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Temperate rainforest lichens in New Zealand: high thallus water content can severely limit photosynthetic CO_2 exchange

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Abstract. CO_2 exchange rate in relation to thallus water content (WC, % of dry weight) was determined for 22 species of lichens, mainly members of the genera *Pseudocyphellaria* and *Sticta,* from a temperate rainforest, Urewera National Park, New Zealand. All data were obtained in the field, either using a standard technique in which the lichens were initially wetted (soaked or sprayed, then shaken) and allowed to slowly dry, or from periodic measurements on samples that were continuously exposed in their natural habitat. A wide range of WC was found, with species varying from 357 to 3360% for maximal WC in the field, and from 86 to 1300% for optimal WC for photosynthesis. Maximal WC for lichens, wetted by the standard technique, were almost always much less than the field maxima, due to the presence of water on the thalli. The relationships between CO_2 exchange rate and WC could be divided into four response types based on the presence, and degree, of depression of photosynthesis at high WC. Type A lichens showed no depression, and Type B only a little at maximal WC. Type C had a very large depression and, at the highest WC, CO_2 release could occur even in the light. Photosynthetic depression commenced soon after optimal WC was reached. Type D lichens showed a similar depression but the response curve had an inflection so that net photosynthesis was low but almost constant, and never negative, at higher WC. There was little apparent relationship between lichen genus or photobiont type and the response type. It was shown that high WC does limit photosynthetic CO_2 uptake under natural conditions. Lichens, taken directly from the field and allowed to dry under controlled conditions, had net photosynthesis rates that were initially strongly inhibited but rose to an optimum, before declining at low WC. The limiting effects of high WC were clearly shown when, under similar light conditions, severe photosynthetic depression followed a brief, midday, rain storm. Over the whole measuring period the lichens were rarely at their optimal WC for photosynthesis, being mostly too wet or, occasionally, too dry. Photosynthetic performance by the lichens exposed in the field was similar to that expected from the relationship between the photosynthetic rate and WC established by the standard procedure.

Key words: Lichen – Water content – Photosynthesis – Rainforest – Diffusive resistance

Lichens are poikilohydric plants with a thallus water content that tends to equilibrate with the water status of the environment. Water contents may alter quite rapidly with wetting or drying phases dependent on the supply of water and on evaporation. Lichen metabolism, especially photosynthetic production, is strongly affected by changes in water content. Air-dry lichens often have only 5–10% water content (on a dry weight basis), with a negative water potential of thousands of bars, and are metabolically inactive. With increase in water content, respiratory processes are initially activated, and then positive net photosynthesis occurs at water contents around 20% for green algal lichens, and over 100% for cyanobacterial lichens (see Lange et al. 1988). Photosynthesis subsequently increases almost linearly with hydration until optimal CO_2 uptake is reached. Water shortage, without doubt, limits primary production of lichens in many habitats, and several experimental field studies have shown special adaptations of lichens to dry conditions in arid and semi-arid habitats (see reviews by Kershaw 1985; Kappen 1988). Lichens can colonise these extreme sites because of their ability to photosynthesise at low water potentials, and to take advantage of minimal water in the form of dew, fog or even, in the case of green algal lichens, high relative humidity.

At thallus water contents greater than those allowing optimal CO_2 exchange, lichens in some laboratory studies (Kershaw 1985) show strongly depressed CO_2 exchange, even to the point of no net photosynthesis or CO_2 release only. This depression is the result of high thallus CO_2 diffusion resistances produced by excess

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water within, or on, the thallus (Lange and Tenhunen 1981; Cowan et al. 1992; Green and Lange 1993). However, in contrast to the considerable information available about photosynthesis at low water contents, little appears to be known about the ecological relevance, if any, of very high water contents (see Green and Lange 1991). It is an important question whether the water content of lichen thalli could become so high under natural conditions as to reduce primary production.

