Photobiont Activity of a Temperate Crustose Lichen: Long-Term Chlorophyll Fluorescence and CO_2 Exchange Measurements in the Field

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Abstract

A novel automatically operating CO_2 gas exchange measuring cuvette ("klapp-cuvette") with integrated MINI-PAM chlorophyll fluorometer has been developed especially for in-the-field monitoring of entire small plants like lichens or mosses over extended time periods. The machinery is very compact and can be integrated into walls or into the ground. The object of investigation is positioned on the base of the open cuvette (set flush with the natural surface) and does not need to be touched or moved again. This minimises disturbances by the process of measurements of the object and of natural habitat microclimate conditions. Two different activity signals can be monitored simultaneously, i.e. chlorophyll fluorescence yield of the photobiont, and photosynthetic or respiratory CO_2 gas exchange of the entire lichen, under quasi-natural conditions for long time intervals (months to years).

Using the saxicolous green algal lichen *Lecanora muralis,* we investigated how well the fluorescence and gas exchange data agreed and if it was possible to deduce carbon gain of the whole lichen from chlorophyll fluorescence yield of the photobiont alone. Additional measurements with the same objectives were made

under controlled conditions in the laboratory. We found that the results from chlorophyll fluorescence and gas exchange measurements correlated well in showing metabolic activity or dormancy phases of the lichen. Chlorophyll fluorescence yield furthermore gave indications on the occurrence of inhibition of photosystem II. However, for calculating CO_2 fixation and carbon balance, fluorescence measurement techniques alone proved insufficient. This most probably can be explained by the ability of algae to also transfer electrons from the photochemical electron transport chain to O_2 and by the poikilohydric life strategy of lichens.

Keywords: field measurement, chlorophyll fluorescence, gas exchange, lichen, photo-biont, algae, carbon gain, photosynthesis, photoinhibition

Abbreviations

PS II = photosystem II, PFD = photosynthetic photon flux density, Fo = minimal fluorescence yield in the dark, Fm = maximal fluorescence yield in the dark, F = minimal fluorescence yield in the light, Fm' = maximal fluorescence yield in the light, Fo' = minimal fluorescence yield immediately after darkening and a 3 second application of far red light, Fv/Fm = PS II quantum yield in the dark, ΔF/Fm' = PS II quantum yield in the light, ETR = electron transport rate through PS II, NPQ and q_N = non-photochemical fluorescence quenching coefficients, q_P = photochemical fluorescence quenching coefficient, GP = gross photosynthesis, NP = net photosynthesis, DR = dark respiration, WC = thallus water content.

1. Introduction

Lichens occupy a wide range of habitats, examples being barren rocks, man-made surfaces, and both hot and cold deserts, many of which are hostile to other plant life. Ecophysiologists with interest in how life can tolerate extreme conditions have long sought to evaluate the performance of lichens in these environments. The classical technique for such measurements has been CO_2 gas exchange. This, however, requires very labor intensive data acquisition with often voluminous equipment and, furthermore, usually frequent disturbance of the specimens because of the necessity to remove them from their natural environment for the duration of measurements in cuvettes. A novel automatic "klapp-cuvette" is now available that allows long-term measurements of CO_2 gas exchange (see Lange et al., 1997). In this system, the lichen is exposed to natural conditions and is only enclosed in the measuring cuvette for three minutes at selected intervals, in our case 30 min. The studied specimens remain almost totally undisturbed and many of the problems

previously associated with gas exchange systems are overcome.

In recent years, progress in the development of automatic chlorophyll fluorometers has made them very suitable for field measurements (Schreiber et al., 1994). Such machines are much smaller, can be battery powered and are less expensive than gas exchange equipment. Furthermore, fluorescence measurements are a form of remote sensing and do not require any contact with the object at any time. This makes them especially interesting for use on organisms like crustose lichens or algae which are very difficult to access with traditional gas exchange techniques.

For higher plants, good correlations between fluorescence quantum yield and CO_2 or O_2 gas exchange quantum yield have been shown in the laboratory (Demmig and Björkman, 1987; Genty et al., 1989; Saeton and Walker, 1990; Genty et al., 1992; Edwards and Baker, 1993; Oberhuber et al., 1993; Schreiber et al., 1994). Lange et al. (1996) found an excellent agreement between CO_2 gas exchange and fluorescence parameters in two different lichen species under controlled conditions and used both techniques together to further clarify the causes of depressed photosynthesis at high thallus water contents. The application of fluorescence techniques to lichens in the field has been successful in the long-term monitoring of activity and in the assessment of photosystem (PS) II performance or damage (Schroeter et al., 1992; Hovenden and Seppelt, 1995; Leisner et al., 1996). However, there is a need to obtain further evidence on the relationship between fluorescence parameters and CO_2 gas exchange in lichens with different types of algal and cyanobacterial photobionts, especially from across a wide range of natural environments. There is an inherent danger in extrapolating from results obtained on higher plants to primitive organisms from different kingdoms, exhibiting different CO_2 uptake systems (CO_2 concentrating mechanisms, see Badger et al., 1993; Palmqvist, 1995), special metabolic properties (e.g. photochemical electron transfer to oxygen, see Scherer, 1990; Schreiber et al., 1995, and nitrogen, see Serrano et al., 1981; Larsson et al., 1985), and a poikilohydric life strategy.

