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Adaptation of an Antarctic lichen to Martian niche conditions can occur within 34 days

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ABSTRACT

Stresses occurring on the Martian surface were simulated in a Mars Simulation Chamber (MSC) and included high UV fluxes (Zarnecki and Catling, 2002), low temperatures, low water activity, high atmospheric CO_2 concentrations, and an atmospheric pressure of about 800 Pa (Kasting, 1991; Head et al., 2003). The lichen *Pleopsidium chlorophanum* is an extremophile that lives in very cold, dry, high-altitude habitats, which are Earth's best approximation of the Martian surface. Samples with *P. chlorophanum* were exposed uninterruptedly to simulated conditions of the unprotected Martian surface (i.e. 6344 kJ m^{-2}) and protected niche conditions (269 kJ m^{-2}) for 34 days. Under unprotected Martian surface conditions the fungal symbiont decreases its metabolic activity and it was unclear if the algal symbiont of the lichen was still actively photosynthesizing. However, under "protected site" conditions, the entire lichen not only survived and remained photosynthetically active, it even adapted physiologically by increasing its photosynthetic activity over 34 days.

1. Introduction

Life could have evolved early in Mars' history, when the planet was warmer and wetter than today, and then retreated to protected (micro-) habitats as Mars became progressively colder and dryer. Likely protected Martian micro-environments which might harbor life include (a) liquid water beneath or in ice, (b) subterraneous aqueous reservoirs (perhaps with elevated heat flow), and (c) openings within rocks [e.g., cracks, fissures, lava tubes, caves (Schulze-Makuch et al., 2005)]. We studied the psychrophilic lichen *Pleopsidium chlorophanum* (Castello, 2003), because it lives in Earth's most Mars-like environmental conditions (low temperatures, high UV fluxes, dryness; see Fig. 1). *P. chlorophanum* preferentially colonizes granites and volcanic rocks of North Victoria Land (Antarctica), at up to 2000 m altitude (Guglielmin et al., 2011). It is mainly found in fissures and cracks, but also on the surfaces of rocks (Fig. 1a–f). The strategy of living in certain specific habitats—especially fissures and cracks—is probably adaptive behavior to protect against desiccation and high UV-fluxes, where just a small amount of scattered photosynthetically active radiation (PAR) can reach the organisms, thus allowing photosynthesis (Fig. 1e and f showing environmental data which were taken in parallel to data of photosynthetic activity of the lichen in niche areas what is shown in Fig. 5a expressed by the column "field conditions: niche site"). The lichen can also resist both temperatures ⩽0 °C, and low water activity (Fig. 1f), as do many species of polar lichens, which remain metabolically active at −17 to −20 °C and can absorb small amounts of liquid water in a snow- and ice-rich environment (Kappen et al., 1996). Extremophilic organisms from various Earth environments have been previously exposed to simulated Martian environmental conditions to study their survival rates and survival strategies (Morozova et al., 2007; Schuerger et al., 2003; Osman et al., 2008; Diaz and Schulze-Makuch, 2006). However, most such experiments were quite short in duration [e.g., 4–7 days (Berry et al., 2010; de Vera et al., 2010; Green et al., 1971)], while ours ran for 34 days, to more closely approximate the "Martian real-world".

2. Material and methods

2.1. The lichen Pleopsidium chlorophanum

The lichen *P. chlorophanum* has been collected at an altitude of 1492 m above sea level at the location "Black Ridge" in the North Victoria Land, Antarctica during the German North Victoria Land Expedition (GANOVEX 10, GPS: 74°23.254′S 163°40.378′E). The lichen was stored at −20 °C before been used in the experiment.

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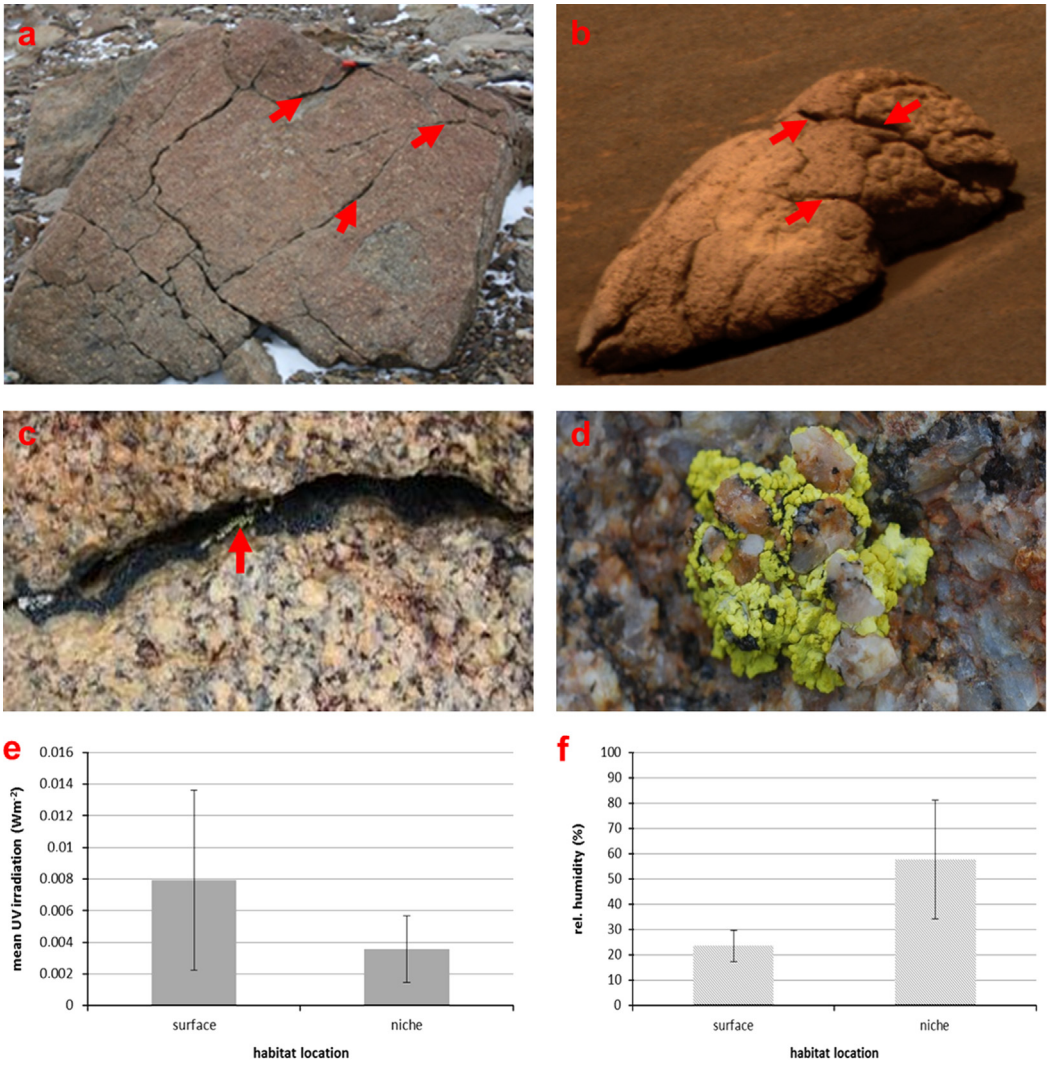

