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Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance?

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Abstract

Extensive investigations made in the past two decades on lichen photosynthesis in relation to water content have shown two features of particular interest: first, the depression of net photosynthesis at high water contents, suprasaturation (i.e. the lichen contains more water than necessary to saturate photosynthesis), and, second, the ability of green algal lichens to regain photosynthetic activity by uptake of water from humid air. Evidence from several investigators is presented to confirm that both phenomena are now well substantiated through laboratory investigations. It has been questioned whether these features do actually occur in nature and, if they do, to what extent. Recent work is summarized that demonstrates that for many of the lichens studied suprasaturation is of major importance and can result in depressed photosynthesis for around a third of the time that the lichens are photosynthetically active. Reactivation of photosynthesis of green algal lichens by high humidity is also, apparently, very common in some environments, for example, humid temperate rainforests, occurring almost every night. It is possible that the dominance of green algal lichens, rather than cyanobacterial species, in these habitats is a result of their ability to utilize water vapour. If so, then the phenomenon must have major ecological importance for lichen productivity. In general, laboratory studies seem to be able to predict extremely well the behaviour of lichens in their natural habitat.

Introduction

Lichens, long acknowledged as being important organisms in extreme environments such as the Arctic, Antarctic, high alpine, and deserts, actually dominate about 8% of the Earth's land surface thus giving them an important role in carbon fluxes at both a local and global level (Ahmadjian, 1995). Interest in the photosynthetic abilities of lichens has grown rapidly in recent decades and, in the 'Recent literature on lichens' database (20 813 references, Culberson *et al*., 2001) published papers with the word-string 'photosynth*' in the title, key words or abstract total 2, 28, 67, 159, and 179 in the decades ending 1960, 1970, 1980, 1990, and 2000, respectively. This rapid growth in interest not only reflects the increasing recognition of the role of lichens especially in sensitive ecosystems like biological soil crusts, but also is an indication of the better availability of portable and more easily used research equipment.

Measurements of lichen photosynthesis have been made in considerable numbers both in the laboratory and in the field, and it has been found that lichens respond to a multivariate complex of environmental factors (Kershaw, 1985; Nash, 1996; Palmqvist, 2000). Lichens are poikilohydric organisms and their water content, therefore, is strongly dependent on environmental conditions. With few exceptions, photosynthetic production

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by lichens is mainly determined by water availability and, in extreme environments like Antarctica or hot deserts, the actual occurrence of lichens reflects the presence of a regular external water supply (Green *et al.*, 1999; Lange *et al.*, 1991). The interaction between thallus water content and net CO_2 exchange in lichens has been found to be rather complex and to differ considerably among species. Two particular phenomena have become established in the literature in the last two decades: the occurrence of depressed net photosynthesis at high thallus water contents, so-called 'suprasaturation', and the ability of some lichens to become rehydrated and photosynthetically active solely through water vapour uptake from humid air. Both phenomena have been amply demonstrated in laboratory studies but questions remain as to whether they are important under natural conditions, i.e. do the phenomena actually occur naturally and, if they do, at a rate that is significant to lichen overall productivity? These questions are founded in part by the obvious difficulties of studying degree of hydration and gas exchange of lichens in nature without impacting the sample and altering its local microclimate condition.

Studies within 'Sonderforschungsbereich 251' (Ökogie, Physiologie und Biochemie pflanzlicher und tierischer Leistung unter Stress) have contributed both to the demonstration and understanding of these phenomena, and in this article present knowledge is consolidated and an attempt is made to evaluate the transference of laboratory experience to the interpretation of lichen performance under their stressful environmental conditions in nature.

Materials and methods

Gas exchange measurements in recent years have almost invariably been made by open, differential systems with CO_2 exchange being measured with infrared gas analysers. The sample has been placed, for variable amounts of time, in a cuvette that is usually conditioned for temperature and, sometimes, humidity and CO_2 partial pressure. Light has been natural or by a controlled artificial source. A typical system is that described earlier (Lange *et al.*, 1984), and used in various forms over time (Kappen *et al.*, 1990; Reiter and Türk, 2000; Schroeter *et al.*, 1992; Sancho and Kappen, 1989). Conditions within the cuvette can never exactly match external, natural conditions, particularly for boundary layer conductance (the cuvettes are normally stirred) and energy balance (which is altered due to the transparent lid of the cuvette, and temperature of the cuvette walls being different from the natural environment). All systems also show some lag between the imposition of new environmental conditions and the occurrence of a steady reading. In spite of these shortcomings, a large amount of reliable information has been produced on lichen CO_2 exchange during the past decades.

Chlorophyll fluorescence allows analysis of the status of the photosynthetic apparatus of lichens *in situ*. It has almost invariably been measured using a modulated system such as the PAM 2000 (Walz Company, Effeltrich, Germany) as outlined earlier (Schreiber *et al.*, 1994) with several typical applications described previously (Schroeter *et al.*, 1992; Lange *et al.*, 1997*b*; Sundberg *et al.*, 1997; Green *et al.*, 2001).

Thallus water content (*WC*) has been calculated as the percentage of thallus dry weight (oven dry under prescribed conditions). CO_2 exchange has been related either to thallus dry weight (g), thallus area (m^2), or thallus total chlorophyll content (mg).

