Fast Reactivation by High Air Humidity and Photosynthetic Performance of Alpine Lichens Growing Endolithically in Limestone

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Fast Reactivation by High Air Humidity and Photosynthetic Performance of Alpine Lichens Growing Endolithically in Limestone

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Abstract

This paper is dedicated to Otto Ludwig Lange on the occasion of his 80th birthday.

Hymenelia prevostii and *H. coerulea* are common endolithic lichen species on limestone outcrops in the eastern alpine mountains. We investigated their photosynthetic properties under controlled laboratory conditions and analyzed them with respect to relevant meso- and macroclimatic parameters to determine specific adaptations to the extreme habitat.

For the first time, it can be shown that endolithically living lichens are able to utilize water vapor alone to gain positive net photosynthesis (NP) rates. With respect to liquid water, both species reached optimum NP rates at low water content (*H. prevostii*: 0.26 mm, *H. coerulea*: 0.07 mm rainfall equivalent), and NP was increasingly depressed at water suprasaturation. In their preferred microhabitat on steep limestone outcrops in alpine regions, endolithic lichens avoid inundation over long time spans, but could perfectly utilize the high air humidity of monthly means between 60 and 80%. Due to light reduction by endolithic growth, both species revealed relatively high photosynthetic light compensation and saturation values, thus avoiding the high light intensities of the alpine habitat. Both lichen species were able to perform 90% of their optimal NP over a wide temperature range and still reached about one-third of their maximum NP rates at 2 °C, demonstrating their capability to cope with the large temperature changes occurring in their habitat.

Introduction

Endolithic organisms, such as cyanobacteria, algae, and lichens, use different habitats within the rock (Golubic et al., 1981). Species that use existing rock fissures to grow in (= chasmoendolithic) are distinguished from other species that actively penetrate into the substrate (= euendolithic; Golubic et al., 1981).

The first descriptions of endolithic lichens were given by Fünfstück (1895), who observed hyphae of *Verrucaria marmorea* Scop. as deep as 19 mm within the rock. More detailed and specific studies on endolithic lichens were conducted by Bachmann, who described the morphology of endolithic species both on granite (1904) and on calcareous rocks (1913). Several types of endolithic lichens and their mechanical effects on different kinds of underlying substrate were investigated by Fry (1924, 1927). Morphological descriptions of endolithic Verrucariaceae were included in a key of that family developed some years later (Zschacke, 1934). Their anatomy, structure, and development were later studied in detail by Doppelbaur (1959). Investigation methods using scanning electron microscopy (SEM) analysis were improved by Golubic et al. (1970), who developed a new casting-embedding method, which allowed for the preservation of either the substrate or the organisms within their natural position. Using transmission electron microscopy, Kushnir and Galun (1977) examined the fungus-alga relationship in endolithic algae and compared it with that in epilithic lichens.

The rock-weathering activity of endolithic lichens was first described by Lindsay (1856). Bachmann (1904, 1916) conducted detailed studies on the effects of both algae and lichens on the surrounding limestone and granite substrate. First experiments on the mechanical weathering activity of lichens were conducted by Fry (1924, 1927), who tested the effect of contraction and expansion of gelatinous material on a variety of substrates. Degelius (1962) investigated a variety of lichen genera containing endolithic species and pointed out that abiotic factors influence the occurrence and weathering activity of endolithic species. Abiotic factors such as temperature, rainfall, and dewfall were measured by Danin and Garty (1983) and were correlated with the occurrence of different organism groups and with the observed weathering patterns. The amount of material being weathered away was considered to be substantial by Danin et al. (1983), who hypothesized eolian transport of the weathered material from the Middle East into the Terra Rossa soil of the Mediterranean region where it forms a major soil component. Detailed measurements by Wessels and Schoeman (1988) indicated a substantial weathering rate by endolithic lichens. In contrast, Hoppert et al. (2004) described stabilizing mechanisms of lithobiontic organisms on carbonate rocks along with the primary deteriorative effects. The two *Hymenelia* species that were used in our study are also understood to dissolve rock material because of their euendolithic growth form. Their anatomy and effect on the surrounding substrate will be treated in a separate publication (Weber et al., in prep.).

Endolithic lichens occur in a large variety of climatic conditions, as they are present both in hot and cold deserts (Friedmann, 1980; Wessels and Kappen, 1993) as well as in temperate regions (Wirth, 1995; Purvis et al., 1992; Esslinger, 2006). They are mainly restricted by the availability of bare rock surfaces and by certain climatic conditions, such as hot and humid weather where rocks are covered with free-living cyanobacteria

and algae (Büdel, 1999). Under these conditions, endolithic lichens seldom occur. In the European alpine mountains, limestones are extensively covered by epi- and endolithic lichens (e.g. Buschardt, 1979; Hofmann et al., 1993; Poelt and Türk, 1984; Poelt and Mayrhofer, 1985; Türk, 1992; Türk and Breuss, 1994; Von Wittmann et al., 1989). The two *Hymenelia* species, which were investigated in this study, occur quite frequently in the alpine habitat on steep or nearly vertical slopes (Türk and Wunder, 1999). Their distribution is restricted to nearly pure calcareous rocks, mainly in upper montane to alpine environments (Eigler, 1969). In Great Britain, *Hymenelia prevostii* was found to occur on more or less horizontal surfaces, including flat tombstones, often in slightly sheltered or moist situations (Purvis et al., 1992). *Hymenelia prevostii* occurs throughout Great Britain, in NW Ireland, Scandinavia (Purvis et al., 1992; Santesson, 1993), Estonia (Randlane et al., 2006), different regions of the Russian Arctic (Komarkova, 2006), and in the United States (Esslinger, 2006). *Hymenelia coerulea* has a more restricted distribution, occurring in the alpine regions of Germany, Austria, and Switzerland (Wirth, 1995; Türk and Wunder, 1999), including records from Romania (Feuerer, 2006), Slovenia (Suppan et al., 2000), and northern Spain (Renobales and Barreno, 1989).

