irradiance increases.

Not only pigments, also structural adaptations of cells have to be considered when discussing how algae cope with UV irradiation. Holzinger & Lütz (2006) reviewed this aspect and showed that mature cysts of *C. nivalis* possess a thick cell wall, covered with inorganic particles (Lütz-Meindl and Lütz 2006), probably shielding the cells and provide inorganic nutrients in a harsh habitat like snow as well. However, such an additional structural shielding seems not to be developed for *Tetracystis* sp. Therefore, this species either must have developed still unknown UV protectants different from flavonoid-like or mycosporin-like compounds, or the repair mechanisms (*e.g.* photolyases, *see* Björn 2008) may work with very high effectiveness.

The long-distance distribution of terrestrial algae, originating from Antarctica, was studied by Marshall and Chalmers (1997). It is an amazing result how many different species had been found as propagules collected from air, including snow algae cysts. During atmospheric transport, the cysts are exposed to much higher UV doses than at the collection sites in the European Alps or even at high latitude origins. In this study, the impaired, but not inhibited photosynthetic activity under high UV irradiance was measured in the liquid phase, while airborne distribution will mostly occur in a dry state of propagules, which should be much more resistant against UV radiation (Crawford 2008).

This study under realistic UV and VIS radiation spectra and close-to-nature climatic conditions has shown that an arctic soil- and an alpine snow algal species can cope with the temporary substantially higher UV irradiation. The reduction in photosynthesis and the increase of secondary pigments suggests a trade-off between autotrophic productivity on the one side and UV-protection of the cells on the other side.

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