In this paper we present concurrent measurements of CO_2 gas exchange and chlorophyll fluorescence made on a lichen in the field using the klapp-cuvette. The data sets produced uninterruptedly over many months provide a good opportunity to compare the two measuring techniques whilst the lichen was effectively under natural conditions.

2. Materials and Methods

Field measurements

CO_2 exchange in the field was measured with a novel fully automatic

cuvette system (klapp-cuvette, Walz GmbH, Effeltrich, Germany; for detailed description see Lange et al., 1997). The cuvette was built into a specially constructed red sandstone wall so that the floor of the cuvette was flush with the surrounding rock surfaces. The measuring site in the Botanical Garden of Würzburg was located at an open plot with other stone walls, rocks, and pavements in the vicinity on which epilithic lichens including *Lecanora muralis* (Schreber) Rabenh. have grown for decades. This species contains a coccoid green alga, probably of the genus *Trebouxia*, as photobiont (Wirth, 1995). A thin piece of sandstone covered with approximately $16 \, cm^2$ thallus surface area was cut from a slab of red sandstone overgrown with *L. muralis* and placed on the base of the open cuvette. The sample was exposed towards the south to exclude shading of the lichen by the measuring equipment. Further slabs of red sandstone covered with *L. muralis* were placed on the wall top around the klapp-cuvette, some of which were used for supplementary laboratory investigations. The control unit of the cuvette was programmed to close the lid every 30 min for about 3 min. During this interval, gas exchange and standard environmental parameters (light intensity, temperatures, relative humidity, ambient CO_2, for details see Lange et al., 1997) were recorded. For the remainder of the 30 min interval the lichen was exposed to natural weather conditions in the open cuvette. Rainfall was measured about 3 m from the klapp-cuvette with an automatic rain gauge (DRG 3, 0.2 mm/tip, Campbell Scientific Inc., Logan, Utah, USA) and registered as 30 min sums simultaneously with the other measurements by a micrologger (21X-Micrologger, Campbell Scientific Inc.).

Chlorophyll fluorescence measurements of the cuvette sample were made at the end of the CO_2 exchange measurement with a MINI-PAM fluorometer (Walz GmbH). A special thin fiber optic (MINI-PAM/F1, 2 mm diameter) was integrated into the klapp-cuvette together with an additional micro light sensor. The fiber optic was fixed about 5 mm above the sample surface. In preliminary laboratory experiments the settings of the MINI-PAM were optimized for measurements on well hydrated *L. muralis*. Saturation pulse was set on 1.0 sec duration and $6000 \, \mu mol/m^2/s$ photosynthetic photon flux density (PFD) at the lichen surface. Fluorescence measurements were triggered by the klapp-cuvette control unit to ensure exact timing immediately after gas exchange measurements had been executed. From the absolute fluorescence signals, Fv/Fm was calculated in the dark, and $\Delta F/Fm'$ in the light. Some calculated values were discarded when absolute fluorescence levels were low and close to the noise level of the instrument. This situation could be easily recognised from the records. The calculated electron transport rates through PS II ($ETR = \Delta F/Fm' \times PFD \times 0.5 \times 0.84$; all calculations according to Schreiber et al., 1994) presented here should be viewed as a relative measure

whose values may be higher than reality because we have no knowledge of the proportion of the light reaching the photobiont; in extreme cases this is known to be as low as 5% of ambient light (Büdel and Lange, 1994). Non-photochemical fluorescence quenching (NPQ) was calculated according to Schreiber et al. (1994), using the last value of Fm before dawn of the respective day. NPQ could not be calculated for days on which the lichen was not fully hydrated and Fm had not reached a constant value before dawn.

Laboratory measurements

CO_2 gas exchange in the laboratory was measured with a mini cuvette system (Walz GmbH) as described by Lange and Tenhunen (1984) and Lange et al. (1995). Chlorophyll fluorescence measurements in the laboratory were conducted with a PAM 2000 fluorometer (Walz GmbH). The fiber optic was fixed in the lid of the cuvette at an angle of 55°, a distance of 5 mm above the sample and so arranged that it did not shade the enclosed lichen during measurements.

A thin stone slab with thalli of *L. muralis* covering about 13 cm^2 surface area was brought in from the field measuring station early in the morning and sprayed with deionized water to activate metabolism. About 2 h later it was submersed in deionized water for 5 min, ensuring suprasaturation (i.e. water content higher than necessary for optimal photosynthesis rates) of the thallus, and then it was placed into the mini cuvette. After darkening the sample for 15 min, respiration and dark fluorescence yield of the lichen were measured. Subsequently it was illuminated and left to slowly dry in a continuous gas stream with 350 ppm CO_2. Light and temperature were kept at constant 1250 $\mu mol/m^2/s$ PFD and 17°C, respectively. CO_2 gas exchange and chlorophyll fluorescence yield were measured simultaneously at 5 min intervals. Once every hour the cuvette was darkened for 5 min to measure Fo', upon darkening after 3 seconds of infra-red light, and respiration at the end of the 5 min interval. These values were interpolated and used to calculate gross photosynthesis (GP = net photosynthesis, NP plus dark respiration, DR), and the fluorescence quenching coefficients q_N and q_P (according to Schreiber et al., 1994).