The lichen samples were collected in an alpine environment characterized by harsh climatic conditions such as high light intensities and strong winds that cause polishing by sand and ice crystals. The endolithic growth form has been interpreted as an adaptation towards extreme abiotic conditions, since it is characteristic for a variety of species within such habitats (Hertel, 1998). The lichens live 1 to 5 mm within the substrate (Pohl and Schneider, 2002) and are only recognized by the slightly different color of the rock surface and by their fruiting bodies.

The special characteristics of different growth forms of lichens that allow them to cope with their habitat conditions have been demonstrated in a multitude of measurements and experiments (e.g. Kappen and Friedmann, 1983; Lange, 1965; Lange et al., 1970; Lange and Kappen, 1972; Lange et al., 1993a, 1997; Moser and Nash III, 1978; Nash et al., 1982). To date little is known about the photosynthetic properties of endolithic lichens. Wessels and Kappen (1993, 1994) measured the photosynthetic properties of endo- and epilithic lichens on sandstone at two different sites in South Africa and correlated them with the local microclimatic conditions. They observed that the lichens were particularly well-adapted to the extremely varying conditions under which they occurred. In arid and semi-arid regions, water was observed to be the key environmental factor limiting photosynthesis (Wessels and Kappen, 1994; Winkler and Kappen, 1997). In contrast to the aforementioned, the photosynthetic properties of endolithic sandstone lichens in the cold desert of Antarctica are mainly limited by low temperatures (Kappen and Friedmann, 1983). Assessment of photosynthetic properties of limestone-inhabiting lichens by gas exchange measurements was considered problematic for a long time, since reactions of the limestone substrate were thought to influence study results (Lange et al., 1970). However, Tretiach and Geletti (1997) excluded an effect of the substrate on the measurements of photosynthetic characteristics of limestone-inhabiting endolithic lichens at the Trieste Karst Plateau, Italy.

Although fog and dew are frequent events in alpine mountain regions, no published data exist on the ability of endolithic lichens to utilize water vapor to gain net photosynthesis (NP). The main focus of this study, therefore, was to examine the ecophysiological role of different water sources for lichens living inside limestone.

In this study, the photosynthetic reaction of two common species of endolithic lichens to the abiotic parameters water, air humidity, light, temperature, and CO_2 concentration was measured under controlled laboratory conditions. On the basis of detailed macroclimatic data from the Untersberg, the results were analyzed and interpreted.

Material and Collection Site

The two crustose endolithic lichen species *Hymenelia coerulea* (DC.) Massal. and *Hymenelia prevostii* (Duby) Krempelh. were collected in the eastern alpine mountains, at the Untersberg (47°43′N, 13°03′E), south of the Austrian city Salzburg. The Untersberg consists of Dachsteinkalk (limestone) and has two summits, the Berchtesgadener Hochthron (1972 m) and the Salzburger Hochthron (1853 m). Samples of about 5–10 cm diameter and 2–5 cm thick were collected with a hammer and chisel at a steep, westward-facing outcrop only a few meters below the Salzburger Hochthron (Fig. 1a). All samples were gathered simultaneously in August, so the samples would not reflect different acclimation stages (Lange and Green, 2005).

The climate of the Untersberg is characterized by an oceanic influence with cold and humid weather throughout the year (Fig. 1b). The average annual precipitation of 1872 mm is distributed relatively evenly throughout the year. The average annual temperature of 3.3 °C is characteristic for alpine environments. From November to April, the mean temperature is below 0 °C, and only on 66 days during the summer an average daytime temperature above 10 °C is observed. Data on the macroclimatic meteorological parameters of air temperature, precipitation, air humidity, global radiation, and wind velocity and direction, measured from 1988 to 1997 on a bi-hourly basis, were supplied by the Department of Environmental Protection of the Federal State Government Salzburg, and by the Austrian Federal Office, Vienna.

To calculate the theoretical time span per month during which the organisms may be activated by water vapor, we summed up the time when the air humidity was above 90% and was not accompanied by rain with a global radiation above 3 J cm^{-2}. The time span after a rain event, during which the thalli were still wet, could not be taken into account since there were no data available.

H. coerulea is characterized by its bluish to bluish-gray thallus, which in some areas remains almost completely below the surface of the substrate. The mostly rimless apothecia are nonpruinose, small (0.2–0.4 mm), and are often situated on small elevations within the limestone. *H. prevostii* has an oval, crustose, whitish-gray thallus. Brownish-pink apothecia are sunk into the thallus, separated from it by a narrow crack. Along the growth zone of this species, the rock surface is marked by a small depression, a feature that may indicate a biological weathering process caused by this species (Fig. 1c). The photobiont of both lichen species was identified as the green algal species *Trebouxia irregularis* Hildreth and Ahmadjian (T. Friedl, personal communication) on the basis of morphological criteria (Ettl and Gärtner, 1995).