Fig. 1. Characterization of habitat niches of the lichen *P. chlorophanum* in Antarctica and possible niches on Mars. (a) Fissures and cracks as micro-niches (red arrows) in a rock (picture taken in characterized Mars-analog field site in the "Black Ridge Mountains" of North Victoria Land/Antarctica). (b) Fissures and cracks as potential micro-niches (red arrows) in the well-studied rock "Wopmay" within the Endurance Crater on Mars (NASA/JPL(Cornell)). (c) Part of the rock (in (a)) enlarged: a fissure is colonized by organisms in a biofilm, and by numerous lichens, e.g. *P. chlorophanum* (red arrow). (d) *P. chlorophanum* grown on the surface of granite rock. Some areas of the surface are showing semi-endolithic growth of the lichen where rocks are fragmented by the lichen due to increase of its size. The pieces of granite are partly covering the lichen possibly offering protection against UV irradiation. (e) Differences of UV-irradiation in the micro-habitat colonized by the lichen *P. chlorophanum*. The measurements were performed during the summer season (circumpolar sun cycle) in the Black Ridge Mountains (North Victoria Land/Antarctica) during the time where the Sun has its right angle directly positioned above the micro-niche and above the neighbor surface area of the rock with a measurement time of 3 h per sample. The protection within micro-niches against UV irradiation gets obvious. (f) Differences of humidity in the micro-niche versus the rock's surface macro-niche: micro-niche→rh (mean relative humidity)= 57, 79% [measured within temperature range of T=267 K (−6 °C) to 265 K (−8 °C)]; macro-habitat→mean rh=23, 48% [T=281 K (8 °C) to 265 K (−8 °C)].

Before the simulation the lichen was removed from the granite rocks and three lichen samples were prepared for *in situ* measurements under Mars-like niche conditions and three additional samples were prepared for Mars-like surface conditions with exposure to the entire simulated irradiation spectrum. The samples were embedded in a Mars analog soil mineral mixture (S-MRS, Table 2) as described later in Section 2.9 and 2 ml of distilled water was provided for each of the tested samples into the soil before the simulation experiment.

2.2. Mars-like environmental conditions

P. chlorophanum was held in the Mars simulation chamber (MSC) for 34 days, uninterrupted. The MSC atmosphere was 95% CO_2, 4% N_2, and 1% O_2, at 800 Pa (see Kasting et al., 1991), with a diurnal cycling of relative humidity between 0.1% and 75% (Fig. 2, Table 1) and diurnal temperature cycling of 294 K (+21 °C) to 223 K (−50 °C), like temperatures observed in equatorial to mid-latitudes on Mars (McEwen et al., 2011; Head et al., 2003). Lichen samples were embedded in a Mars analog mineral mixture (S-MRS, Table 2) and three of the six lichen samples were also exposed to Xenon lamp radiation that simulated the complete Martian solar spectrum (Fig. 3). Details and information of the MSC and S-MRS are described in Sections 2.3–2.9.

2.3. Experimental description

The experiment was carried out at the Mars Simulation Facility (MSF) of the DLR Institute of Planetary Research in Berlin. The MSF is part of the Department of Experimental Planetary Physics and is used to perform laboratory experiments with controlled time-profiles (e.g., simulated diurnal variations) of temperature down to about 198 K

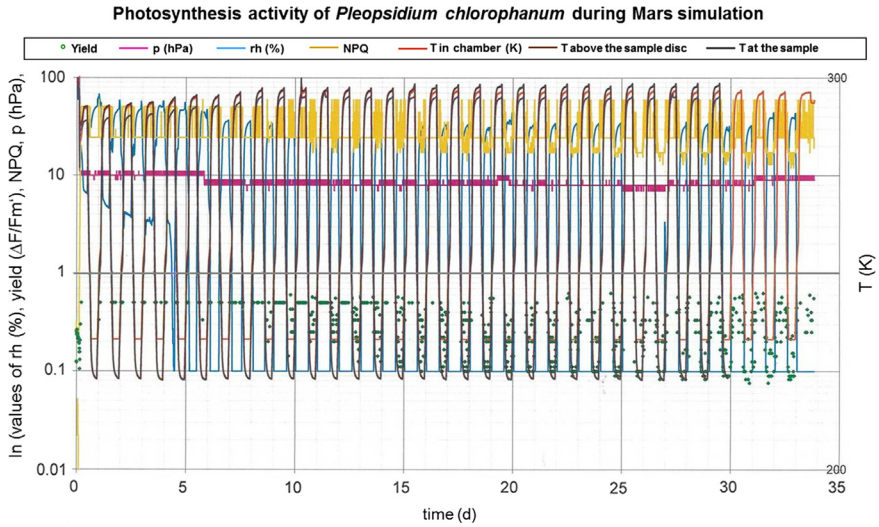

Fig. 2. The protocol of the environmental data during the whole test series given as representative data set. Data collected and monitored in an overview profile during the 34-day simulation. Pink=pressure, blue=humidity, red=temperature at three positions (chamber (10 cm above the sample), 1 cm above the sample, and directly at the sample), green=PS II activity, and yellow=NPQ/stress values.

Table 1
Experimental parameters and measurement categories as provided in the experiment.

Measurement category	Trace humidity in gases, relative humidity/water activity, pressure, temperature, photosynthetic activity, gas analyses
Ranges of experimental parameters	
Relative humidity	0.1–75%
Pressure	Mean pressure p=800 Pa
Temperature (climate chamber)	223–294 K (above sample)
Gas mixture (GMS)	Three gases mixed (95% CO_2, 4% N_2, and 1% O_2, as best approximation to Mars-like conditions)
Controlled time-profiles	Humidity, gas mixture, temperature, pressure, LED-illumination for Mars-like niche conditions with scattered UV light (final dose of 269 kJ m^{-2}), UV-irradiation for Mars-like surface conditions (final dose of 6344 kJ m^{-2})
Irradiation with Xenon lamp via fiber (inside the experimental chamber)	Spectral range from 200 nm to 2200 nm on a 13 mm diameter spot (cf. Fig. 2a)

Table 2
Mineralogical composition of Sulfatic Mars Regolith Simulant (S-MRS) in weight percent [wt%].