Idealized photosynthetic response to thallus water content

An idealized photosynthetic response to *WC* is given in Fig. 1. It has been constructed from a large number of response curves generated for the desert lichen, *Ramalina maciformis* (Del.) Bory. Other lichens will differ in detail because of species-specific differences in the ranges for *WC* and net photosynthesis (*NP*). In particular, cyanobacterial lichens commence positive *NP* at a higher *WC* and can reach higher maximal *WC*; especially the homoiomerous species. The overall form of the response is typical for all lichens. A rise in *NP* occurs with an increase in *WC* until a maximum is reached (NP_{max}, marked by a vertical line in Fig. 1) at WC_{NPmax}. With further increases in *WC*, *NP* can remain relatively constant at close to NP_{max}, or decline to a variable extent depending on the lichen species. A large number of photosynthetic response curves have been summarized earlier (Lange and Matthes, 1981; Kershaw, 1985). At water contents above WC_{NPmax} the lichen is said to be water suprasaturated with respect to *NP*, i.e. there is more water in the thallus than is necessary to achieve NP_{max}. Above WC_{NPmax} free water is present and the lichen has a water potential close to zero (Green and Snelgar, 1982). *WC* below WC_{NPmax} can be attained by equilibrating the lichen with air at the appropriate relative humidity indicating that the lichen water potential must be below
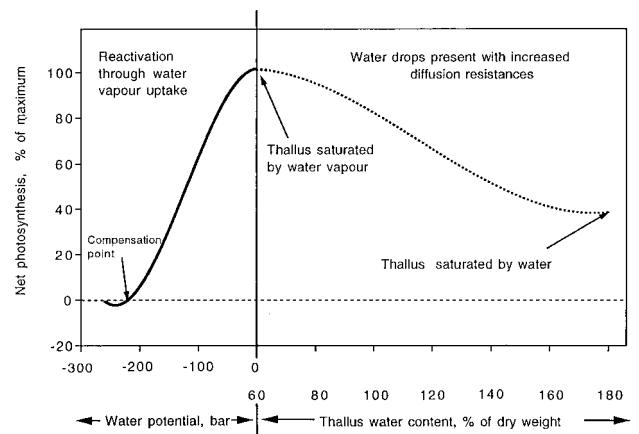

Fig. 1. Idealized response of photosynthesis to thallus water content for *Ramalina maciformis* (Del.) Bory (see text).

zero and that the water is confined to cell walls and cell contents (Cowan *et al.*, 1979). This is indicated in Fig. 1 by *WC* below WC_{NPmax} being given in water potential units and those above WC_{NPmax} as percentage thallus water content. A similar division of thallus water content into a free-water zone (near-zero water potential) and a limited-water zone with increasingly negative water potential has been proposed for bryophytes (Proctor, 1982).

NP_{max} (at WC_{NPmax}) also divides the response curve neatly into the two zones that have raised most interest as to whether laboratory studies actually are applicable to the natural situation. The suprasaturation zone, $WC > WC_{NPmax}$, has received most interest because it is relatively easy to obtain high water contents in the laboratory which, it can be claimed, may not be attainable in nature. It is also counter-intuitive that a poikilohydric organism, totally dependent on water from the environment, should be so negatively affected by high *WC*. The water-limited zone, $WC < WC_{NPmax}$, has received considerable interest because lichens with green algal photobionts are able to achieve positive *NP* when taking up water vapour and can eventually reach water potential equilibrium with humid air. Lichens with cyanobacterial photobionts seem not, in general, to have this ability (see review in Nash, 1996). The relevance of this phenomenon in the field and its ecological importance is at present in question (Palmqvist, 2000).

Photosynthetic response at high, suprasaturating, thallus water contents

Laboratory demonstration and analysis

The occurrence of photosynthetic depression at high *WC* has been amply demonstrated in the laboratory for a range of species (Green *et al.*, 1994). The depression is most pronounced at saturating PPFD (photosynthetic photon flux density) and may be smaller, but nevertheless substantial at low, non-saturating PPFD (Fig. 2). The discovery that increased CO_2 levels could ameliorate the depression and, if high enough, remove it completely (Lange and Tenhunen, 1981) suggested that it was being produced by increased diffusion resistances. However, because water and CO_2 do not follow identical pathways in the thallus it was not possible to apply classical resistance analysis procedures as used for higher plant leaves. This problem was solved by the use of helox (helium and oxygen) mixtures to alter the CO_2 diffusion constant and allowing the diffusion resistances to be calculated (Cowan *et al.*, 1992) and the presence of increased CO_2 diffusion resistances at suprasaturation to be confirmed. Both the occurrence and mechanism of *NP* depression at high *WC* have, therefore, been fully established in laboratory studies.
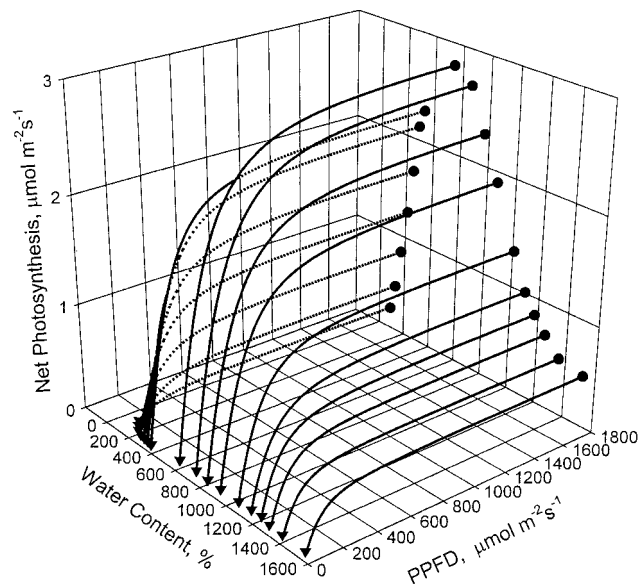
Collema cristatum