3. Results

Field measurements of lichen CO_2 exchange and chlorophyll fluorescence

Throughout the year *L. muralis* remained desiccated for a considerable proportion of time, around 65% of total hours (see Lange and Green, 1997). In

this paper, in order to compare chlorophyll fluorescence and gas exchange under field conditions, we have concentrated on typical diel courses with frequent thallus hydration, which have been selected from a plentitude of similar examples.

A spring series of five diel courses from April 3rd through April 7th is depicted in Fig. 1. The period provided a wide range of activity events during which there was a fairly good coincidence between CO_2 exchange and chlorophyll fluorescence parameters. The lichen was activated either by rain, as on the first three days, or from dew, as on the last day. There were two dull days (April 4th and 5th) with the lichen being continually active, and two bright days (April 6th and 7th) when the lichen dried out. Quantum yield efficiency (Fv/Fm and $\Delta F/Fm'$) declined as the lichen desiccated in the dark (see night before morning of April 6th, Fig. 1) and in the light (see daytime of April 6th and 7th, Fig. 1). The decline, however, was much more pronounced in absolute signal intensity which could be used as a rough indicator of thallus water content (WC). After desiccation the thallus temperature of the lichen rose considerably above air temperature on sunny days (top panel). Dark adapted quantum yields at night are an excellent measure of PS II status and, for adequately moistened thalli, indicated whether any inhibition of PS II was present. This was the case on the evening of April 4th. Here, Fv/Fm immediately after sunset showed substantially lower values than e.g. on the evening of April 5th. Also NPQ did not relax back to zero by sunset on April 4th as was the case on the following day (Fig. 1, 4th panel). This indicates cold-induced photoinhibition of the wet lichen during comparatively low PFD on April 4th. At first inspection, ETR showed a good agreement with CO_2 exchange (bottom panel in Fig. 1), the ETR pattern coinciding well with that of CO_2 exchange during daylight hours. When respiration occurred, fluorescence signals also indicated activity (see the nights in Fig. 1).

There were days that generated an excellent relationship between ETR and photosynthesis. Such an example is shown in Fig. 2 for July 11th 1996, when the thallus was wetted during the night by dewfall. Positive ETR was already reported shortly before 4 a.m. while CO_2 exchange of the lichen had still not yet reached the light compensation point. Dawn was followed by a four hour peak of CO_2 uptake which ended when the lichen dried out as PFD increased. ETR almost exactly tracked NP, and a good linear relationship existed between the two parameters (Fig. 2, bottom panel; $r^2 = 0.991$).

There were also many occasions, however, when there was little to no agreement between ETR and CO_2 exchange. A typical example is given in Fig. 3 for July 25th 1996 when the lichen was thoroughly wetted by heavy rainfall. It is known for this species that net photosynthesis becomes severely reduced at high thallus water contents because of increased CO_2 diffusion resistances

within the thallus (Lange and Green, 1996, 1997) and, indeed, net photosynthesis was strongly depressed and did not respond to changing light for most of the day. ETR, in contrast, showed clear light responses, was not depressed and mostly remained at levels expected for higher CO_2 exchange rates. When plotted against NP, ETR values obtained during thallus suprasaturation with water formed a vertical line clearly showing its large changes at almost constant low NP (Fig. 3, bottom panel).

To show the distribution of the two phenomena described above over a longer period, all the data generated on days during March 1996 when *L. muralis* showed some activity are depicted in Fig. 4. On ten days no suprasaturation-caused depression occurred (open symbols). On four days NP was severely depressed due to high WC (solid symbols). Although a relationship between ETR and NP is evident in Fig. 4, forming a right-hand envelope of the data, there was considerable spread of data points to the left of this line, representing high ETR at low NP especially on the four days with high WC causing NP depression.