Methods

STORAGE AND PREPARATION OF THE LICHEN SAMPLES

After transport, the dry samples were stored in a freezer at –25 °C. Five days before they were used the samples were
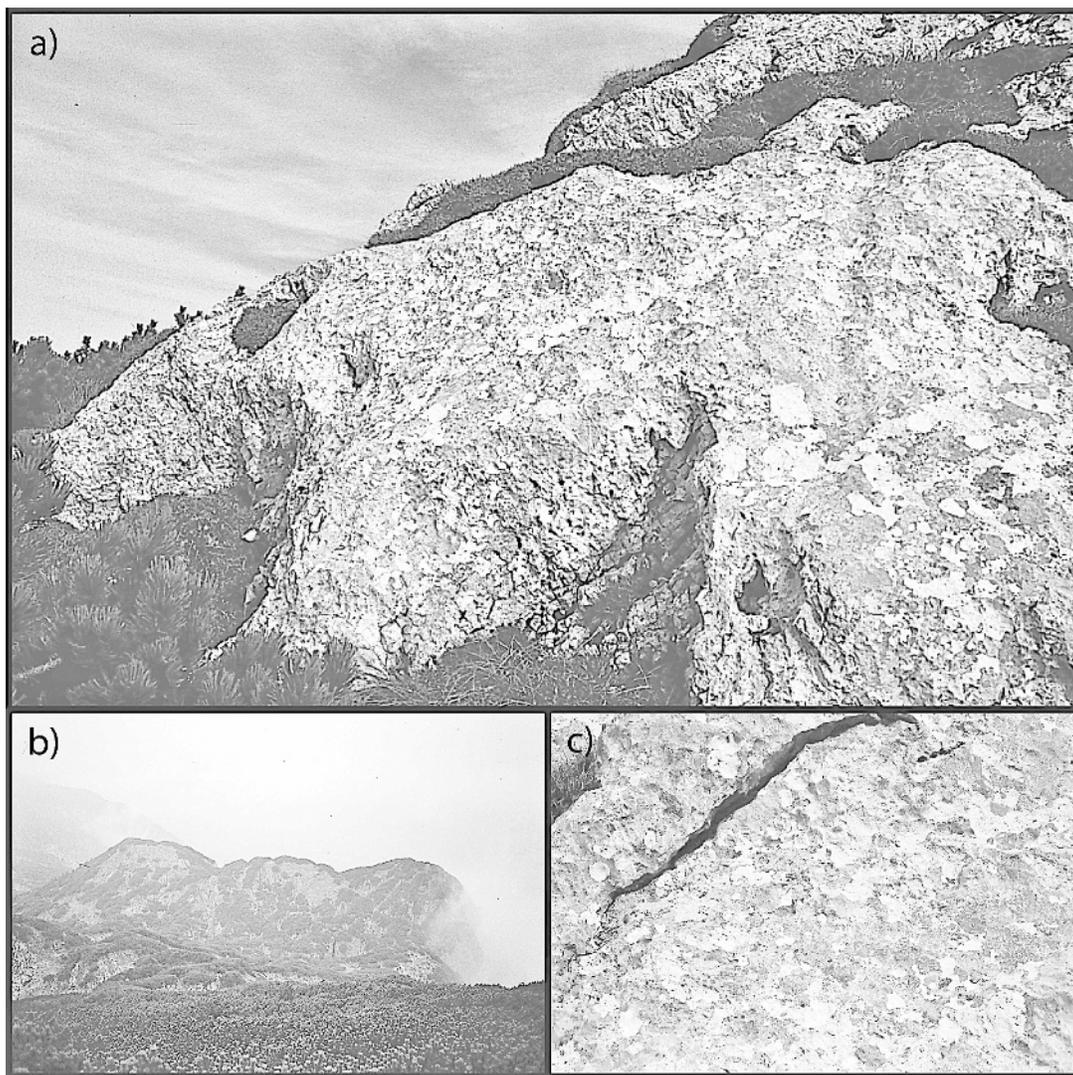
FIGURE 1. (a) Collecting site of the investigated organisms. A steep, westward-facing limestone outcrop only a few meters below the Salzburger Hochthron. (b) The climate on the Untersberg is characterized by an oceanic influence, with cold and humid weather throughout the year. (c) In the area where *Hymenelia prevostii* grows, the rock surface is marked by a small depression, a feature that may indicate a biological weathering process caused by this species.

acclimated in a small transparent chamber at 17 °C with light of approximately 60 μE m^{-2} s^{-1} photon flux density (PFD) and a light-dark regime of 14:10 hours. With hammer and chisel, the samples were reduced to pieces small enough to fit in cuvette baskets (about 3 cm diameter, 0.5–1 cm high), containing only a single lichen species. Once a day the samples were sprayed with aqua dest to activate their metabolism.

On the day of the experiment, all breakage zones of the samples were covered with CO_2 inert terostat (Teroson, Heidelberg) to avoid simplified CO_2 release along the broken thallus zone. The samples were fixed in small stainless steel wire baskets and adapted to the desired temperature. They were submersed in distilled water for 3 hours, thus ensuring that they were fully saturated with water. Prior to the measurements, water droplets on the wire basket were carefully removed.

GAS EXCHANGE MEASUREMENTS

CO_2 gas exchange measurements were conducted under controlled laboratory conditions in a minicuvette system (Walz Company, Effeltrich, Germany).

To test possible influences of the limestone substratum itself (i.e., release of CO_2), rock samples with and without lichens were autoclaved and then measured in pilot tests at 17 °C in dry and wet stages. Additionally, one non-autoclaved rock sample that was formerly not exposed to the rock surface but taken from the inner part of the rock was measured in the same way as the lichen samples (at temperatures of 2, 17, and 27 °C, and CO_2 concentrations of 250, 350, and 800 ppm).