Fig. 2. Response of net photosynthesis to PPFD (photosynthetic photon flux density) at several thallus water contents for *Collema cristatum* (L.) Wigg. (17 °C, from Botanical Garden, Würzburg).

Demonstration of depressed NP at high WC in the field

The simplest demonstration that suprasaturation occurs under natural conditions can be achieved by placing a naturally wetted specimen, i.e. collected from the field, immediately into a cuvette and monitoring both its *NP* and *WC* as it dries. An example from a New Zealand temperate rainforest is given in Fig. 3A and it can be seen that, as the specimen progressively loses water, the initially low and almost constant *NP* suddenly rises and shows a clear maximum before declining as it dried out completely. Similar performance is depicted for a lichen from the montane tropical rainforest in Panama (Fig. 3B), that had received light–dark cycles during drying. Dark respiration remained almost constant whilst *NP* strongly increased as the thallus lost water. One sample of the lithophytic *Xanthoria calcicola* Oxner (Fig. 3C, 'field') was continuously suprasaturated during a wet day in a habitat in Germany and showed, *in situ*, constant and only low rates of *NP* even at high PPFD. Another sample from the same site was initially suprasaturated in a similar manner, however, its *NP* increased almost 7-fold after removal and drying under laboratory conditions (Fig. 3C, 'experimentally drying'). These examples from contrasting habitats prove, in general, that these lichens must have been naturally raised to a *WC* that substantially depressed *NP*, i.e. they were suprasaturated in the field. The high *WC* achieved in the laboratory are similar in magnitude to, and have the same effect as, those under natural conditions (Lange *et al.*,
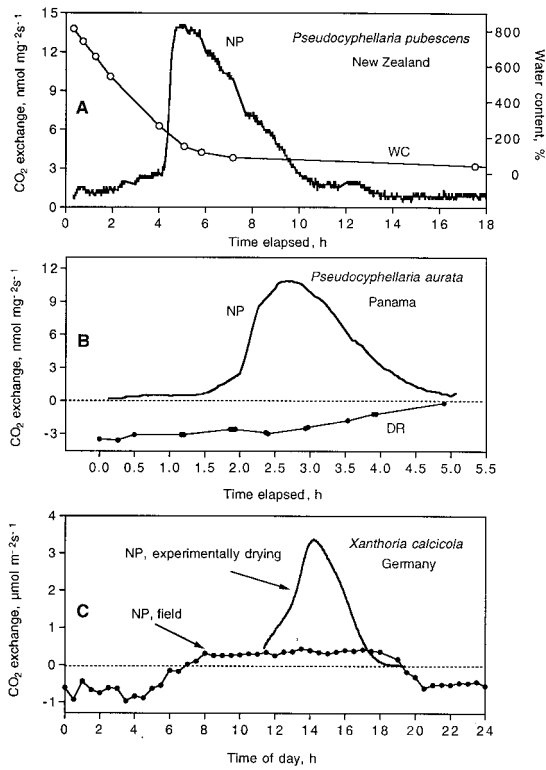
Fig. 3. Suprasaturation demonstrated by CO_2 exchange measurements as the naturally wetted lichen (time 0 in A and B) dries down experimentally, see text. (A) *Pseudocyphellaria pubescens* (Müll. Arg.) D. Galloway et P. James, temperate rainforest, New Zealand; *NP*: net photosynthesis (modified from Lange *et al.*, 1993). (B) *Pseudocyphellaria aurata* (Ach.) Vainio, montane tropical rainforest, Panama; *NP*: net photosynthesis, DR: dark respiration. (C) *Xanthoria calcicola* Oxner, wall-top, Botanical Garden Würzburg, Germany. Diel course of CO_2 exchange of one sample under field conditions (line with solid circles); a second sample was transferred for experimental drying (modified from Lange and Green, 1996).

1993). There remains the question, whether this phenomenon occurs so frequently that it is of ecological relevance.