Component	S-MRS [wt%]
Pyroxene, plagioclase, amphibole, ilmenite (gabbro)	32
Olivine	15
Quartz	3
Hematite	13
Goethite	7
Gypsum	30

(−75 °C). Atmospheric pressure and composition (including humidity) can be modified to simulate conditions on Mars. In particular, the MSF can be set for thermo-physical conditions typical of Martian mid- and low latitudes. The MSF's measurement categories and controllable parameters are summarized in Table 1.

2.4. Description of experiment equipment

The main part of the MSF is a climate chamber (CC) with inside dimensions of 80 cm height, 60 cm depth, and 50 cm width. The experiments were performed in the "experimental chamber" (EC: inside the climate chamber), which can be cooled separately from the CC. The EC is of stainless steel, forming a cylinder with a volume of 10.3 L with an inner diameter of 20.1 cm and an inner height of 32.4 cm. There are connections through the top plate for gas flows and electrical contacts (50 pins each in two D-Sub connectors; 100 total) inside the top plate to connect to internal sensors or devices.

A "Gas-mixing system" (GMS), which includes controls for humidity, and a PC-based data and control unit completes the system. The GMS was developed to simulate planetary atmospheric conditions with respect to varying compositions and amounts of gases. The equipment is computer controlled to actively selected dew points (198–283 K), gas mixing rates, and flow rates. The experimental setup allows the mixing of five gases, at three flow rates (1.5–75 l/h at standard ambient temperature and pressure). Six flow controllers regulate the flow of the different gases into the GMS. The gases are mixed in a pipe system and can be hydrated in a wash bottle. Mass flow controllers regulate the flow of the (moistened) mixture. Air or any other gas can be used as a carrier. Air is provided by a compressor and dried out in two steps at a frost point temperature of 199 K (−74 °C) using a permeation dryer. The other gases are bottled. Humidity, which can be directly provided, corresponds to a partial water vapor pressure of about or slightly less than 0.15 Pa at 101,325 Pa and 0.00113 Pa at 800 Pa, to approximate the mean water vapor pressure on Mars. Two membrane vacuum pumps regulate pressure (from 200 to 101,325 Pa). All experimental parameters and data are PC-controlled and logged (using LabView). Fig. 4 is a schematic of the system.

2.5. Configuration inside the experimental chamber

Six biological samples were placed inside the chamber (Fig. 4a–c). Three of the six samples were placed in a Petri-dish on a sample stage

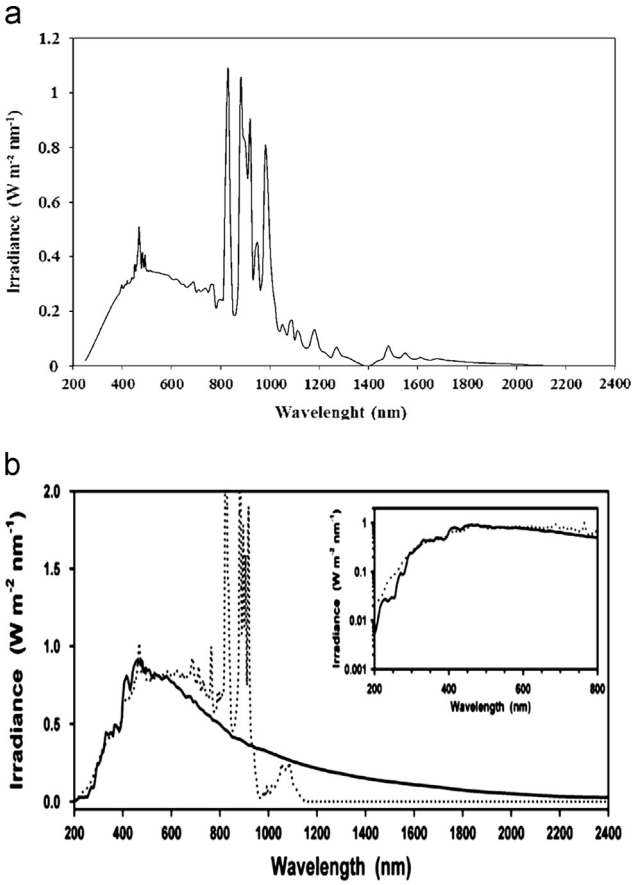

Fig. 3. (a) Irradiance of the spectra range of 150 W Xenon lamp measured at the light fiber optic. A 6.6 A current was used to get this irradiation. The distance of the fiber optic during measurements was 7.5 cm from the sample site. The calibration was performed at the Kalibrierlabor für optische Strahlungsmessgrößen der Gigahertz-Optik GmbH (test equipment is reducible to national and/or international normal). (b) Mars spectrum (Schuerger et al., 2003) for comparison to the applied lamp in (a).

and exposed to Mars-like surface radiation (see also Zarnecki and Catling, 2002) produced by the Xenon lamp (Fig. 4b). The other three were placed in a sample holder exposed to the Martian atmosphere with LED-PAR/UV B radiation and scattered Mars-like radiation from the Xenon lamp (Fig. 4c), thus simulating Martian "physical-niche" conditions. The sample in the sample holder was 1 cm from the photosynthesis-sensor of the Mini-PAM, which emitted actinic light during measurements of photosynthetic activity and detected the resulting fluorescence of each of the three photosynthetically active samples. Three additional sensors were also arranged inside the chamber at the sample holder, two Pt100 temperature sensors and one capacitive humidity sensor. The humidity sensor and a Pt100 were attached close to the biological samples (1–2 cm, Fig. 4c). The second Pt100 was affixed in the middle of the chamber (Fig. 4b).

2.6. Implementation of the experiment

At the beginning of the experiment a bowl of water was left to evaporate in the chamber (see Figs. 2 and 4). This water delivered additional humidity on the first days of the experiment. After about 6 days, the water in the bowl evaporated completely and the humidity was solely delivered by the gas flow of 10 l/h. The constant mixed-gas flow was 95% CO_2/vol and 5% air/vol (4% N_2/vol and 1% O_2/vol). The humidity of the gas flow was ca. 270 ± 2 K (-3 ± 2 °C) frost point temperature at 101,325 Pa (which coincides with a frost point temperature of ~222 K (−51 °C) at 800 Pa inside the chamber) over the entire experiment (34 days) with one exception: between the 25th and 27th day the CO_2 gas bottle was empty and therefore had to be changed (only the dry air gas flow of 0.5 l/h was inserted with a frost point temperature of ca. 173 K (−100 °C) at 800 Pa (see Fig. 4)). This decrease of the humidity could have led to the described stress reaction of the samples.