Desiccation characteristics under controlled conditions in the laboratory

Chlorophyll fluorescence and gas exchange were measured at constant temperature and near saturating light on a thallus of *L. muralis* (Fig. 5). Measurements started at maximal WC and the lichen was allowed to dry slowly in the gas exchange cuvette. Whilst the thallus was suprasaturated, GP remained low (top panel). As the thallus dried its WC passed through the optimal range for photosynthesis and GP rose about eightfold to a maximum. With further desiccation it gradually fell to zero. The absolute fluorescence signal intensity showed a similar pattern but with smaller differences (center panel). At the start of the experiment it took 20 min until the absolute signals reached steady state conditions. Then both minimal (F) and maximal (Fm') fluorescence signal intensity were slightly depressed during suprasaturation, rose to maxima at the same time as CO_2 uptake and declined to zero with further desiccation. The minimal fluorescence signal after a brief application of far red light in darkness (Fo') remained constant until all fluorescence signals started to decrease together (data not shown). The fluorescence signal maximum, reached at optimal thallus hydration, lasted longer than the peak for GP and signal intensities only started to decrease when GP had already diminished by almost 40% because of water limitation. Despite the large changes in absolute fluorescence signals, fluorescence quantum yield (and consequently also ETR at constant PFD) remained stable over a wide range of thallus water content (top panel). Only when GP had almost reached zero (and the thallus was nearly air-dry) did the quantum yield decrease steeply. As
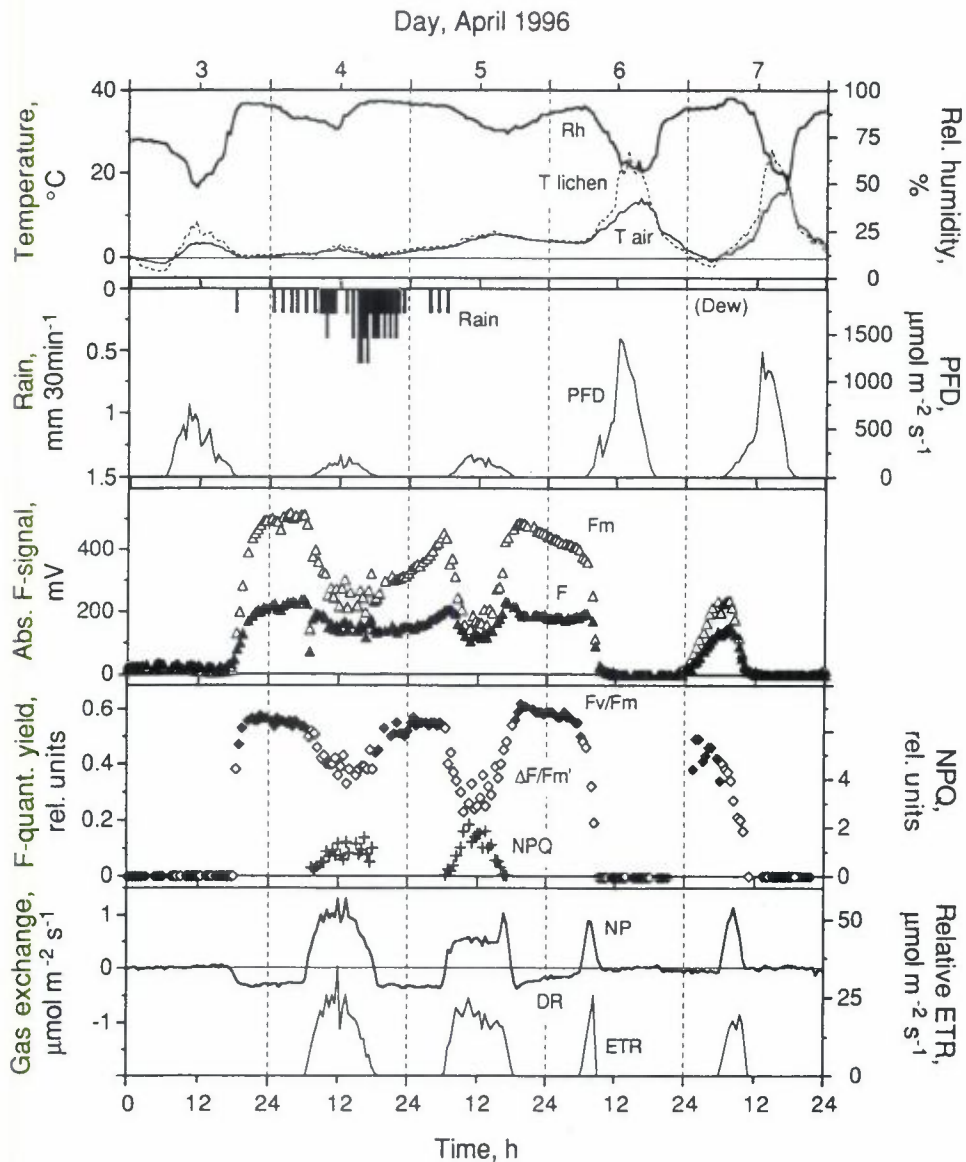
Figure 1. Time course of microclimate (top two panels), chlorophyll fluorescence
parameters (next two panels), and net CO_2 gas exchange and ETR (bottom panel)
of *L. muralis* at a semi-natural growing site in the Botanical Garden of
Würzburg from April 3rd through April 7th 1996. In the top panel the
temperatures of ambient air (thin solid line) and thallus surface (thin dashed
line) are scaled on the left, and relative air humidity (Rh, fat solid line) is scaled
on the right. In the second panel, rainfall as bars of 30 min sums is scaled on the