The influence of water, light, temperature, and CO_2 on the NP and dark respiration (DR) of the lichens was determined in two (*H. coerulea*) and three (*H. prevostii*) replications. Measurements were started after sample and cuvette had reached the same temperature. The light dependency was measured with fully watered samples that were exposed to light cycles between 0 and 1300 μE m^{-2} s^{-1} (*H. coerulea*) and 0 and 1500 μE m^{-2} s^{-1} (*H. prevostii*). The light curves had to be repeated for a full desiccation cycle, since it was not possible to keep the thalli at a constant water status for a long time. One light cycle took about 35 minutes, and after 3.5–4 hours the samples had lost about 100 mg (*H. coerulea*) and 140 mg (*H. prevostii*) of water and reached constant weight again.

The influence of water on the gas exchange of the samples was measured at ambient CO_2 content, under optimal light regime (800 µE m^{-2} s^{-1} [*H. coerulea*], and 1000 µE m^{-2} s^{-1} [*H. prevostii*]), and at 17 °C in order to have water as the only limiting factor. Again, full desiccation cycles were conducted. Additionally, the lichen species ($n = 1$) were tested for their capability to utilize water vapor for NP at two different air humidities (rH = 80 and 90%). They were dried over silicagel for 26 hours and then measured at 17 °C and the requested air humidity. In an automated experiment over 48 hours with a light-dark cycle of 20:100 minutes, a measuring point was taken every 5 minutes. Besides the lichen samples, a bare rock sample without lichens was autoclaved and then measured in the same experimental design over 22 hours. During the experiment, special care was taken to avoid condensation of water at the lichen surface or the translucent cover of the cuvette. This was also controlled by temperature probes measuring the sample surface and the air within the cuvette at an accuracy of 0.1 °C. The samples were weighed every 12 hours. In order to study the influence of temperature on the lichen samples, the experiments were carried out in the same manner as those with respect to light and water but at temperature steps of 5 °C between 2 and 27 °C. The CO_2 dependency of the lichens was measured under optimal light regime (see above) and at 17 °C. Desiccation curves were conducted at 250, 350, 500 ppm (both species), and 800 ppm CO_2 (only *H. prevostii*).

CO_2 gas exchange was related to the surface area and the chlorophyll content of the samples. The surface area was measured with an area meter (LI-3100; LICOR, Lincoln, Nebraska). For chlorophyll determination, the samples were reduced to small pieces with hammer and chisel and then extracted two times with di-methyl-sulfoxide (DMSO) at 60 °C for 90 minutes. The chlorophyll$_{a+b}$ content was determined and calculated according to Ronen and Galun (1984).

The water content (WC) of the thalli could not be determined for the endolithic species. Therefore, the WC was expressed as millimeters of rainfall equivalent, calculated from the WC per surface area of the sample.

Results

The chlorophyll content revealed large differences between the species. The samples of *H. prevostii* contained 322.4 mg Chl m^{-2} (std dev.: 39.4 mg Chl m^{-2}, $n = 3$), whereas for *H. coerulea*, only 141.7 mg Chl m^{-2} were determined (std dev.: 2.2 mg Chl m^{-2}, $n = 2$).

In pilot tests, an influence of the calcareous substrate on the gas exchange measurements was excluded. CO_2 uptake or release was not found for wet and dry sterilized samples or for the non-sterilized rock sample.

LIGHT

At optimal thallus WC, both lichen species reached gas exchange values of similar magnitude at saturating light intensities [mean values: *H. prevostii*, 10.3 nmol CO_2 (mg Chl)$^{-1}$ s^{-1} and 3.3 µmol CO_2 m^{-2} s^{-1} with respect to surface area; *H. coerulea*, 8.7 nmol CO_2 (mg Chl)$^{-1}$ s^{-1} and 1.2 µmol CO_2 m^{-2} s^{-1} with respect to surface area (Fig. 2)]. However, *H. prevostii* reached the light compensation point earlier (75 µE m^{-2} s^{-1} vs. 150 µE m^{-2} s^{-1}) and was saturated (90% of maximum NP) at higher light intensities than *H. coerulea* (1000 µE m^{-2} s^{-1} vs. 800 µE m^{-2} s^{-1}).
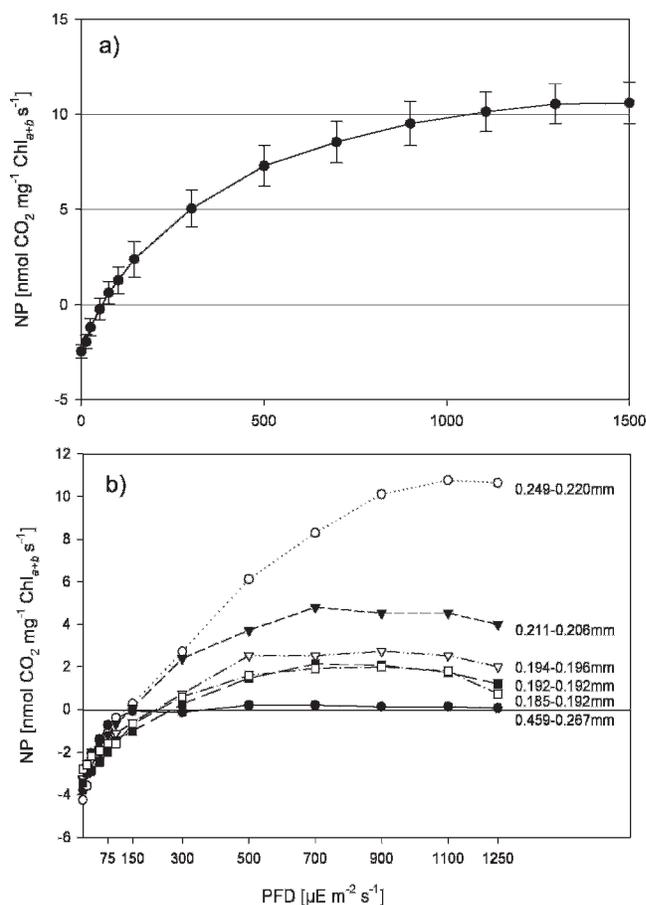