Pressure in the experimental chamber was ~1000 Pa during the first 6 days and after the evaporation of water in the bowl the pressure decreased to about 800 Pa over the rest of the experiment's life. The temperature was varied in a diurnal cycle between 223 K (−50 °C) at night and 294 K (21 °C) at day time with corresponding slopes and decays of the curve.

2.7. Irradiation time of the samples

The LED-unit was activated for 16 h and switched off 8 h every day to simulate the diurnal cycle of the Sun. In the same time range the Xenon lamp was switched on and off with the exception that on weekends the UV-lamp remained off, because only manual operation was possible. Total radiation dose was 6344 kJ m^{-2}(Mars-like surface conditions) and 269 kJ m^{-2} (protected niche conditions). The photosynthetic activity of the lichen sample was measured every 10 min by the Mini-PAM instrument (see Section 2.11).

2.8. Mars analog mineral mixture "Sulfatic Mars Regolith Simulant" (S-MRS)

Sulfatic Mars Regolith Simulant (S-MRS, Table 2) for the Mars simulation experiments was provided by Dr. Jörg Fritz of the Naturkundemuseum Berlin. The mineral mixture reflects current understanding regarding environmental changes from a phyllosilicate rich deposit- phase to a more sulfate-rich epoch on Mars (Poulet et al., 2005). The minerals and rocks were chosen to be structurally and chemically similar to those identified in Martian meteorites (McSween, 1994) and by recent Mars orbiter and rover missions (Bibring et al., 2005; Chevrier and Mathé, 2007; Morris et al., 2010; Poulet et al., 2005), which are giving insights into weathering and hydrothermal alteration of crustal rocks, and about secondary mineralization during part of the Noachian and Hesperian epoch followed by the prevailing cold and dry oxidizing conditions with formation of anhydrous iron oxides.

S-MRS contains igneous rocks with a mineral composition similar to those of Martian meteorites, e.g. composed mainly of pyroxene and plagioclase (gabbro), and olivine. Both quartz and anhydrous iron oxide hematite (Fe_2O_3) were added. Hematite forms under various environmental conditions and is the only thermodynamically stable iron oxide under present Martian conditions (Gooding, 1978).

The S-MRS serves as an analog for a more acidic environment with sulfate deposits. In addition to igneous rocks and anhydrous iron oxides it includes goethite [α-FeOOH] and gypsum [$CaSO_4 + 2H_2O$].

The materials were crushed to obtain a grain size distribution like that of mechanically fragmented regolith. Only fragments < 1 mm were used. The components were stirred thoroughly, and particulate size distribution was measured by sieving.

2.9. Dosimeter

The Optometer X92, a UV Curing Irradiance Meter of Gigahertz-Optik with a RCH-106-UV-Curing detector head was used for measurements on UV fluxes and doses. It is a UV-broadband irradiance meter (250–400 nm), Typ. Sensitivity: 0.3 nA/mW/cm^2 and is able to measure at a maximal irradiance of 35,000 mW/cm^2. The sensor measured to the same level of accuracy for both sample

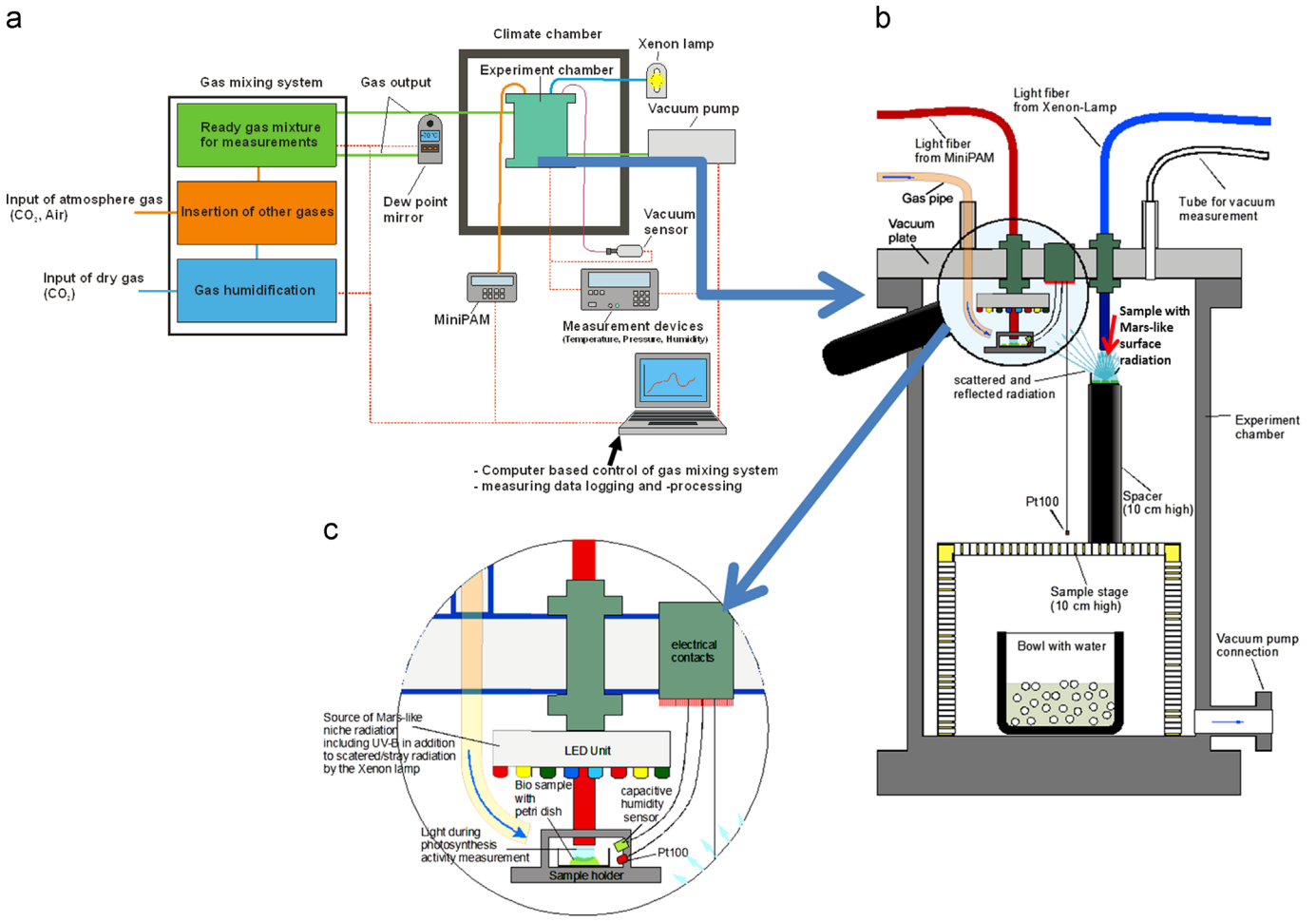

Fig. 4. (a) Experiment configuration of the biological experiment. (b) Experiment arrangement inside the experiment chamber showing sample areas with Mars-like surface conditions and samples placed in Mars-like niche conditions. (c) Magnified insert from (b) showing the area of simulation realizing Mars-like niche conditions.

devices (Petri-dish on spacer under the Xe-lamp and sample holder under the LED light source; see Fig. 4).