For desert, semi-desert and even mediterranean sites, either suprasaturation of lichen thalli does not occur, or periods of maximal hydration are so short that their negative impact on CO_2 fixation can probably be neglected (see Lange et al. 1985; 1991). For foliose and fruticose lichen species growing in a local xerothermic steppe formation near Würzburg (Hahn et al. 1989), and in a dune habitat at an island in the North Sea (Bruns-Strenge and Lange 1991; Lange and Bruns-Strenge 1991), the highest water contents that occur in the field approach the range for photosynthetic depression but do not greatly influence the annual carbon balance of these lichens. Longer periods of supraoptimal water content in lichen thalli occur in the arctic tundra, and may be more important as a factor affecting photosynthetic production in those habitats (Hahn 1991; Hahn et al. 1993). If high thallus water content really is a problem of ecological significance for lichens one would expect it to be most obvious in very wet terrestrial lichen habitats, such as in tropical or temperate rainforests.

In contrast to lichens of arid and polar regions, there is little literature on the physiological or ecophysiological aspects of photosynthesis of rainforest lichens. Green and his coworkers have addressed various aspects of water relations and CO_2 exchange of rainforest species from New Zealand. Some lichens were found to have a strong depression in net photosynthesis when very wet (Green and Snelgar 1981a, b), which seemed to be related to thallus structure (Snelgar et al. 1981a, b; Green et al. 1985). Low discrimination against ^{13}C, especially in cyanobacterial rainforest lichens, was interpreted as being the result of high thallus diffusion resistances during photosynthesis after hydration with liquid water (Lange et al. 1988). In all of these laboratory experiments, no general differences were found between photosynthesis in relation to water content of rainforest lichens and species from other regions. Whether increased diffusion resistances in constantly moist conditions in the rainforest would actually be detrimental could only be resolved by field studies. However, no work is known to us in which water contents and CO_2 exchange of lichens have been measured under such field conditions.

During a period of wet spring weather in October/November 1991, we recorded microclimate, water content, photosynthesis and respiration of many typical lichen species in the temperate rainforest of the Urewera National Park, North Island, New Zealand. CO_2 exchange was continuously monitored under natural conditions, and its dependence on water content, light and temperature was also determined. Comparison of the two sets of results allows conclusions to be reached about the relative importance of the different environmental and plant parameters for primary production of the different lichen species. Here we report on the effect of thallus water content on the CO_2 exchange of 23 typical macro-lichens, focusing especially on thallus suprasaturation which was found to occur commonly in the rainforest.

Materials and methods

Research site

Measurements were carried out in the spring (last days of October and first half of November 1991) in the temperate forest in the Aniwaniwa valley, Urewera National Park, Waikaremoana, New Zealand. The forest was dominated by *Nothofagus menziesii, Ixerba brexioides* and *Dacrydium cupressinum* and supported a high diversity of well-developed macro-lichens. Annual precipitation at this site is about 3000 mm. As normal for that time of year, the period of our measurements was wet with rain every day except one and, on two days, the rainfall was more than 175 mm. Average air temperature was around 10° C, ranging from near zero to almost 20° C. The measuring period was divided into two parts: initially, the instrumentation was set up within the forest, and typical shade species were studied. Subsequently, lichens from the edge of the forest and from the open were investigated. The experimental lichens are listed in Table 1; nomenclature follows Galloway (1985).

Methods

Three different instruments were used to measure lichen CO_2 exchange, climate parameters such as air temperature, air humidity, photosynthetic active photon flux density (PFD) and atmospheric CO_2 partial pressure (Tarnawski et al., unpublished data). Diel courses of CO_2 exchange were monitored by means of a CO_2/H_2O-porometer (Walz Company, Effeltrich, FRG), and adjacent to this apparatus, a click-cuvette (Walz Company) was also installed. Rapid measurement of CO_2 exchange was possible in both systems. The air stream in the small gas exchange chamber could be kept at ambient temperature conditions or controlled at constant temperatures near ambient. A third minicuvette system (Walz company) was operated in a tent nearby. The latter instrument allowed CO_2 exchange measurements under fully controlled temperature, light and humidity conditions so that photosynthesis could be investigated in relation to various factors. When required, artificial illumination was provided by lamps with fibre optics (KL 1500 electronic, Schott Glaswerke, Wiesbaden, FRG). All CO_2 measurements were done by Binos infra-red analysers (Rosemount, Hanau, FRG) operated in the differential mode. Open, flow-through systems were used and CO_2 could be measured to ±0.1 ppm, or to within 0.1 ppm CO_2 for the minicuvette. CO_2 exchange of the lichens was related to thallus dry weight (4 days at 70° C), projected area (whenever possible, determination by an area meter, LiCor, Lincoln, Nebraska, USA), thallus carbon content (elementary analysis; CHNO-Rapid, Foss Heraeus, Hanau, FRG), and chlorophyll *a* (cyanobacterial lichens) or total chlorophyll $a+b$ (green algal lichens) content (as determined by Ronen and Galun 1984; see Arnon 1949; Arnon et al. 1974). All the techniques used, together with general methodological considerations, are described in more detail in Lange et al. (1985), Bruns-Strenge and Lange (1991), and Lange et al. (1991).