Recognizing suprasaturation in field measurements

No data sets seem to exist where the *WC* of a lichen has been monitored in conjunction with regular measurements of *NP* over long periods in the field. The closest approximation are studies where lichen samples had their *NP* and *WC* determined at regular intervals in a cuvette but were replaced in their normal environment between measurements. As an example, Fig. 4 shows ranges of *WC* of *Pseudocyphellaria faveolata* (Del.) Malme in a temperate rainforest during daylight hours as compared with its *WC*-related *NP* response: during most of the time the thallus appeared to be suprasaturated. By selection of data under conditions where PPFD no longer limits *NP*, it is possible to demonstrate and to quantify the effect of suprasaturation on carbon acquisition from such results (see Figs 4 and 5 in Green *et al.*, 1993).
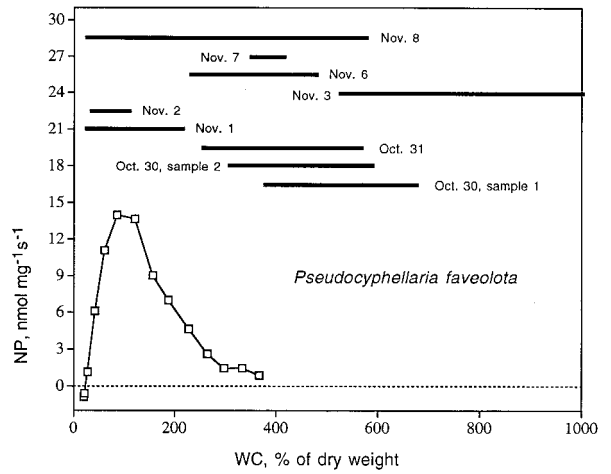

Fig. 4. Net photosynthesis (*NP*) of *Pseudocyphellaria faveolata* (Del.) Malme in relation to water content (*WC*), temperate rainforest, NZ. The horizontal bars show the range of *WC* of the same sample under natural conditions during daylight hours (modified from Lange *et al.*, 1993).
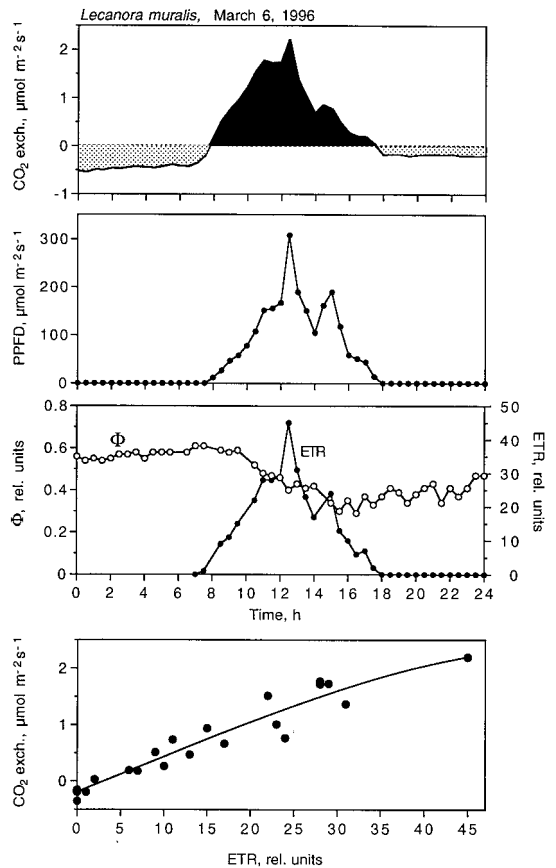

Fig. 5. *Lecanora muralis* (Schreber) Rabenh., top-wall, Botanical Garden, Würzburg, Germany, 6 March, 1996. Diel courses of CO_2 exchange (upper panel), corresponding photosynthetic photon flux density (PPFD, second panel) and apparent PSII photosynthetic quantum yield (Φ) and calculated relative electron transport rate (*ETR*, third panel). Bottom panel: lichen net photosynthesis (CO_2-exchange) in relation to electron transport rate.

The occurrence of depressed *NP* at suprasaturation can be recognized *in situ* by identifying relevant patterns, or 'signatures', in daily courses of *NP* and chlorophyll fluorescence. A typical signature for suprasaturation is a time sequence during a day with non-limiting PPFD and drying conditions for an initially wet thallus, when a relatively constant and low *NP* is followed by a period of much higher *NP* before eventual quiescence due to progressive desiccation. Calculated electron transport rate (*ETR*) is usually considered to reflect rates of photosynthetic carbon fixation, if CO_2 is available (Schreiber *et al.*, 1994). High rates of calculated *ETR* with simultaneously low rates of *NP* indicate a limitation in CO_2-supply most probably caused by high diffusion resistances due to suprasaturation (Leisner *et al.*, 1997). Signatures for the different environmental situations can be identified in the time sequences produced using an automatically operating klapp-cuvette (Lange *et al.*, 1997*b*), in which the lichen *Lecanora muralis* (Schreber) Rabenh. was, for most of the time, exposed to natural conditions untouched on a piece of stone for longer than an annual course. Brief CO_2-exchange and fluorescence measurements took place every 30 min.

In the first daily course, presented for 6 March 1996 (Fig. 5), the lichen had been wetted the previous day and had a relatively constant respiratory rate throughout the night. During the day the lichen maintained a moderate *WC*. Apparent photosynthetic quantum yield (Φ, 1 s saturating light pulses; methods see Leisner *et al.*, 1997) remained high and *NP* tracked incident PPFD reaching a maximal value around 2 μmol m^{-2} s^{-1} at about 300 μmol m^{-2} s^{-1} PPFD. Respiration continued the following night showing that the lichen had not dried out. Calculated *ETR* was almost linearly related to *NP* during this day, when the lichen was not suprasaturated (Fig. 5, lower panel).