FIGURE 2. Net photosynthesis (NP) related to chlorophyll content of *Hymenelia prevostii* (a) and *H. coerulea* (b) as function of light intensity. The curves were acquired at optimal water content (WC), ambient CO_2, and 17 °C. For *H. prevostii*, means and standard deviations of three thalli are shown. For *H. coerulea*, the representative curves of one specimen are presented. Curves of the second thallus have the same characteristics and similar values. PFD = photon flux density.

WATER

Both species were suprasaturated at high water contents and showed a clear depression in CO_2 uptake (Fig. 3). The range of WC that could be used for positive NP was small for both species and smaller for *H. coerulea* (0.1 mm vs. 0.2 mm rainfall equivalent). *H. coerulea* reactivated NP at 0.05 mm H_2O, whereas *H. prevostii* started NP at 0.2 mm H_2O. The optimum WC was by far higher for *H. prevostii* (0.26 mm vs. 0.07 mm).

ACTIVATION BY AIR HUMIDITY ALONE

In a pilot test, a reaction of bare rock to exposure of 90% air humidity over 22 hours was excluded.

When exposed to 80% air humidity, first respiration rates of the initially dry lichens could be measured after 30 minutes. The respiration rates then remained constant [*H. prevostii*, –0.4 nmol CO_2 (mg Chl)$^{-1}$ s^{-1}; *H. coerulea*, –1 nmol CO_2 (mg Chl)$^{-1}$ s^{-1}] and no positive NP rates were achieved. However, when exposed to an air humidity of 90%, the lichens reached positive NP rates. After 4 hours, *H. coerulea* reached the compensation point (NP equals DR) and after 10 hours NP rates stabilized around 0.5 nmol CO_2 (mg Chl)$^{-1}$ s^{-1} (Fig. 4b). *H. prevostii* reached the
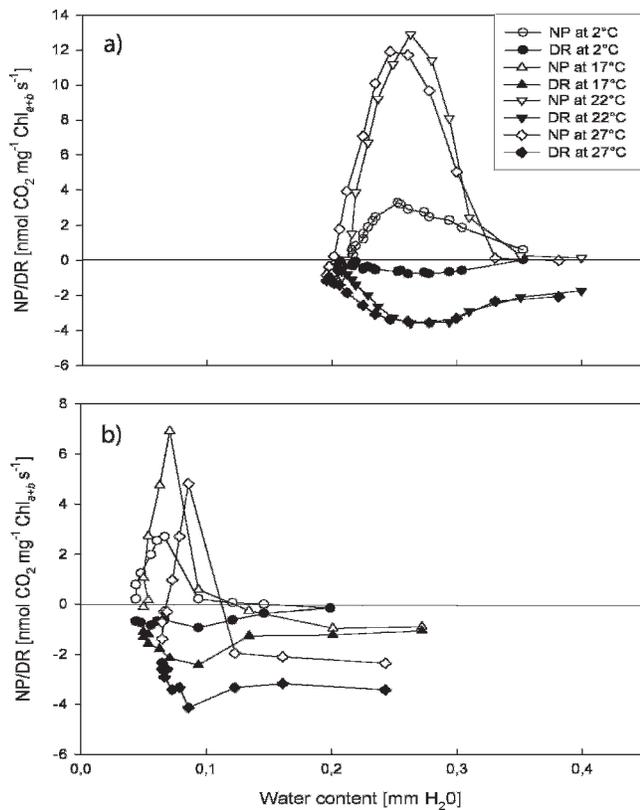
FIGURE 3. Net photosynthesis (NP) and dark respiration (DR) of *Hymenelia prevostii* (a) and *H. coerulea* (b) as a function of water content (representative curves of one specimen). Measurements were conducted at optimal temperature (*H. prevostii*: 22 °C; *H. coerulea*: 17 °C), ambient CO_2 concentration, and saturating light intensity (*H. prevostii*: 1000 μE m^{-2} s^{-1}; *H. coerulea*: 800 μE m^{-2} s^{-1}).
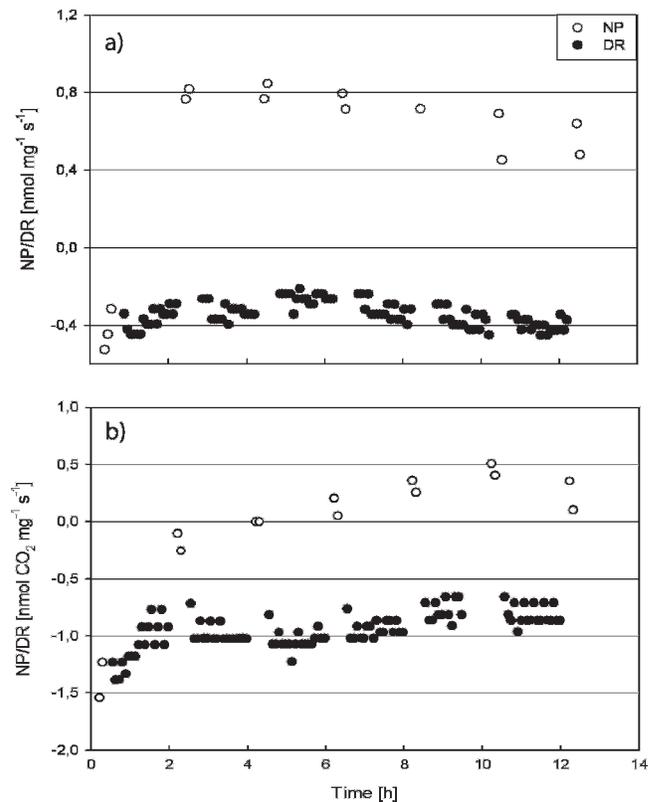
FIGURE 4. Reactivation of net photosynthesis (NP) and dark respiration (DR) of dry samples of *Hymenelia prevostii* (a) and *H. coerulea* (b) at a relative air humidity of 90% (representative curve of one specimen). Measurements were conducted at 17 °C, ambient CO_2 and a photon flux density of 1000 μE m^{-2} s^{-1} (*H. prevostii*) and 800 μE m^{-2} s^{-1} (*H. coerulea*).