For measurement of photosynthetic and respiratory CO_2 exchange, the lichen thalli were fixed in wire-mesh baskets (3.8 cm diameter, 1–1.5 cm height, mesh width 1.6 mm). In these dim evergreen rainforests, foliose lichen thalli tend to grow horizontally, either on tree trunks or branches, thus maximising light interception. We tried hard to expose the experimental samples together with their wire-mesh baskets in their natural position on a fallen tree log which had a dense cover of (partly identical) lichens and mosses. Thallus water content (WC) was determined by quickly weighing trays plus lichens on an electronic digital balance (PM 460, Mettler, Giessen, FRG; accuracy: 1 mg), and was related to the thallus dry weight as a percentage. High thallus water content is a critical measure with lichens (see Lange and Matthes 1981) because water droplets can externally adhere to the thalli, for instance at the branchings of fruticose species. Therefore, maximal water contents can vary greatly from sample to sample. For standardization, in all of our experiments in which we studied the dependence of photosynthesis on water content, maximal hydration was achieved by first submerging the thalli in, or spraying them with, rain water, followed by vigorous shaking to remove water drops and, finally, blotting the baskets with paper tissue. When monitoring the diel CO_2 exchange responses, the samples were not shaken before they were exposed in the porometer cuvette. This was considered necessary because some of the species, e.g. *Pseudocyphellaria rufovirescens,* can store water externally as droplets in small cavities on the upper surface (Green et al. 1985). As a result, much higher water contents (termed water holding capacity) were reached during the CO_2 exchange measurements in the field than with the specimens prepared for other experiments. Whenever possible, several replicates of a species were used. Time constraints and the overall aim of an investigative, general survey, did not always allow this, but when several replicates were studied they gave similar results.

In addition to the porometer measurements in which the natural responses of the lichens over diel courses were studied, two types of experiments were carried out in order to establish the relationship between CO_2 exchange and thallus water content. (1) Maximally moistened, freshly collected lichen samples were enclosed in the minicuvette at 15° C and 150 μmol m^{-2} s^{-1} PFD. CO_2 exchange was measured as soon as steady-state conditions had been attained; samples were then weighed, and exposed for a short time outside the cuvette in order to cause some water loss. CO_2 exchange was then once again determined. This procedure of decreasing water content stepwise was continued until no further water loss occurred. Typically, such an experiment lasted for several hours. (2) In other, equivalent, experiments the samples remained enclosed in the click-cuvette for longer periods, up to 24 h, at selected temperature and light intensity, and their CO_2 exchange was monitored every minute. Relative humidity was decreased in steps so that very slow water loss took place. The thallus was weighed at appropriate intervals to monitor water content. This method generated WC-dependent courses of net photosynthesis for which the water content had changed only very slowly with time thus avoiding large gradients in water potential across the lichen thalli. In the event, both methods produced similar response curves, indicating that the quicker water loss in the first approach did not cause irregular drying of the lichen thalli.

Results

The range of lichen thallus water content

Lichen species showed very large differences in thallus water contents even when under apparently identical rainforest conditions (Table 1). Fully soaked and subsequently shaken thin, heteromerous, foliose species such

Table 1. Description of the experimental lichens and the extremes of their water content (in % of thallus dry weight): the species are arranged according to increasing values of maximal experimental water content. "Maximum experimental" is the highest water content when the thalli are soaked and subsequently shaken, so that adherent water drops were removed. "Maximum field" is the highest water content of the thalli found under natural conditions in their trays, including adhering drops and water at the surface of the thalli. "Minimum field" is the lowest water content found naturally during the measuring period. Water relation types are listed according to the definition given in the text. Description of the species follows Galloway (1985). G indicates green photobionts without or with (G^+) cephalodia, BG indicates cyanobacterial photobionts. PS means that the species can be a partner of a photosymbiodeme. Structure of the different species is characterised with respect to the upper (us) and for the lower surface (ls) of the thalli. Whether the experimental material was studied and collected in the shaded habitat of the forest of or in the open clearing is also indicated