On the second day presented, 2 July 1996 (Fig. 6), the lichen was rewetted from a non-active state (no detectable respiration or Φ) by dew or high humidity at dawn. The *WC* reached would not have been high and this is confirmed by the rapid desiccation and suspension of activity by the lichen (Fig. 6, upper panel). The lichen would not have been suprasaturated. *NP* reached a high value of about 4.2 μmol m^{-2} s^{-1} at about 750 μmol m^{-2} s^{-1} PPFD. *ETR* effectively tracked *NP* also on this day with low *WC*.

The signature for strong *NP* depression at suprasaturation is clearly identifiable in the daily course of *NP* for 27 May 1996 (Fig. 7). First the lichen had been substantially wetted by rainfall overnight leading to a strong respiration rate and high Φ. After dawn the *NP* was low and constant, only reaching about 0.2 μmol m^{-2} s^{-1} at PPFD between 300 and 750 μmol m^{-2} s^{-1}. The lichen was slightly rewetted again by rain at about 10.00 h. At about 13.00 h a large peak in *NP*
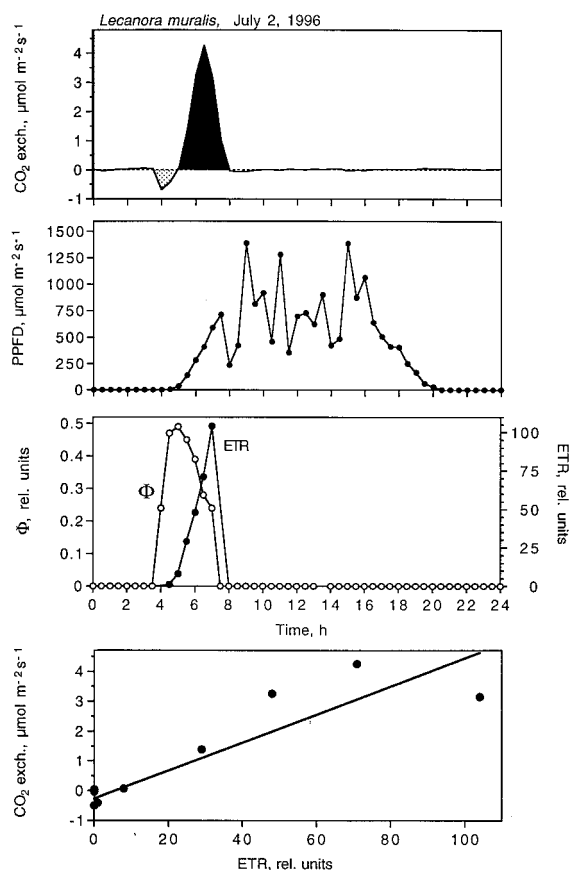

Fig. 6. *Lecanora muralis* (Schreber) Rabenh., top-wall, Botanical Garden, Würzburg, Germany, 2 July, 1996. Diel courses of CO_2 exchange (upper panel), corresponding photosynthetic photon flux density (PPFD, second panel) and apparent PSII photosynthetic quantum yield (Φ) and calculated relative electron transport rate (*ETR*, third panel). Bottom panel: lichen net photosynthesis (CO_2-exchange) in relation to electron transport rate.

suddenly occurred, reaching about 1.5 μmol m^{-2} s^{-1} at 400–750 μmol m^{-2} s^{-1} PPFD, until the lichen dried out causing both zero *NP* and zero Φ at about 14.00 h. This is the signature one would expect from suprasaturation when, despite sufficient PPFD, for a large part of the day *NP* was depressed in combination with high rates of *ETR*. Substantial rates of *NP* only occurred for a brief period when the initially increased CO_2 diffusion resistances disappeared before complete desiccation. When the lichen was rewetted again by a heavy rain shower at 16.00 h, *NP* was at first high and then was depressed with increasing *WC*, i.e. a reverse signature. When *NP* is compared to *ETR* (Fig. 7, bottom panel) it can be seen that during most of the day when the lichen had high *WC*, *ETR* remained high but *NP* was depressed. Three outlier values occurred which coincided with the *NP* peaks (labelled with arrows in Fig. 7, upper and lower panel). These values are comparable to those in the *NP*/*ETR* responses from the other days. When suprasaturation is present it seems that *ETR* no longer reflects *NP* and that an
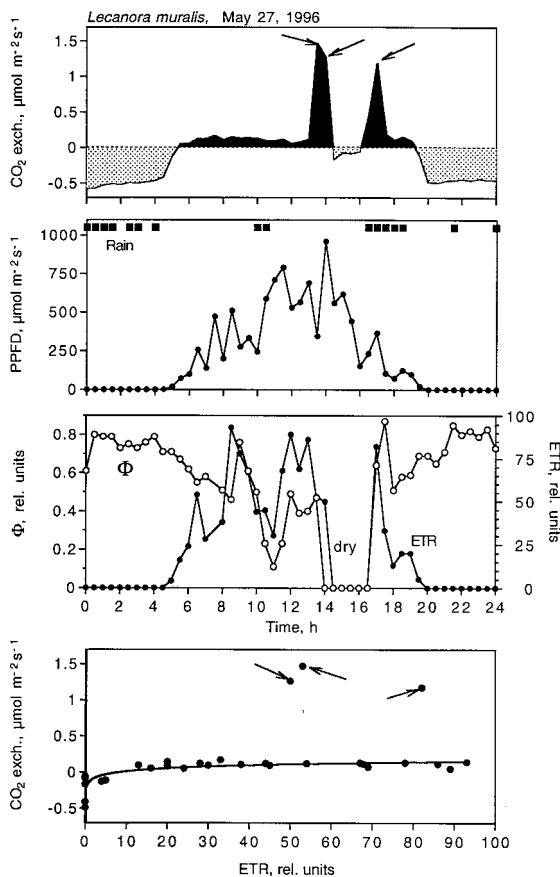
Fig. 7. *Lecanora muralis* (Schreber) Rabenh., top-wall, Botanical Garden, Würzburg, Germany, 27 May, 1996. Diel courses of CO_2 exchange (upper panel), corresponding photosynthetic photon flux density (PPFD, second panel) and apparent PSII photosynthetic quantum yield (Φ) and calculated relative electron transport rate (*ETR*, third panel). Bottom panel: lichen net photosynthesis (CO_2-exchange) in relation to electron transport rate.