2.10. Measurements of photosynthetic activity

Photosynthetic activity was inferred from the fluorescence behavior of chlorophyll-a as an indication of the activity of Photosystem II (Ben-Haim et al., 1999; de la Torre et al., 2010; Fleck et al., 1998; Sancho et al., 2007). We used a Mini-PAM instrument (Heinz WALZ GmbH, Effeltrich, Germany), which measures fluorescence and expresses rates (Y) via the following equation:

$$\text{for light-adapted lichen}: \quad Y = (Fm' - F)/Fm' = \Delta F/Fm', \tag{1}$$

and

$$\text{for dark adapted lichen}: \quad Y = Fv/Fm, \tag{2}$$

where F (day time) and Fv (night time) are the natural fluorescences of the sample measured briefly before the saturation pulse is triggered by the Mini-PAM device. Fm is the maximum fluorescence value measured after dark adaptation. Fm′ is the maximum fluorescence reached during the saturation pulse, as measured under light conditions when all reaction centers of Photosystem II are open (i.e., equivalent to oxidation of all primary electron acceptors). By incorporating the effects of Fm or Fm′ and Fv or F (according to light conditions) one obtains a calculated effective rate of photochemical energy conversion, displayed on the Mini-PAM as a "yield" value (Y). Heat dissipation during this process is minimal and monitored as low NPQ values expressing the low degree of stress reaction during non-photochemical quenching. It is calculated thus by

$$NPQ = Fm - Fm'/Fm \tag{3}$$

2.11. Confocal laser scanning microscopy (CLSM) imaging: viability of P. chlorophanum analyzed by LIVE/DEAD staining kit FUN 1

Adult lichen thallus and young thallus of *P. chlorophanum* were stained by FUN 1 to determine their viability. A green and yellow color of the cells indicates that they still maintain vitality. A change from green to yellow in the cytoplasm and from green to red in the vacuoles is an indication of physiological activity expressed by accumulation of the dye in the vacuoles. Dead cells cannot be stained; therefore, red crystals are not formed in the vacuoles. Instruments used for imaging are as follows: TCS SP5 of Leica Microsystems Heidelberg GmbH with objective lenses $20\times$/oil immersion, scanning resolution 1024×1024 pixels and time 60.6 μs, scan zoom 1.0, image bit depth 8 bit, experimental temperature condition 20 °C, UV-laser diode with excitation wavelength 405 nm, Argon-VIS-laser, DPSS-561-VIS-Laser with 561 nm excitation, HeNe 633-VIS-Laser with 633 nm excitation and 5 mW power, and filters with emission bandwidth PMT 2: begin 510–end 610 nm. For operation of the instrument, the Leica Microsystems software at the Institute of Environmental Technology in the department of Environmental Microbiology was used.

3. Results and discussion

3.1. Photosynthetic activity of P. chlorophanum

Measurement of the irradiation, relative humidity and temperature on the original habitat of *P. chlorophanum* of the Mars-analog field site in Antarctica (Fig. 1a–f) clearly showed differences between surface and niche conditions of the investigated rocks. The irradiation on the surface of the rock was about twice to three times higher if taken into account the standard deviation (Fig. 1e) compared to the conditions we have observed in the niches, as there are the fissures, fractures and micro-caves of the rock which were colonized besides other micro-organisms by the investigated lichen *P. chlorophanum*. Because of this reason, a differentiation between Mars-like surface and Mars-like niche conditions was tested during the Mars simulation experiments.

Irradiation experiment #1 was designed to reach a final cumulative radiation dose of 6344 kJ m^{-2} (with a spectral irradiance of 0,4 W/m^2 nm), equivalent to normal (that is, fully-exposed, unprotected) surface conditions in Mars' equatorial regions if exposed 1 month to Mars-like irradiation. Photosynthetic activity dropped to 17.5% of pre-experiment levels (~37.2% of that measured in Antarctica; Fig. 5a). The photosynthetic yield value was $Y < 0.1$ (within the 95% error limits), thus raising doubts whether the lichen was effectively active, photosynthetically.

In experiment #2, the lichen was exposed to a "protected environment", with a cumulative dose of ~269 kJ m^{-2}, representing the low radiation dose that would be encountered by the lichen in near surface semi-protected loci (e.g., fissures and cracks in rocks; polygon-rich permafrost soil), Under "protected" conditions, the lichen increased its photosynthetic activity by 17% over that measured in the field in Antarctica (Fig. 5a–c). But those experimental values were 45% lower than those measured in the lab (pre-experiment) under control conditions (T=25 °C, PAR light 131.67 μmol quanta m^{-2} s^{-1} during 2 h/no Mars spectrum).

Photosynthetic activity did not remain constant during the protected experiment—rather, it increased over the 34 days as evidenced by increasing rates ($\Delta F/Fm' \geq 0.1$, i.e., 1/3 of the rate found during field studies). The increase continued up to the experiment's end (day 34: Fig. 6a). Also, while the lichen attained its maximum yields for only 20–30 min at the beginning of the experiment, towards the end of the experiment the maxima tended to be stable for 2–3.4 h, clearly suggesting considerable (and rapid) physiological (photosynthetic) adaptation by the lichen to the simulated "semi-protected" conditions. There did seem to be something like a period of initial "shock" (see Fig. 6a), which the lichen quickly ($< \sim 7$ days) overcame. The reality of this "shock" effect has other support: the calculated and monitored NPQ values, expressing shock-effects or stress-effects where the energy is ineffectively captured by chlorophyll and transformed directly into heat (Guralnick et al., 1992; Herzog et al., 1999; Scholes and Rolfe, 2009) rather than being used in photosynthesis decreased over time (Fig. 6b) as the lichen adapted to its new environment. [note—NPQ=Fm−Fm′/Fm (Logan et al., 2007; Walz, 1999)].