	Maximum experimental	Maximum field	Minimum field	Type
Pseudocyphellaria colensoi, G^+ foliose, large lobes, yellow medulla, pseudocyphellae (ls), isidia, soredia; australasian; forest, tree trunk and branches	189	1175	19	D
Sphaerophorus macrocarpus, G fruticose, crowded branches, cortex continuous; endemic; forest, corticolous, tree trunk	222	1022	37	A
Sticta latifrons, G^+ foliose, large thallus lobes stalked, cyphellae (ls) numerous; endemic; forest, tree trunk	261	949	24	B
Pseudocyphellaria coronata, G^+ foliose, like *P. colensoi;* australasian; forest, tree trunk and branches	269	–	–	D
Usnea xanthophana, G fruticose, erect or pendulous, no isidia, pseudocyphellae or soredia; endemic; forest, branches, lower canopy	340	496	11	B
Pseudocyphellaria homoeophylla, G^+ foliose, large orbicular thallus, pseudocyphellae (ls); endemic; forest, tree trunk	352	824	23	C/D
Pseudocyphellaria delisea, G^+ foliose, orbicular, lower surface tomentose, pseudocyphellae (ls); austral; forest, branches of trees	357	706	5.4	C

Table 1. (continued)

	Maximum experimental	Maximum field	Minimum field	Type
Pseudocyphellaria faveolata, G^+ foliose, spreading, loosely attached, upper surface reticulate-faveolate, pseudocyphellae (ls) verruciform; austral; forest, tree trunk and lower branches	367	1318	24	C/D
Usnea rubicunda, G fruticose to subpendulous, reddish, spinules and fibrils, pseudocyphellae, isidia; cosmopolian; forest, branches, lower canopy	398	478	14	B
Pseudocyphellaria dissimilis, BG foliose, linear-laciniate, isidia, pseudocyphellae (ls) minute, deep shade; australasian; forest, tree bole	417	1841	32	D
Pseudocyphellaria lividofusca, G^+ PS; foliose, thallus thick, loosely attached, lower surface tomentose, pseudocyphellae (ls, us); endemic; forest, tree trunk	476	1679	33	C
Cladina leptoclada, G fruticose, multi-branched podetia in tufts, no soredia, cortex cottony; palaeotropical; clearing, on soil	489	680	14	C
Cladia sullivanii, G fruticose, pseudopodetia branching dichotomous, walls perforate, dark medulla filling central canal; australasian; clearing, on soil	494	358	9.0	C
Sticta caliginosa, BG foliose, stalked, fragile, isidiate, cyphellae (ls) sunk in tomentum; australasian; forest, tree bole	502	955	28	A
Pseudocyphellaria rufovirescens, G^+ PS; foliose, thallus spreading, shallowly faveolate, pseudocyphellae (ls); endemic; forest, tree trunk	613	3116	71	D
Menegazzia pertransita, G foliose, closely attached to bark, thallus perforated, without isidia or soredia; endemic; forest, tree branches	747	2310	20	B
Pseudocyphellaria murrayi, BG PS; lobate-foliose, loosely attached, lower surface tomentose, pseudocyphellae (ls), maculate (us); endemic; forest, tree trunk	759	2307	34	C
Pseudocyphellaria pubescens, G^+ foliose, loosely attached, lower surface densely tomentose, pseudocyphellae large (ls); endemic; forest, tree trunk	811	1899	44	B/D
Cladia retipora, G fruticose, pseudopodetia coralloid, in cushions, walls perforated, forming network; australasian; clearing, on soil	813	668	16	B
Sticta filix, G^+ foliose, lobes palmate, almost erect, stalked, cyphellae (ls) pock-like; endemic; forest, tree trunk	1011	1663	32	D
Coccocarpia palmicola, BG foliose, orbicular, adjacent, isidia becoming coralloid, rhizines dense; pantropical; forest, tree trunk	1175	2451	92	C
Collema laeve, BG foliose, membranous, gelatinous, homoiomerous, thallus irregularly folded; australasian; forest, base of tree boles	2237	3357	563	B
(*Nephroma australe,* G^+) foliose, lobate, without isidia, soredia or pseudocyphellae; australasian; forest, tree trunk	–	928	50	–