expected, q_N (bottom panel) started out very high at thallus suprasaturation and reached a minimum when GP was maximal, indicating that the excitation pressure in PS II was mitigated by the high photosynthetic rates. However, once absolute fluorescence signals had started to decrease due to water limitation it was no longer valid to calculate q_N. During desiccation alterations in Fm' need not be due to light-induced changes in PS II and thus cannot be related to the Fm value measured at high thallus hydration. Uncertainty concerning absolute thallus hydration is the major problem with the calculation of non-photochemical fluorescence quenching parameters from field data. Unexpectedly, and in spite of the high q_N, q_P remained maximal over the entire period when GP was depressed at high thallus WC. Like the quantum yield, q_P only started to decrease during the final phase of desiccation. During the laboratory measurements there was no indication of photoinhibition, which is a function of PFD and exposure time (Henley, 1993), despite the long periods of reduced NP and saturating PFD. In contrast, GP and Fm' reached maxima after 2.5 h and fluorescence quantum yield showed a slight tendency to rise during the course of the experiment.

4. Discussion

A general but weak relationship between ETR and CO_2 exchange was found on the basis of long-term field measurements on *Lecanora muralis* (Fig. 4). The right hand boundary of a large data set was defined by data obtained on days with low levels of hydration when there was good agreement between ETR and CO_2 exchange (Fig. 2). There was an obvious spread of data points to the left of this line, i.e. higher than expected values for ETR at any particular value of NP, with a left hand boundary formed by data obtained from very wet thalli (Fig. 3, Fig. 4: solid symbols). It is this spread that weakens the relationship

Figure 1.   Continuation.   ... left from top to bottom, and PFD (thin solid line) is scaled on the right. In the third panel absolute fluorescence signals are depicted where, for simplicity, the same symbols were used for values measured in the light and in the dark, and all measuring-light-fluorescence is being called F, and all saturation-pulse-fluorescence Fm. In the fourth panel chlorophyll fluorescence yield of the photobiont (diamonds, scaled on the left), and NPQ (crosses, scaled on the right) are depicted. Open diamonds represent $\Delta F/Fm'$ in the light, filled diamonds represent Fv/Fm in the dark. In the bottom panel net CO_2 gas exchange is depicted (fat solid line scaled on the left) together with relative ETR (thin solid line scaled on the right). Negative values of gas exchange represent respiration, positive values net photosynthesis of the lichen.
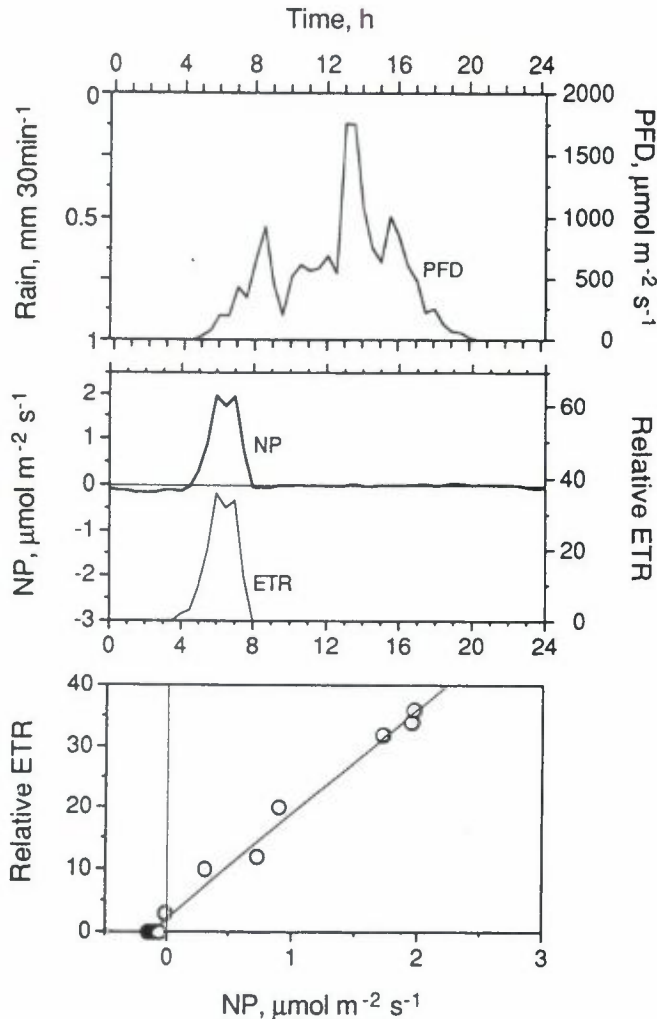
Figure 2. Diel course of PFD (top panel), and CO_2 exchange (NP) and relative ETR (center panel) of *L. muralis* in the field on July 11th 1996. The lichen was hydrated by dewfall during the night and showed a 4 hour peak of CO_2 uptake during the morning. Under these conditions, NP and ETR correlated well ($r^2 = 0.991$, bottom panel).