compensation point already by 1 hour and an hour later it gained 0.8 nmol CO_2 (mg Chl)$^{-1}$ s^{-1} (Fig. 4a). After the 48-hour experiment, the samples of both species had a load of 12 mg of water. An uncolonized rock sample took up 5 mg of water during a 22-hour experiment. At no time during the experiment was the temperature of the sample lower than that of the surrounding air, so that condensation at the sample surface was avoided at all times.

TEMPERATURE

The mean optimum temperature for three replicates was found at 22 °C for *H. prevostii* and for two replicates at 17 °C for *H. coerulea* (Fig. 5). At higher temperatures, *H. coerulea* especially showed strongly increased respiration rates, which caused a clear decline of the NP values. It is striking that at 2 °C both organisms still achieve about one-third of their maximum NP rates.

CO_2

An elevated CO_2 content of 500 and 800 ppm CO_2 had no positive effect on the NP of the endolithic lichens at their optimal water content (Fig. 6). At 500 ppm CO_2, the maximum NP value of *H. coerulea* was clearly lower than at 350 ppm, since the DR was markedly higher. *H. prevostii* showed slightly lower maximum NP values at 800 ppm CO_2. However, in a supersaturated stage, *H. prevostii* showed higher NP rates at elevated CO_2 concentrations compared to ambient CO_2 concentrations (Fig. 6).

Discussion

The two endolithic lichen species showed characteristic physiological reactions to variations of abiotic factors such as light, water, temperature, CO_2, and air humidity. Their maximum NP rates accorded well with those measured for endolithic lichens of South Africa [*Lecidea confluenta* Müll. Arg., *Lecidea* aff. *sarcogynoides* Körb., *Lithoglypha aggregata* Brusse, *Sarcogyne* cf. *austroafricana* (Zahlbr.) H. Magn.: 1.14–2.47 μmol CO_2 m^{-2} s^{-1}; Winkler and Kappen, 1997]. The rates of *H. coerulea* (1.2 μmol CO_2 m^{-2} s^{-1}) were also in the same range, whereas those of *H. prevostii* (3.3 μmol CO_2 m^{-2} s^{-1}) were markedly higher than those found by Tretiach (1995) for endolithic lichens of the Trieste Karst Plateau. In Tretiach's (1995) experiments, lichens with both green algal and cyanobacterial partners [*Acrocordia conoidea* (Fr.) Körber, *Petractis clausa* (Hoffm.) Krempelh., *Rinodina immersa* (Körber) Zahlbr., *Verrucaria marmorea* (Scop.) Arnold, and *Verrucaria* sp.] only gained maximum NP rates between 0.2 and 1.5 μmol CO_2 m^{-2} s^{-1}. Kappen and Friedmann (1983) also measured far lower NP rates on endolithic lichens in the dry valleys of Antarctica [*Buellia* sp. and an unidentified sample: 0.42–1.15 nmol CO_2 (mg Chl)$^{-1}$ s^{-1}].