as *Pseudocyphellaria colensoi* and *Sticta latifrons* contain water equivalent to about twice their dry weight. In contrast, after the same experimental treatment, the homoiomerous *Collema laeve* contained 10 times more water. These differences remain when water content is related to thallus area instead of thallus dry weight. *Collema laeve* holds water in its thallus equivalent to more than 2 mm of precipitation.

There is no doubt that all the species studied, under natural conditions, reached the maximal hydration which could be attained experimentally by submerging and soaking the thalli. The maximum natural water holding capacity was, in almost all cases, much higher than maximal content after removing the externally adhering water. *Collema laeve* holds more than 33 times its dry weight of water. During the measuring period, times of suprasaturation with water were interrupted by short phases of dehydration. All species investigated experienced low water contents, at least briefly, and most became so desiccated that the lower moisture compensation point of net photosynthesis was reached or at least approached.

Photosynthetic responses of three species, with extreme differences in maximal water contents, are illustrated in Fig. 1. Maximal rates of chlorophyll-related net photosynthesis (NP) of around 15 nmol CO_2 mg_{chl}^{-1} s^{-1} were similar for all three species. However, optimal rates of CO_2 fixation were reached at very different water contents. *Pseudocyphellaria faveolata* attained maximal rates of photosynthesis at a WC of 86% (Fig. 1), whereas the thallus was water-saturated at 367% (see Table 1). This water content was lower than the moisture compensation point of *Collema laeve,* which achieved positive CO_2 exchange only at a WC higher than 433%, and maximal CO_2 exchange rates at water contents greater than 1300%. *Coccocarpia palmicola* showed an intermediate response.

Dry-weight related water content has most often been taken to be the most suitable basis for representing the physiological effect of water content in lichens. Nevertheless, it can be influenced by thallus dry matter density, and for instance, a high thallus mineral content can impact calculations of WC. Therefore, water content of the experimental lichens was also related to other bases. Area-related water content (Fig. 1, middle) can be calculated as a precipitation equivalent (mm water) held in the lichen thallus. When thallus water is related to chlorophyll content (Fig. 1, bottom), it is an indication of water available per unit of photobiont. In both of the latter cases, the general pattern for the three species displayed remains the same as when water content was related to thallus dry weight.