and requires explanation. From the analysis given above it would seem that periods of thallus suprasaturation (WC above that required to attain optimal CO_2 exchange) and associated depressed photosynthesis accounted for much of the data spread. However, it is reasonable to assume that some variability in the ETR/CO_2 exchange relationship was due to times when the thallus was
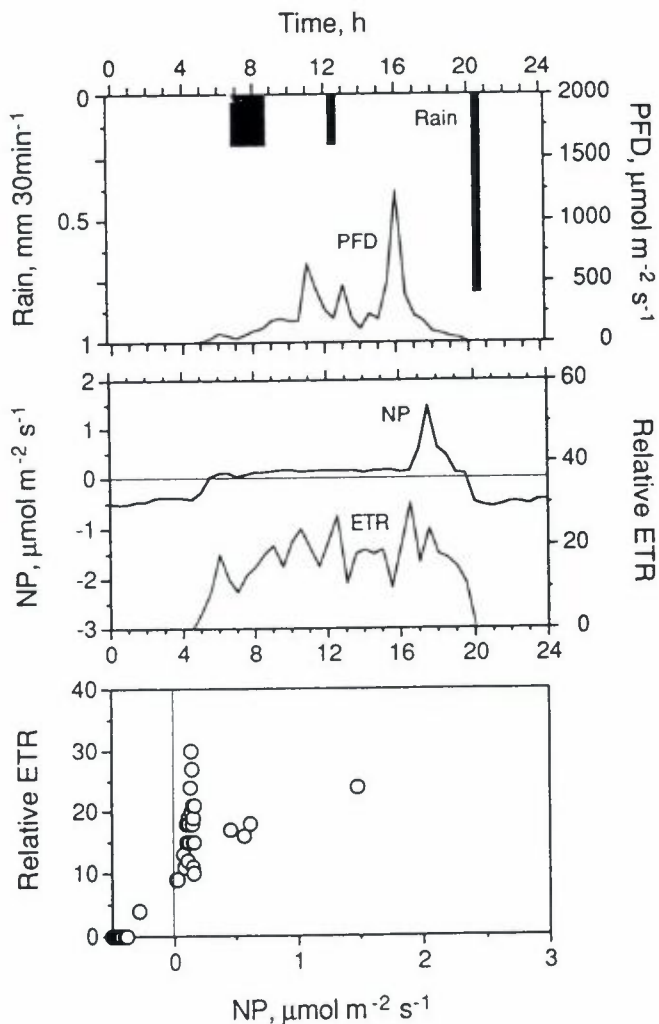
Figure 3. As Fig. 2 but on July 25th 1996. In addition, rainfall is depicted in the top panel as bars of 30 min sums (scaled from top to bottom). The lichen remained hydrated throughout the diel course and showed diffusion limited low NP because of thallus suprasaturation for most of the light phase (center panel). Only in the late afternoon a brief peak of CO_2 uptake independent from PFD indicated no further suprasaturation. The depression in NP is not reflected in ETR, leading to the vertical arrangement of data points in the bottom panel. The data points produced during non-depressed photosynthesis in the afternoon fall near the regression line from Fig. 2, bottom panel.
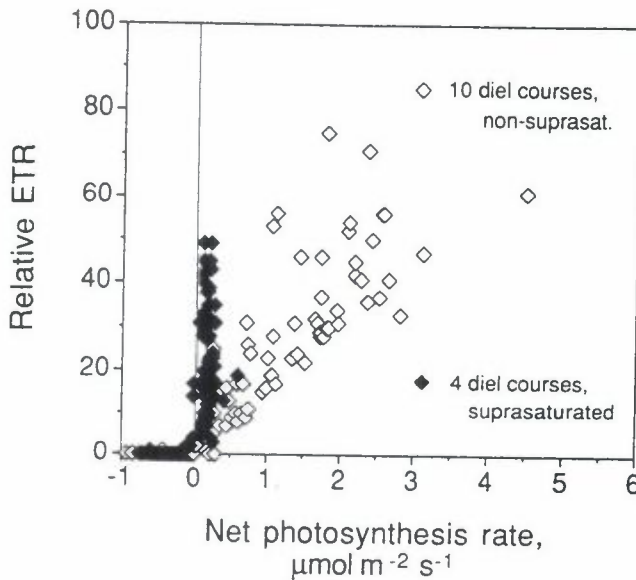
Figure 4.   Relative ETR plotted against NP from field measurements. Depicted is the complete set of data points from all days with metabolic activity of *L. muralis* during March 1996 (total 14). Four diel courses differed clearly from the rest in showing high ETR at low NP which was depressed because of high diffusion resistances due to suprasaturation of the thallus (solid symbols).

in the process of desiccation. Jensen and Feige (1991) also found that Fv/Fm was little affected during considerable water loss in two green algal lichens. It is furthermore possible that also measurements taken while the lichen was in the final stages of desiccation contributed to variability. It has already been shown that lichen thalli dry unevenly, i.e. that some parts of a thallus can still be active whilst others are already desiccated (Schroeter et al., 1992). Therefore, in the final stages of desiccation the fluorescence probe may be reading from a small area that is not at the same water status as the remainder of the thallus. This could lead to both higher or lower than expected values of ETR in comparison to CO_2 exchange which is measured over the total sample.