alternative electron sink such as O_2 is in operation (Schreiber and Neubauer, 1990; Heber *et al.*, 1996). Confirmation that this signature does indeed represent suprasaturation was obtained when two specimens of *Xanthoria calcicola* that had been held under identical natural conditions in a klapp cuvette, were separated and one allowed to dry out under controlled laboratory conditions (Fig. 3C).

Frequency of occurrence of suprasaturation under natural conditions

Use of the signature defined above allows the periods of time during which a lichen is suprasaturated to be recognized from daily courses of *NP*. Analysis of 12 months of continuous measurements (June 1995 to June 1996) using the klapp cuvette in Würzburg (Bavaria) found that the lichen *Lecanora muralis* was metabolically inactive (dry or totally frozen) for 64.4%, and it was hydrated and metabolically active for 35.6% of the total time; it photosynthesized 16.7% and respired 18.9% of time.

During more than one-third of its photosynthetically active time (37%) *NP* was depressed due to high *WC* (OL Lange, unpublished data). This indicates clearly that suprasaturation is a common phenomenon at least with this and with many other lichen species, and that thallus water contents required to produce it are not confined to the laboratory or to exceptional situations in the field. However, it should be remembered, as stated before, that not all lichen species show depressed *NP* at high *WC*.

Photosynthesis following rehydration by humid air

Laboratory results

Subsequent to the pioneering discovery of Butin (Butin, 1954), numerous laboratory studies have produced a consensus that photosynthesis in green algal lichens can be rapidly reactivated from humid air (Lange and Kilian, 1985). Green algal lichens placed in a relative humidity of 96% show *NP* activity within a few hours and reach maximal rates within 20 h (Lange *et al.*, 1988). During the same time period *WC* stabilizes at about 50–70%, the actual *WC* reached depending on the relative humidity. Use of frozen–hydrated specimens in a low-temperature scanning electron microscope shows that the visual turgor of the lichen photobiont and mycobiont follow the changes in *WC*. At low humidities the photobiont cells are contracted but are nearly at full turgor at 96% relative humidity (Büdel and Lange, 1991; Scheidegger *et al.*, 1995). *NP* declines with thallus water potential to reach zero at almost −400 bar (Nash *et al.*, 1990). The rate of decline is independent of the method used to achieve the low water potentials, i.e. relative humidity or sorbitol or salt solution.

Cyanobacterial lichens do not share this ability to achieve positive *NP* after water vapour hydration (Lange *et al.*, 1986). Studies using sectors with different primary photobionts from a photosymbiodeme which were held at >95% relative humidity have fully confirmed this (Lange *et al.*, 1988). Both green algal and cyanobacterial lichens reach approximately the same *WC* in equilibrium with humid air (Schlensog *et al.*, 2000). However, cyanobacterial species seem to require much higher *WC* than green algal species for positive *NP*, and these *WC* cannot be attained in equilibrium with humid air but require additional liquid water (see review by Kappen and Valladares, 1999). The physiological basis for this difference is not yet clear.

Field studies on reactivation at high relative humidities

Is the ability of green algal lichens to use water vapour for hydration of ecological importance for their existence? Early studies on *Ramalina maciformis* in the Negev Desert (Lange *et al.*, 1968) clearly showed that hydration

through high air humidity alone and without dew condensation during the night resulted in a short peak of positive *NP* in the morning hours before the thallus dried again. Similar performance in the morning or during the late afternoon hours was shown for lichens from other arid areas, for example, for the Namib Desert (Lange *et al.*, 1991) even if the photosynthetic gain after humidity reactivation remained rather small. More frequent and longer-lasting phases during which photosynthesis was activated and subsequently maintained through high ambient air humidity was found for the epiphytic species *Ramalina menziesii* Tayl. which was studied for two morphological forms at a coastal and an inland site in central California (Matthes-Sears and Nash, 1986*a*; Matthes-Sears *et al.*, 1986).