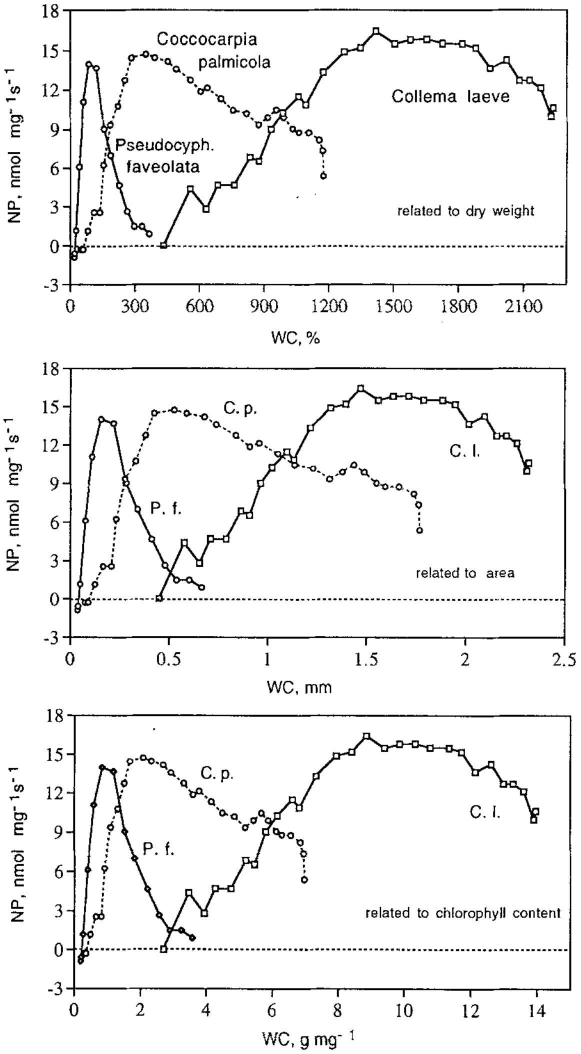

Fig. 1. Chlorophyll-related net photosynthesis (*NP*; CO_2 uptake positive) in relation to water content (*WC*) for the three extremely different species *Pseudocyphellaria faveolata, Coccocarpia palmicola,* and *Collema laeve.* Water content is related to thallus dry weight (percentage, *top*), to projected thallus area (volume water per area, i.e. precipitation equivalent, *middle*), and to thallus chlorophyll content *(bottom)*. The last data point for each response curve represents the maximal water content attained after soaking and subsequent removal of adhering droplets

Response of net photosynthesis at supraoptimal water contents

There are large and, in terms of primary production, very important differences between the species with respect to their gas exchange performance at supraoptimal thallus water content. It was found possible to subdivide these performance differences into four types that were independent of the absolute water-holding capacity (Fig. 2). Although there is always the risk that future, more extensive work will require a revision of this grouping, which is the product of a relatively small number of species sampled, this subdivision was found to be very helpful in the physiological and morphological analysis of the lichens' response to high levels of hydration. Only two species, *Sphaerophorus macrocarpus* and *Sticta caliginosa,* did not show depressed photosynthesis at their highest water contents and retained maximal rates of CO_2 fixation from about 60% to maximal thallus water content (Fig. 2, Type A). A slight depression near thallus saturation occurs in the species of type B but, in group C, depression begins at around 50% of maximal water content and increases steadily until almost no positive CO_2 exchange was found at maximal water content. *Cladia sullivanii* respires even at the highest water contents. These changes are the consequence of decreasing thallus CO_2 conductance. The release of respiratory CO_2 at extremely high water contents can be explained as the result of the development of a high CO_2 diffusion resistance between a CO_2 source and the photobiont (Cowan et al. 1992). The latter workers studied *Ramalina maciformis* where a similar net CO_2 loss was achieved by infiltration of a thallus with water. In the fourth group (D), a depression of net photosynthesis is also present at thallus water contents greater than optimal. However,