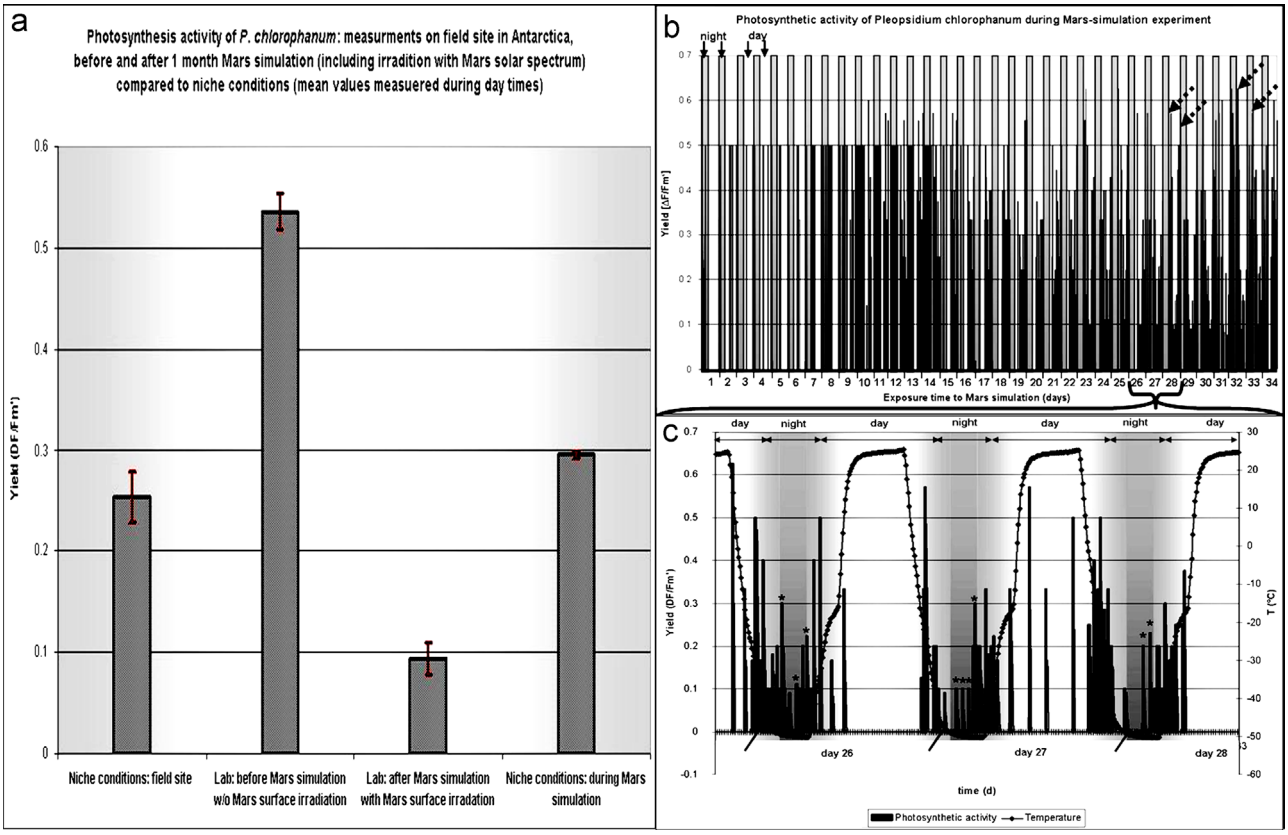

Fig. 5. Complete data set for *P. chlorophanum* (including Photosystem II activity) as collected during Mars simulation in the Mars simulation lab. (a) Photosynthetic activity (PS II) of *P. chlorophanum* (data from the field, lab, before and after the simulation, and mean values from the simulations). PS II activity is clearly reduced when the lichen is exposed directly to surface irradiation, but is not reduced in surface niches (in fact, it is higher than measured in the species' normal Antarctic habitat). (b) One-month profile of the same experiment showing night and day cycles (white and grayish areas, respectively) and PS II activity (dark black columns). The arrows indicate the highest $\Delta F/Fm'$ values (at the beginnings and ends of simulated Martian days). (c) Detail of (b) after the lichen was accidentally even more strongly stressed (at day 26) with very low humidity (rh=0.1%) due to temporary problems with the gas supply and humidity control. Normal photosynthetic activity re-started soon after humidity returned to "Mars normal" on day 27. Highest rates (yield values) occur at the end and at the beginning of the day. (the day 27 anomaly is explained by humidity stress: see Fig. 4). Most photosynthetic activity occurs at 233 K (−40 °C) to 258 K (−15 °C). Bars marked by (*) show a cessation of photosynthesis (i.e., dark control values) induced by the saturation impulse from Mini-PAM. The values are lower than observed during starting day times because of the very low temperatures (~223 K/−50 °C).