However, there are habitats where water vapour uptake seems to be an essential parameter for lichen productivity and where the difference in response between green algal and cyanobacterial lichens is extremely important for their performance. A 7-week continuous study of the components of a photosymbiodeme, involving separated segments with green algae and with cyanobacteria combined in the same thallus, was performed on a *Nothofagus* tree in New Zealand (Green *et al.*, 2001). *In situ* chlorophyll fluorescence measurements showed that reactivation occurred every night with a regular pattern for the green algal sector of overnight rehydration and subsequent morning desiccation (Fig. 8, panel B). More importantly, the extent of the reactivation depended on the humidity and temperature reached overnight. Activation did occur, but to a lesser extent, on nights when humidity did not reach 100% which suggests
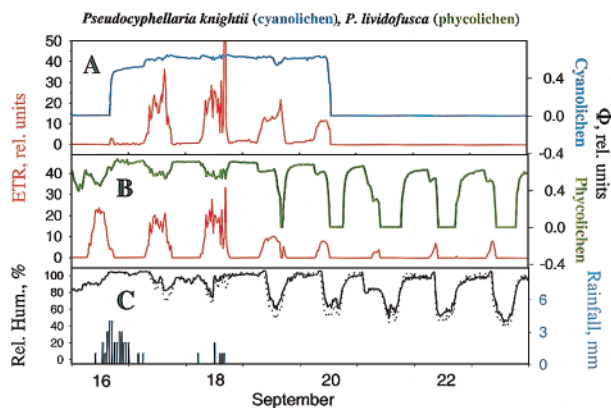
that dew was not involved. For the cyanobacterial sectors, reactivation occurred only after rain (Fig. 8, panel A) although apparently higher *WC* maintained *NP* for longer in these sectors.

'Optimal' rates of photosynthesis

Many response curves of net photosynthesis to factors such as temperature, PPFD and thallus water content have been constructed in the laboratory. When a maximal rate for *NP* has been achieved the value for the parameter at that time is defined as being 'optimal'. For instance, in Fig. 1, a *WC* of about 60% would be said to be optimal for *NP* of *Ramalina maciformis*. It is possible to construct a set of conditions for PPFD, temperature and *WC* which should produce maximal *NP* for the lichen. For *Collema cristatum* (L.) Wigg. (Fig. 2; 17 °C) this would be a *WC* of about 600% and PPFD >1000 µmol m^{-2} s^{-1}. There is, however, growing evidence that lichens, in nature, are rarely active under such 'optimal' conditions. Green *et al.* showed for several *Pseudocyphellaria* species that in the shade of the temperate rainforest, the lichens were almost never active under PPFD and *WC* that would be defined as optimal under controlled conditions (Green
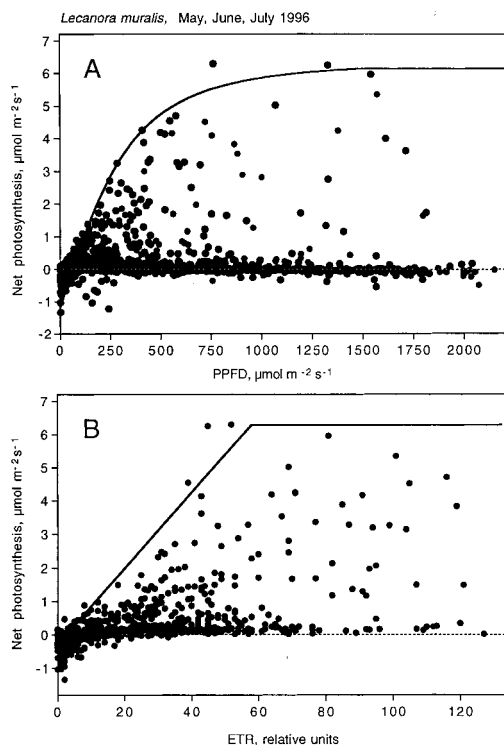
Fig. 8. Chlorophyll fluorescence and microclimate measurements for green algal [*Pseudocyphellaria lividofusca* (Krempelh.) D. Galloway; 'phycolichen'] and cyanobacterial (*P. knightii* D. Galloway; 'cyanolichen') sectors of a photosymbiodeme growing on a *Nothofagus* tree at Waikaremoana, New Zealand. The results presented are for 8 d from a 7-week continuous investigation. (A) Relative electron transport rate through photosystem II (*ETR*, red) and photosynthetic quantum yield (Φ, blue) for the cyanobacterial sector; (B) *ETR* (red) and Φ (green) for the green algal sector; (C) relative humidity (%) adjacent to the lichen and rainfall (mm, columns) outside the tree canopy. (Modified from Green *et al.* (2001), see this publication for full data set.)

Fig. 9. *Lecanora muralis* (Schreber) Rabenh., top-wall, Botanical Garden, Würzburg, Germany. (A) Net photosynthesis (*NP*) in relation to incident photon flux density (PPFD). Plotted are all measurements which took place every 30 min during daylight hours on days in May, June and July 1996, when metabolic activity of the lichen occurred. (B) As (A), however *NP* is shown in relation to calculated relative electron transport rate (*ETR*).

et al., 1995). Long-term monitoring of *Usnea antarctica* Du Rietz in the Maritime Antarctic using chlorophyll fluorescence also showed that activity was rare at the *WC* and temperature required for maximal *NP* in the laboratory (Schroeter, 1997; Green *et al.*, 1999).