   The laboratory data generated under controlled conditions confirmed the information from the field measurements. When allowed to dry from maximal thallus water content the lichen *L. muralis* showed practically constant fluorescence quantum yield (which resembles relative ETR at constant PFD) until just before complete desiccation (Fig. 5). Higher than expected ETR was particularly clear in the suprasaturated condition, where photosynthesis was very depressed due to high diffusion resistances (Lange and Green, 1997). The photobiont was obviously using another energy consuming process than photo-
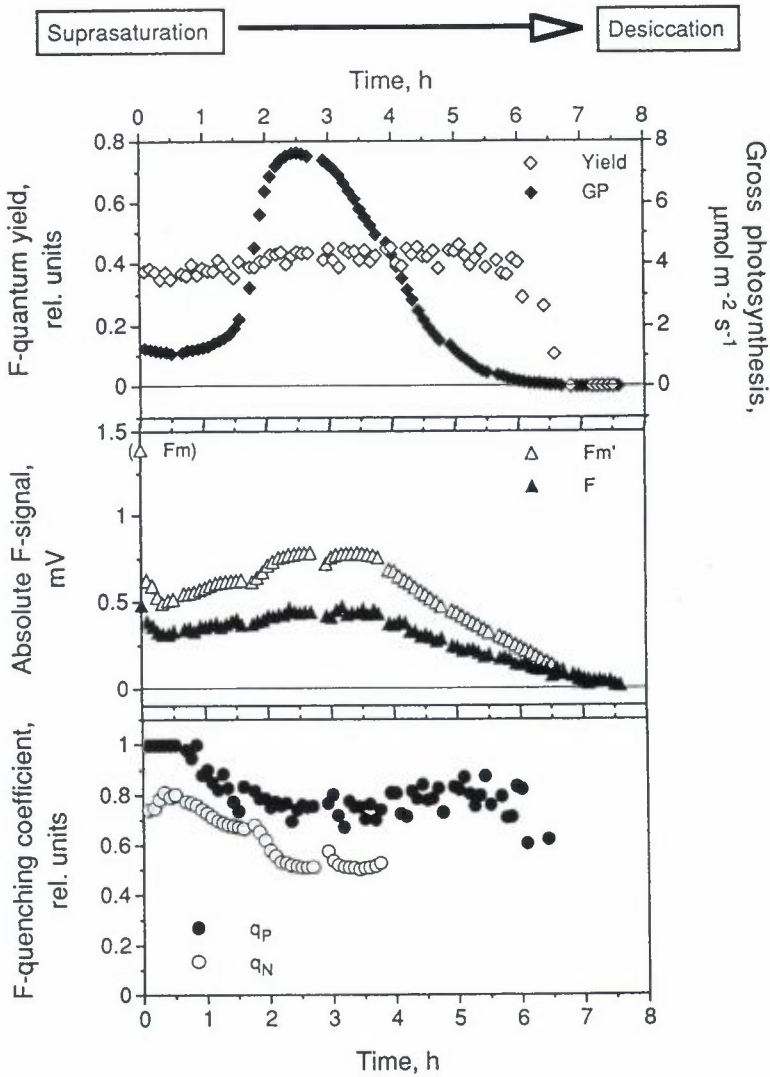
Figure 5.   Chlorophyll fluorescence data and gross photosynthesis during progressive water loss (represented by the time axis) from suprasaturation until desiccation of *L. muralis* under controlled conditions. Measurements were conducted at 1250 μmol/m^2/s (near saturating according to unpublished results) PFD, 17°C and 350 ppm CO_2 in air. In the top panel fluorescence quantum yield (open diamonds scaled on the left) and gross photosynthesis (filled diamonds scaled on the right) are depicted. In the center panel absolute fluorescence signals, and in the bottom panel the fluorescence quenching coefficients qP and qN are depicted. Fluorescence quantum yield (being equivalent to ETR under these constant conditions) remained unaffected of thallus water content over a wide range while gross photosynthesis varied by a factor of about 8. See text for further explanations.

synthesis to remove excess absorbed light energy after it has passed through PS II, one indication being the high q_P. It has long been known that algae are capable of effective photorespiratory activity (e.g. Gfeller and Gibbs, 1985; Peltier et al., 1987; Rebeille and Gans, 1988; Scherer, 1990). Thus, the high ETR at diffusion limited low photosynthetic rates could be explained by the ability of the photobiont to transfer electrons from the photochemical electron transport chain to oxygen (non-assimilatory electron transport, Osmond and Grace, 1995; Schreiber et al., 1995; Heber et al., 1996).