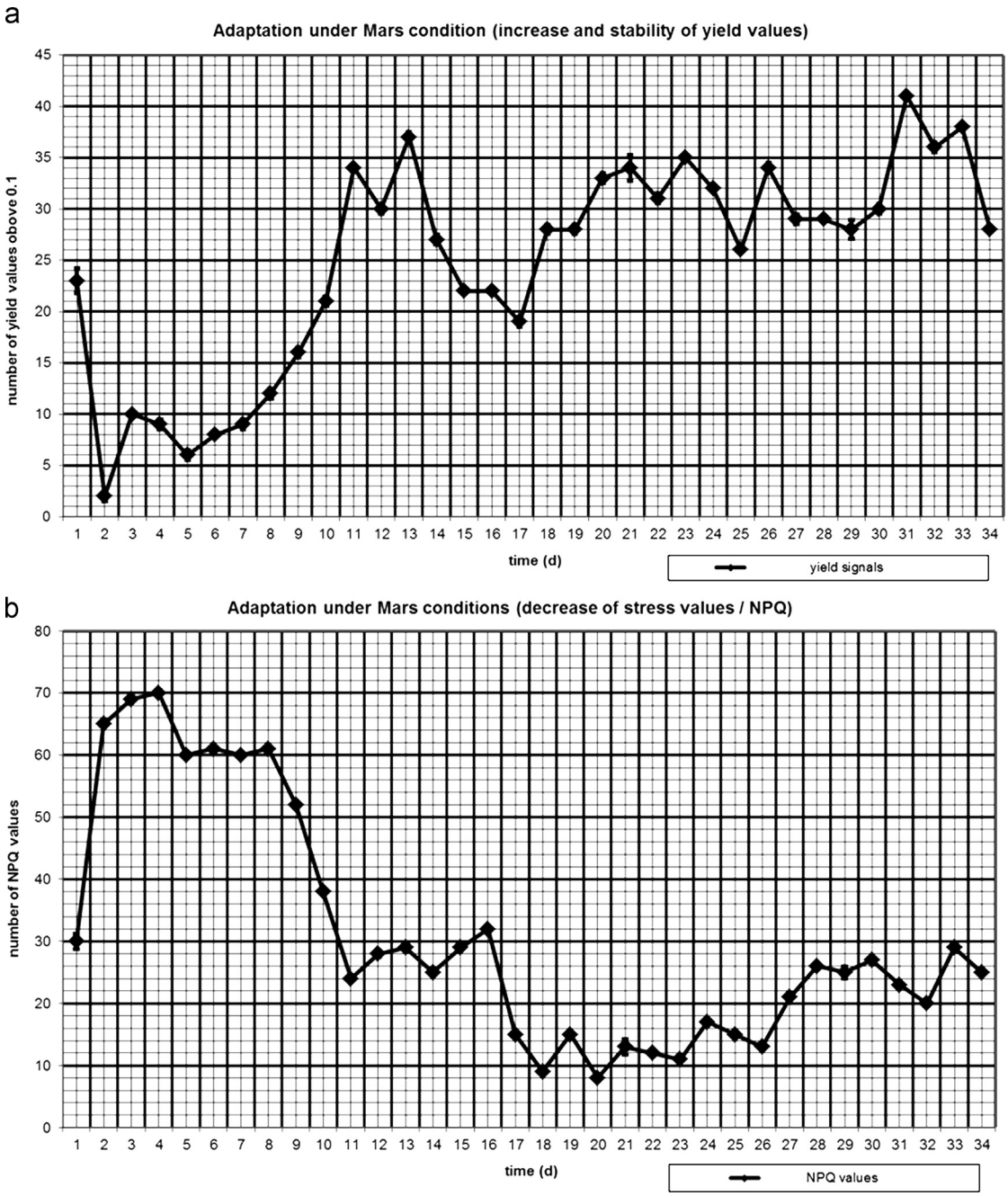

Fig. 6. Adaptation of *P. chlorophanum*: increase of photosynthesis and decrease of stress values (NPQ). (a) The number of photosynthetic rates >0.1 (which rate is taken as a critical value of effective photosynthesis in *P. chlorophanum*), plotted across the 34-day experiment. An increase of PS II activity with time is clear. (b) NPQ "stress" values plotted across the 34-day experiment: note the decrease of stress over time.

The lichen apparently was able to optimize its photosynthetic activity to Mars-like surface conditions remarkably quickly. We found a strong correlation of photosynthetic activity (as $Y=\Delta F/Fm'$) with the humidity and temperatures at the beginning and end of simulated Martian days (e.g., just after the light switched on and before the light switched off: Fig. 5b and c), which correspond to the highest photosynthetic rates. At those times, condensing and adsorbed atmospheric water in the soil might be available to the lichen as liquid solution probably forming also cryobrines in the surrounding Mars analog soil (Möhlmann, 2010; Möhlmann and Thomsen, 2011) near the mineral surface. The liquid phase of water was also very likely present in the soil because of the pressure of 700–800 Pa used in the Mars simulation experiment. This pressure was above the triple point of pure water at 600 Pa and was also currently confirmed by measurements performed on the Mars rover Curiosity in the Gale crater (see NASA/JPL-Caltech/CAB(CSIC-INTA), 2013).

3.2. *Viability of P. chlorophanum stained with LIVE/DEAD staining kit FUN 1 and analyzed by CLSM*

Differences were notable after screening for vitality of the lichen if compared between the lab control, Mars-like niche and Mars-like surface conditions. The three samples of the control (Fig. 7a–c) indicated that 2/3 to the entire lichen thalli were stained green, yellow and red by the Live-marker FUN 1 including algal cells (photobiont) and fungal cells (mycobiont). No differences occurred between adult thallus (Fig. 7a), subdividing thalli on old dead unstained lichen cell material and rocky substrate