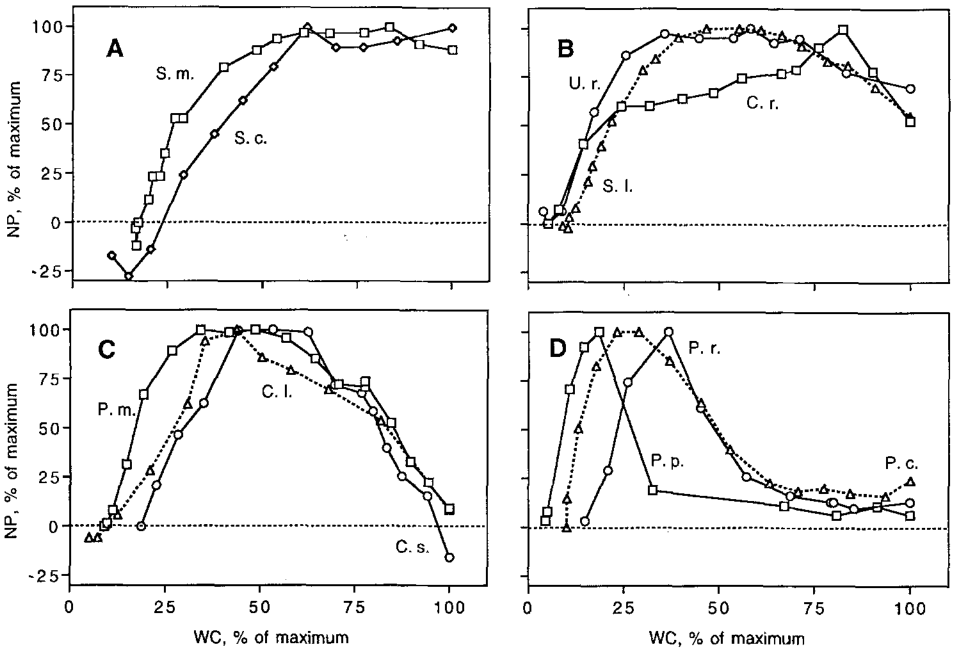

Fig. 2A–D. Net photosynthesis (*NP*, % of maximum) in relation to water content (*WC*, % of experimental maximum; see Table 1) for a selection of species. The four diagrams show the different types of water content responses discussed in the text. **A**: *S.m.*, *Sphaerophorus macrocarpus*; *S.c.*, *Sticta caliginosa*. **B**: *U.r.*, *Usnea rubicunda*; *C.r.*, *Cladia retipora*; *S.l.*, *Sticta latifrons*. **C**: *P.m.*, *Pseudocyphellaria murrayi*; *C.l.*, *Cladina leptoclada*; *C.s.*, *Cladia sullivanii*. **D**: *P.r.*, *Pseudocyphellaria rufovirescens*, *P.p.*, *Pseudocyphellaria pubescens*; *P.c.*, *Pseudocyphellaria colensoi*

the response curve shows an inflection point so that the low rates (10–25% of the optimum) attained at water contents around 60% of maximum remain almost constant until thallus saturation. The affiliation of all studied species to these groups is included in Table 1.

Occurrence of high thallus water contents in the field

Species belonging to the response types C and D, show a very strong depression of their net photosynthesis at high water contents. Not only can these water contents be attained by artificial soaking of the lichen thalli, they are also substantially surpassed by maximal lichen water contents under natural conditions in the field (Table 1). High water contents in the range that would be expected to cause strong photosynthetic depression were certainly not rare or exceptional events. They occurred frequently during our measuring period for the species of the forest interior and could last for long periods.

The range of thallus water content for the daylight (i.e. potentially photosynthetic) hours of 8 days are depicted for the cyanobacterial *Pseudocyphellaria dissimilis* (Fig. 3) and for the green algal *P. faveolata* (Fig. 4). Data are missing for November 4 and 5 when rainfall was so heavy that the instrumentation was flooded and no readings could be taken. However, thallus water contents of the lichens on those days must have been at least equal to those on the preceeding, drier day. During these 8 days, both lichens had water contents optimal for net photosynthesis only for brief periods. For most of the daylight hours they were far too wet for optimal CO_2 exchange, reaching more than 1000% WC on 3 November, with drops of water standing on the thalli. The lowest WCs on this day were 664% and 526% for *P. dissimilis* and *P. faveolata*, respectively.

Diel courses under natural conditions for *Pseudocyphellaria pubescens* (type D) are analysed in Fig. 5.

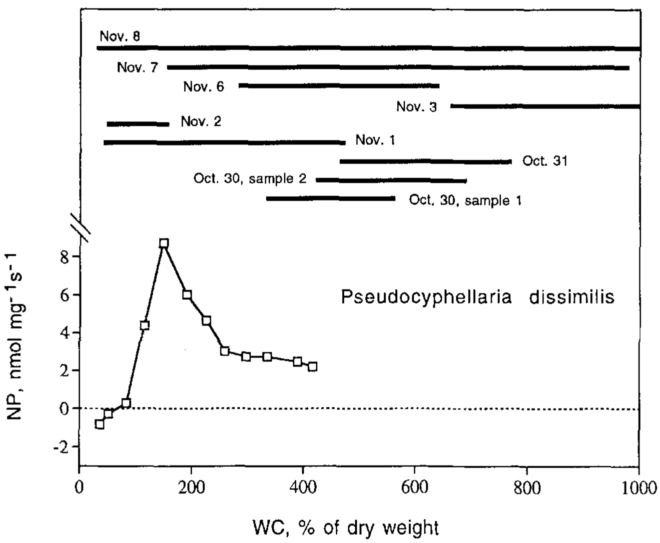

Fig. 3. Chlorophyll-related net photosynthesis (*NP*) of the cyanobacterial *Pseudocyphellaria dissimilis* in relation to water content (*WC*; dry weight basis). The horizontal bars show the range of water content of the same species under natural conditions during the daylight hours on the days indicated during the measurements in 1991