The maximum NP values related to chlorophyll content of the lichens were relatively similar for the two species studied. Related to the surface area, however, the NP values for *H. prevostii* were about twice as high as those measured for *H. coerulea*. This phenomenon largely results from morphological differences between the two species, since *H. coerulea* contains approximately half the amount of chlorophyll$_{a+b}$ per surface area compared to *H. prevostii*.
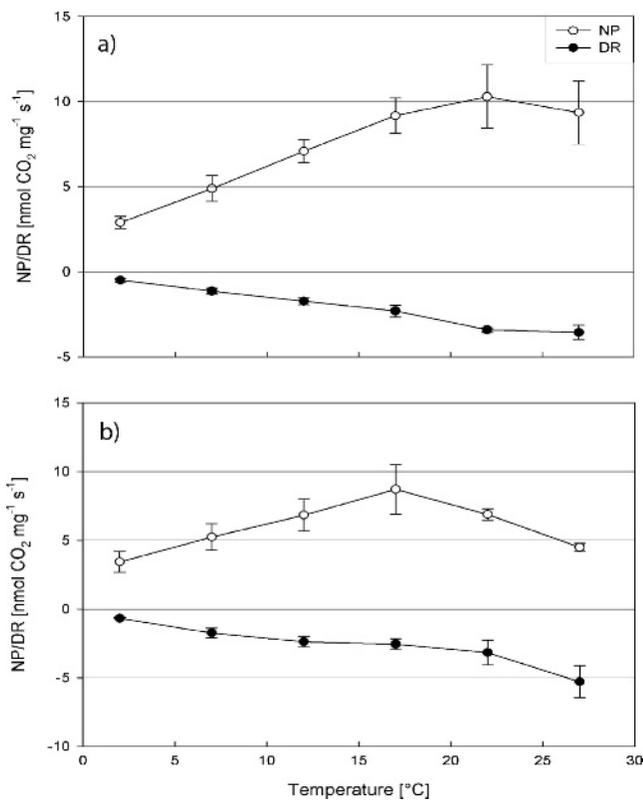
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FIGURE 5. Net photosynthesis (NP) and dark respiration (DR) of *Hymenelia prevostii* (a) and *H. coerulea* (b) as a function of temperature. Measurements were conducted at optimal water content and saturating light intensity (*H. prevostii*: 1000 μE $m^{-2} s^{-1}$; *H. coerulea*: 800 μE $m^{-2} s^{-1}$). The curves depict means and standard deviations of maximum NP values of two (*H. coerulea*), and three (*H. prevostii*) thalli.
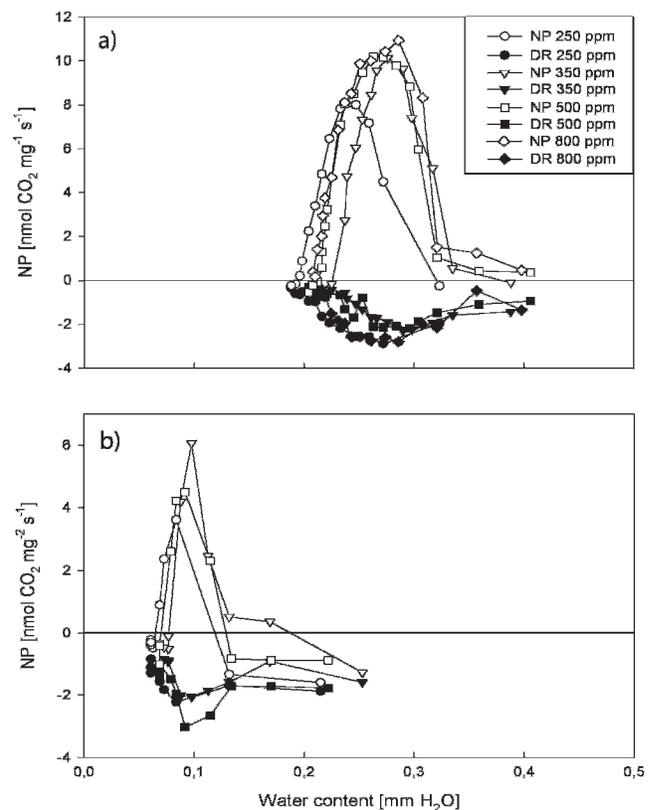

FIGURE 6. Net photosynthesis (NP) and dark respiration (DR) of *Hymenelia prevostii* (a) and *H. coerulea* (b) as a function of water content measured at various CO_2 concentrations (representative curves of one specimen). Measurements were conducted at 17 °C and saturating light intensity (*H. prevostii*: 1000 μE $m^{-2} s^{-1}$; *H. coerulea*: 800 μE $m^{-2} s^{-1}$).

Both species needed higher light intensities to reach compensation and saturation points of NP compared with epi- and endolithic lichens from the Trieste limestone plateau, as measured by Tretiach (1995), but they were comparable to those of endolithic lichens in South Africa (Winkler and Kappen, 1997). The relatively high light compensation points and saturation values are most probably due to the endolithic growth, where light penetration to the photobiont is drastically reduced by the layer above it that is formed by rock particles and fungal hyphae (Nienow and Friedmann, 1993; Kühl et al., 1994). Endolithic growth provides light protection in the high alpine mountains and thus can be seen as one mode of adaptation to high light environments. The lichens do not have to invest in a dense upper cortical layer.

Concerning water relations and NP, it was not possible to determine the lower water content related to the compensation point of NP, since both species took up water vapor from the air within the cuvette. Net photosynthesis was clearly depressed at excess water. This is due to decreased diffusion rates of CO_2 (10,000-fold lower in water than in air), which leads to a lack of CO_2 when water films spread over the upper surface of lichen thalli (Golubic et al., 1979; Snelgar et al., 1981; Cowan et al., 1992; Badger and Price, 1992). This phenomenon has been shown in a study on lichens of a temperate rain forest in New Zealand (Lange et al., 1993a), where the lichens were classified into four response types: type A, no depression at supraoptimal WC; type B, small depression; type C, clear depression and CO_2 release also in the light; type D, clear depression and constant low photosynthesis

values. Both species examined here can be related to type D. A reduction of NP of *H. coerulea* and *H. prevostii* was already observed at a WC, where epilithic species like *Rhizocarpon umbilicatum* from the same habitat did not show a reaction at all (Scherr, 2000). We therefore conclude that the two endolithic species are best adapted to activation by dewfall or fog. The results are consistent with those presented by Wessels and Wessels (1995), who found that sandstone humidity was highly correlated with air humidity, which allowed the endolithic lichen *Lecidea* aff. *sarcogynoides* to thrive in that habitat.