Also, when water availability limited CO_2 exchange, ETR remained stable until GP was very low (Fig. 5). Although absolute signal intensities decreased markedly, fluorescence quantum yield and also q_P persisted at a constant high level, indicating no gradual mitigation of PS II efficiency *per se*. One likely explanation for this is that an increasing number of complete algal cells ceased to transfer energy between the light-harvesting complex and PS II (Bilger et al., 1989). The diminishing number of active photobiont cells would produce lower fluorescence signals but, because they remained fully functional, the fluorescence ratios would remain constant at the pre-desiccation levels. Alternatively, since the light reactions are physical, in contrast to the enzymatic CO_2 fixation and CO_2 concentration, they could well be maintained for longer under desiccation stress. In that case the light energy from PSII would again have to be passed to a non-assimilative sink such as oxygen. This would also explain the maintained q_P and ETR as the CO_2 exchange declined. Lack of experimental evidence prevents any firm conclusions as to the actual processes at the moment, further analyses are needed.

The pronounced ability of the photobiont of *L. muralis* to apparently utilise an alternative sink for excited electrons from PS II while photosynthesis is limited at high WC or possibly also under desiccation could be regarded as a special adaptation to its environment. The epilithic lichen frequently becomes suprasaturated with water in its natural habitat and, on occasions, has to tolerate high light intensities in this state (Lange and Green, 1996, 1997). The ability to effectively dissipate electrons from the photochemical electron transport chain to oxygen would enable *L. muralis* to avoid overexcitation of PS II, and generate a high ΔpH which promotes q_N, both mechanisms to prevent photoinhibition. The overall result was a lack of relationship between ETR and photosynthetic CO_2 uptake.

It is possible now, from both laboratory and long-term field studies on *L. muralis*, to say that the use of fluorescence signals to predict photosynthesis has inherent problems, at least for this species. Certainly, on many occasions, the relationship between ETR and NP was excellent and almost linear (Figs. 2 and 4); at other times, especially at high thallus WC, the relationship was non-existent (Figs. 3 and 4). Unfortunately it is very difficult in the field to

discern exactly what the water status of a lichen is. In addition to their different life strategies (i.e. poikilohydry versus homoiohydry), a major difference between lichens and higher plants is the relatively high rate of DR in the former. It is still uncertain exactly how DR should be incorporated into photosynthesis measurements for the comparison with ETR. Traditionally, GP is taken to equal [NP + DR], but it is not certain if DR also resembles respiration in the light especially in algae. In addition, DR in lichens varies in a complex manner with factors such as WC, previous WC and previous NP, and respiration rates in the light are rarely known during CO_2 exchange measurements.

However, the long-term data set does confirm that chlorophyll fluorescence has an important and useful role in ecophysiological studies of lichens. The excellent relationship between the presence of a chlorophyll fluorescence signal and metabolic activity of the lichen has been well proven. With only minor exceptions, probably relating to patchy thallus desiccation, there was an almost perfect agreement between the occurrence of CO_2 exchange and detectable fluorescence. The results support the use of fluorescence monitoring techniques as performed e.g. by Schroeter et al. (1992) in Antarctica. In addition, the status of PS II is provided by nighttime fluorescence measurements, which gives the possibility to monitor the occurrence of photoinhibition or other damage to PS II (Leisner et al., 1995).

It has been suggested that, while moist in high light, lichens protect themselves against light damage by the use of screening pigments (Green and Lange, 1994). Although this could well be true also for *L. muralis*, the studies presented here show that this lichen also has a metabolic method, i.e. photochemical quenching possibly by photorespiration, for handling excess absorbed light energy. It is interesting to speculate whether the occurrence of such mechanisms is related to the ecology of the individual species. Certainly Lange et al. (1996) found no evidence for its occurrence in the partial shade lichens *Peltigera neckeri* and *P. leucophlebia* which may only rarely get suprasaturated in nature, probably because of their morphology and the highly water conductive moss substrates they grow on. *Lecanora muralis*, in contrast, grows in the open and tightly appressed to compact stone surfaces on which a water film may persist during and after rainfall, and it will often be exposed to high PFD when wet. The answer to this speculation will be important to all lichenologists hoping to use chlorophyll fluorescence for anything beyond an activity or PS II status monitoring system.

The large and still growing data set for lichens being continually monitored in the field in the klapp-cuvette will allow us to more thoroughly investigate the factors that cause the lack of relationship between ETR and CO_2 exchange for a variety of different species. In particular, evidence will be sought from

absolute fluorescence signals and associated CO_2 exchange to possibly allow the thallus water status to be estimated. However, a general caution must be taken because chlorophyll fluorescence analysis originally was developed for steady state studies on higher plants. Eucaryotic algae and cyanobacteria differ in their fluorescence performance in many respects from phanerogamous leaves (Schreiber et al., 1995). One of these differences is their ability of a very rapid induction of oxygen dependent electron flow, and to effectively utilise energy sinks alternative to CO_2 fixation. Another important difference is their poikilohydric life strategy. Further investigations about the fluorescence peculiarities of green algal and cyanobacterial photobionts in comparison with higher plant leaves would seem essential before general application of chlorophyll fluorescence data for ecophysiological analysis of lichen photosynthesis in the field is reasonable.

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