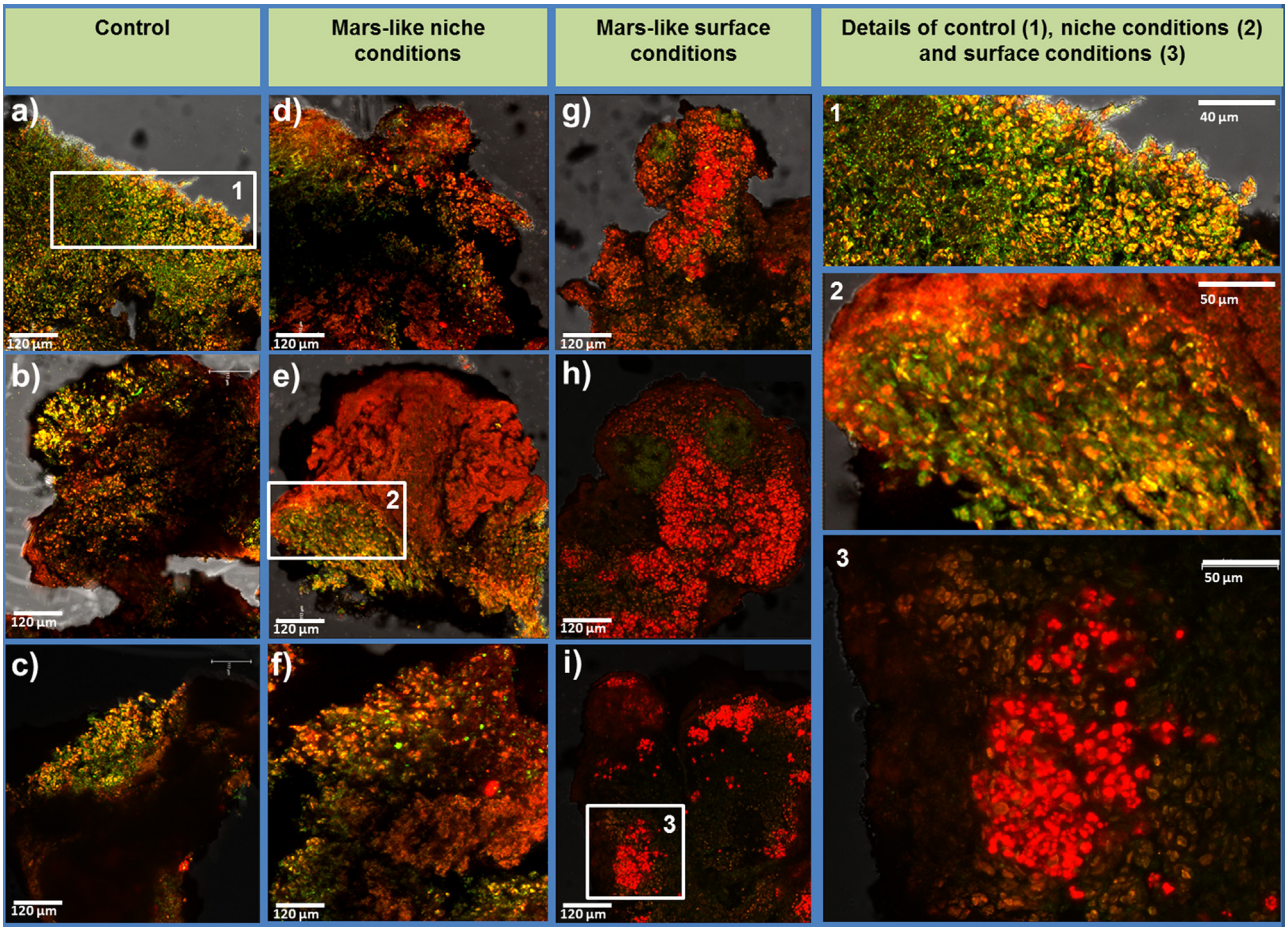

Fig. 7. CLSM-images of cross-sections through the investigated lichen of Pleopsidium chlorophanum stained by LIVE/DEAD-staining dye FUN I. Cells stained green are vital but not metabolic active. Cells stained yellow are metabolic active and changing from green to red color. Cells stained red are metabolic active and have transferred the staining dye into red fluorescing crystals within the vacuoles. (a)–(c), Control samples with (a) adult thallus, (b) adult thallus on substrate consisting of dead cells and which is subdividing into two areolic thalli, and (c) young thallus on dead cells of previously developed lichen generation. (d)–(f) Different thalli of different samples of the lichen exposed to Mars-like niche conditions. No significant differences in vitality occur if compared to the control. (d) Adult thallus producing areols (sub-thalli), (e) cross-section of adult thallus (image in pseudo-3D), and (f) young thallus on dead cell material of previously formed lichen generation. (g)–(i) Different thalli of different samples of the lichen exposed to Mars-like surface conditions. The samples are much more affected and mainly the fungal cells are dead and the majority of the algal cells are vital (red colored cells). (g)–(h) Two adult thalli of the lichen where mainly algal cells are colored by FUN 1 indicating vitality and the surrounding fungal cells in close contact to the alga. (i) Little young areols of the lichen on old dead lichen material. The majority of algal cells and a few fungal cells in between the algal packages are vital (see red colored cells). (1)–(3) Details of (a)–(i), (1) and (2) adult thalli clearly showing the maintained vitality of control- and exposed samples to Mars-like niche conditions respectively. (3) Thallus of a young lichen areole exposed to Mars-like surface conditions with red colored vital algal cells and a few red colored vital fungal cells in close connection to the alga. The majority of the fungal cells are dead and affected by the Mars-like surface conditions.

(Fig. 7b) and young thallus (Fig. 7c). The green color indicated vital but not metabolic active cells; the yellow color showed the metabolic activity of the cells changing from green to red. The red color was the final state of metabolic activity where accumulation of the dye in the vacuoles by crystallizing in bright red colored crystals occurred (Fig 7.1 as detail of Fig. 7a). By comparing the control samples with the samples exposed to Mars-like niche conditions, no significant damage has been observed. The subdividing adult thallus (Fig. 7d) which had grown on unstained dead cell material substrate as well as the adult thallus taken as a picture of pseudo-3D with showing the cross-section and the view to the surface covered with red auto-fluorescent secondary lichen metabolite (Fig. 7e) showed also that 2/3 of the thalli was still vital and metabolic active. In Fig. 7.2 a picture is showing the cross-section area as a detail of Fig. 7e. The cells were stained green and mainly yellow to red by FUN 1. The same was obvious for a young thallus growing on dead pseudo-tissue structures of an older previously formed layer of lichen thalli (Fig. 7f).

In contrast to these findings the lichen thalli which were exposed to Mars-like surface conditions have lost significantly their vitality. Between 1/3 and 1/2 of the thallus was vital and the rest of the associated lichen symbionts died. There was no clear difference in the status of vitality of old and subdividing thalli (Fig. 7g and h) if compared to young thalli (Fig. 7i). A detail of Fig. 7i shows that the majority of the fungal cells were not stained indicating they are dead. But the majority of the algal cells and a few fungal cells closely connected to the algal cell clusters were still vital (detail of Fig. 7i (3)). The analysis of the CLSM-images of the investigated lichens might lead to the conclusion that the alga embedded and surrounded by the fungus are much more protected and therefore less affected if compared to the fungal cells which are directly exposed to the Mars-like surface conditions. The fungus seems to be much more sensitive to UV and Mars-like solar irradiation. Other Mars-like parameter such as high CO_2 concentration, very low temperatures and extreme humidity fluctuations because of extreme periodicity changing with diurnal long desiccation times seemed not to influence the degree of the fungus' activity (see niche conditions Fig. 7d–f). Because the fungus is an aerobe heterotroph organism its maintained vitality during exposure to Mars-like niche conditions was remarkable,

because in an environment saturated with 95% CO_2 atmosphere it might not survive. The same results were previously observed during investigations performed with the lichen *Xanthoria elegans* (de Vera et al., 2010). An explanation could be that the still photosynthesizing and metabolizing algae within the lichen thallus, as shown by the performed fluorescence measurements on photosynthetic activity and by the vitality check, were still able to produce oxygen what could have been directly provided to the closely connected fungus. Therefore it is also comprehensive that the less radiation affected vital fungal cells were observed in locations close to the algal cells (Fig. 7i, 3).

The two combined methods are very good tools and provide high confidence in the results when an oxygen sensor cannot be employed to measure for this specific by-product of photosynthesis, either because of technical reasons or because tests are conducted with a lichen where any oxygen that is produced by the algal component will immediately be consumed by the fungal component of the organism.

4. Conclusion

This work strongly supports the interconnected notions (i) that terrestrial life most likely can adapt physiologically to live on Mars (hence justifying stringent measures to prevent human activities from contaminating/infecting Mars with terrestrial organisms); (ii) that in searching for extant life on Mars we should focus on “protected putative habitats”; and (iii) that early-originating (Noachian Period) indigenous Martian life might still survive in such micro-niches despite Mars' cooling and drying during the last 4 billion years.

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