The effects of different CO_2 concentrations are closely related to the WC of the samples. At low water contents, maximal NP rates increased with higher CO_2 concentrations in the air. This means that even at very low thallus water contents (0.08 mm water column for *H. coerulea*) the diffusion resistance for CO_2 uptake is already relevant. The increase of CO_2 concentrations, however, did not result in higher rates of NP at optimal water contents for *H. coerulea* and gave only a slight increase of NP for *H. prevostii*, a result also found by Lange et al. (1999) for *Fulgensia fulgens*. These authors additionally found that 4500 ppm CO_2 were necessary to compensate for the depression of NP during water suprasaturation. Tretiach and Pecchiari (1995) found that at 1300 ppm CO_2 the NP of the endolithic lichen *Petractis clausa* was not yet saturated. Since the NP rates of the two *Hymenelia* species could not be increased or could be only slightly increased by higher CO_2 concentrations, the carboxylation reaction seems to be nearly saturated at ambient CO_2 concentration. This suggests the existence of morphological structures or physiological mechanisms that cause an accumulation of CO_2 within the photobiont. The
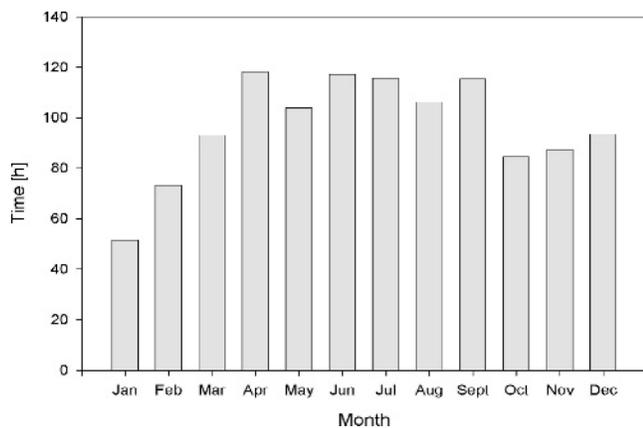
FIGURE 7. Potential water vapor uptake during daytime. Number of hours per month with air humidity above 90% (without precipitation) and light intensity above 3 J cm^{-2} (calculated from macroclimatic meteorological data measured on the Untersberg on a bi-hourly basis between 1988 and 1997).

presence of a CO_2-concentrating mechanism (CCM) was proven for the photobionts of both species (Scherr, 2000).

Probably the most astonishing result is the short time span needed by both lichens to reach maximum DR rates after exposing dry thalli to high air humidity. This is in contrast to all other findings (e.g. Lange and Kilian, 1985; Lange et al., 1993b). However, we do not have a sound explanation for this result. With the uncolonized rock sample, we excluded CO_2 outgassing from the rock during uptake of water. By drying the air-dry samples over silica gel for 26 hours prior to the experiment, humidity of the rock was lower than 5%. Finally, condensation on the rock surface was avoided at all times by maintaining higher temperatures of the rock compared to the cuvette temperature. After 1 hour *H. prevostii*, and *H. coerulea* after 4 hours had gained enough water from the air to reach positive NP rates at 90% rH. It is striking that *H. prevostii* reached the compensation point after a shorter time span, although it needed larger amounts of water for activation. This could probably be explained by differences in thallus morphology and deserves further investigation. The capability to utilize water vapor alone is common and typical for most green algal lichens (Lange and Kilian, 1985; Lange et al., 1986, 1991; Larson et al., 1985), but this phenomenon is here shown for the first time for endolithic lichens. Fruticose species growing under arid climatic conditions, such as *Ramalina maciformis* in the Negev desert (Lange et al., 1970) and *Ramalina capitata* from a rain-protected habitat in central Spain (Pintado and Sancho, 2002), reached CO_2-fixation rates at optimal thallus WC similar to those with high air humidity as the only water supply. Lichens with cyanobacteria as photobiont, however, almost exclusively need liquid water to be activated (Lange et al., 1993b; Büdel and Lange, 1991; Lange et al., 1994).

According to the local climate records for the Untersberg, periods with high air humidity occur regularly throughout the year. Monthly means during the year fluctuate between 60 and 80%, with higher values being reached during the summer months. The time span during which the lichens theoretically could be activated by high air humidity is largest from April to September, since the air carries enough humidity during that time (Fig. 7). However, the values given should only be seen as crude approximations. Field measurements are necessary for improvement.

Both lichen species were able to perform 90% of optimal NP over a wide temperature range. Their temperature optima are within the range of values recorded for endolithic lichens in South Africa (Winkler and Kappen, 1997). Both species are quite well-adapted to low temperatures and still reached about one-third of their maximum NP rates at 2 °C. This is typical for lichens from the alpine habitat (Lange, 1965).

The ability to gain positive NP rates over a wide temperature range makes them well-adapted to their habitat on steep rock surfaces in areas where temperature changes occur quickly and drastically, depending on the influence of sun, wind, rain, and ice. Lange et al. (2001) showed in their review that laboratory results concerning the reactivation of green algal lichens by high air humidity can be used to successfully predict their behavior in their natural habitat. With their capacity to become activated by high air humidity alone, the two *Hymenelia* species are perfectly adapted to the damp weather that often occurs in their habitat. Their high sensitivity to a supraoptimal WC probably only plays a minor role in their natural habitat, since superficial water immediately runs off the steeply exposed rock surface. Along with this, their CCM enables an efficient CO_2 fixation, also at low CO_2 concentrations within the thallus.

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