The response of *NP* and *ETR* to PPFD for the months May, June and July 1996, for *Lecanora muralis* on its wall-top habitat in a klapp-cuvette is presented in Fig. 9. All the readings (one every 30 min) that are plotted took place when light >1 µmol m^{-2} s^{-1} PPFD on days with metabolic activity. Only a small number of the *NP* measurements lie on the envelope curve for optimal response of *NP* to *PPFD* (Fig. 9A). There are many measurements at high PPFD but most have low or zero *NP* and are data generated when the lichen could not respond to the available PPFD because it was too dry or suprasaturated. When *NP* was related to *ETR*, only those situations were considered where positive quantum yield of the lichen indicated sufficient hydration for metabolic activity, i.e. all *NP* values when the lichen was dry are now excluded. Even then a similar pattern is found (Fig. 9B). The huge majority of all *NP* rates attained were lower than the potential rates which seem to be possible for a particular *ETR*; there are many measurements with *NP* close to zero but with high *ETR*. This is exactly what would be expected for suprasaturation (Fig. 7). The optimal combinations of light and hydration for maximal rates of *NP* were very rarely reached. In addition to desiccation, suprasaturation was the main factor preventing the lichen from making optimal use of the light available.

Discussion

Over the past two decades, the importance of thallus suprasaturation and reactivation from humid air for lichen CO_2 exchange has been amply demonstrated in laboratory studies. What is, perhaps, surprising is the extent to which these phenomena also determine carbon gain of lichens under natural condition. It is estimated from extensive daily courses of *NP* that suprasaturation limits *NP* for about one-third of the metabolically active daytime hours in *Lecanora muralis* under temperate conditions, and similar numbers apply to other species such as *Fulgensia fulgens* (Swartz) Elenkin and *Collema cristatum*. This has a major impact on the carbon income for these lichens. At present there is not much known about the structural causes of suprasaturation. Increased CO_2 diffusion resistances are present and are most probably produced by blockage of previously air-filled capillaries. Only a very short section of such a capillary needs to become water-filled for there to be a large increase in CO_2 diffusion resistance. This has been calculated for the pore system of *Sticta latifrons* A.Rich. and

Pseudocyphellaria amphisticta Kremp. by Snelgar *et al.*, who found that a layer of water as thin as 1 µm caused a large increase in CO_2 diffusion resistance (Snelgar *et al.*, 1981*a, b*). It remains somewhat a puzzle as to why such capillaries are not waterproofed by hydrophobin proteins, as suggested for the medulla of lichens (Honneger, 1997; Scherrer *et al.*, 2000). One result of the depressed *NP* is that the lichen must possess suitable systems to protect against light damage to photosystems (Heber *et al.*, 2000). It is noticeable that electron flow through PSII is not affected by high PPFD during suprasaturation but is maintained by other electron sink than CO_2 fixation (Leisner *et al.*, 1997; Heber *et al.*, 1996, 2000).

Carbon gain due to reactivation by humid air of photosynthesis in green algal lichens seems to be relatively small, but, nevertheless, ecologically important under arid and semi-arid conditions. Matthes-Sears *et al.* modelled CO_2 exchange of *Ramalina menziesii* and estimated that water vapour uptake accounts for about 11% of gross annual carbon gain of this species in central coastal California (Matthes-Sears *et al.*, 1987; Matthes-Sears and Nash, 1986*b*). Reactivation by humidity appears to be extremely common in some of the other habitats studied, i.e. in temperate evergreen rainforests. Here, the frequency of the reactivation, apparently almost every night, is far more than anticipated and means that a phenomenon that was, perhaps, a laboratory 'curiosity' is actually of major ecological importance. The full significance cannot yet be judged but the huge preponderance of green algal lichens in the evergreen rainforests of New Zealand, northwest United States and Chile may well be due to *NP* activity under humid conditions. The ability would not only allow reactivation in the absence of rainfall but would also allow prolongation of *NP* in the humid air once wetted.

Despite the evidence from, for example, temperate forests and hot deserts there does seem to be evidence that rehydration from humid air may not always result in photosynthetic productivity where it might be expected. Cuvette studies in the laboratory have clearly established the ability of lichens to rehydrate at subzero temperatures in the presence of snow but the absence of any liquid water (Kappen, 1993; Schroeter *et al.*, 1997). However, under natural conditions, activity was found to be minor or totally absent in antarctic lichens under snow where they would have been expected to be able to take up moisture (Hovenden and Seppelt, 1995). In the case of suprasaturation, not all lichens show the phenomenon even when at very high *WC* (e.g. *Pseudocyphellaria amphisticta*, Snelgar *et al.*, 1981*b*; *Diploschistes* species, Lange *et al.*, 1997*a*). In addition, other lichens seem to exist, for example, *Peltigera* species which show suprasaturation under experimental conditions in the laboratory, but where high *WC* was found only very occasionally in the field (Leisner *et al.*, 1996). In their

natural habitats, the extra free water needed to produce the depressed *NP* was wicked away by moss shoots on which the thalli were growing. More investigations will, no doubt, reveal a richness of responses by various lichens and in different habitats.

According to the authors' experience, lichens photosynthesize in the field rarely under so-called optimal conditions. Almost always they are limited by factors such as low and high *WC* or low PPFD. These observations need further investigation. Suprasaturation and humid air reactivation are very difficult to incorporate in carbon budget models and cast doubts that such models can be effectively applied without knowledge of actual thallus water content. Development of an effective carbon model and a structural understanding of lichen water relations remain major research goals for lichen ecophysiologists. It is encouraging, however, that laboratory studies have been able to identify and quantify unusual responses of *NP* to *WC* well before they were known from the natural habitat and thus provide a useful guide to the interpretation of lichen field performance.

Acknowledgement

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