The thallus water content (ordinate) has been subdivided into sections according to net photosynthetic rates, and then overlain with the diurnal courses of WC for 7 days. On 4 days (31 October, 6, 7, 8 November) and most probably also on the very wet days 4 and 5 November, WCs were so high that the lichen never had a water content which would have allowed more than 25% of optimal photosynthesis (at saturating light and at the given temperature). On 2 days, optimal water contents did occur, however only temporarily for a few hours, until the thallus either became too dry (1 November) or was again too wet due to rain (8 November). On the driest day, 2 November, optimal water content was reached only during the early morning hours. The lichen then dried out but, even on this day, the lower moisture compensation point for CO_2 exchange was not reached. This dry phase lasted only for the remainder of the day until it rained, and the water content increased again to more than 600% during the night. For the majority of the measuring period, the net photosynthetic rate of this species would be expected to have been substantially limited by the high thallus water content.

Demonstration of net photosynthesis limitation by high water content in the field

Proof that net photosynthesis was actually depressed by high water contents under field conditions was provided by the following experiment, carried out on a day when *Pseudocyphellaria pubescens* had attained a water content of more than 1000% after rain in the night and then dried to about 800% at midday. A sample was tranferred into the cuvette, where it was subsequently kept at constant illumination (about 150 μmol m^{-2} s^{-1}), air temperature between 11 and 12° C and at a relative humidity which was slowly decreased from 87 to 72%. For the first 4 h, WC decreased from 811 to 265% (Fig. 6, lower panel) with little change in lichen net photosynthesis (Fig. 6, upper panel). But, with further drying NP suddenly increased from 3 to more than 13 nmol mg^{-1} s^{-1} in little more than 20 min. Maximal net photosynthesis was reached at a WC of about 150%, and further drying of the sample resulted in loss of photosynthetic activity. The time course clearly shows that, when collected from the field, the lichen was incapable of high rates of photosynthesis because of the unfavourable thallus water content. *P. pubescens* has a type D water-content response (see Fig. 2). The time course also shows the small range of water content over which water films, which initially greatly depress photosynthesis, appear to break, allowing optimal rates of carbon gain to be achieved.

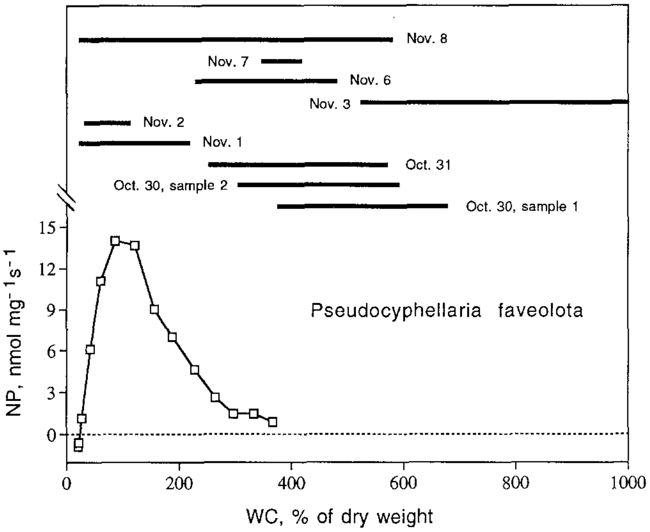

Fig. 4. Chlorophyll-related net photosynthesis *(NP)* of the green algal *Pseudocyphellaria faveolata* in relation to water content (*WC*; dry weight basis). The horizontal bars show the range of water content of the same species under natural conditions during the daylight hours on the days indicated during the measurements in 1991

Changes in CO_2 exchange rate with change in thallus water content during normal diel measurement courses also demonstrated the deleterious effect on photosynthesis of supraoptimal water contents. Conditions on 7 November were especially suitable for such a comparison because of a sudden increase in thallus water contents in the middle of the day. Temperature varied only between 7.9 and 10.9° C and relative humidity was close to saturation. Before noon, lichen water content stayed almost constant at moderately low levels, allowing rather high rates of net photosynthesis for most of the species. At noon, heavy rain quickly soaked the thalli to almost maximal water-holding capacity and they remained in that condition until night fell. Figure 7 shows the dependence of CO_2 exchange on incident PFD for four species, as extracted from the diurnal courses of photosynthetic activity. The light responses of CO_2 exchange are com-

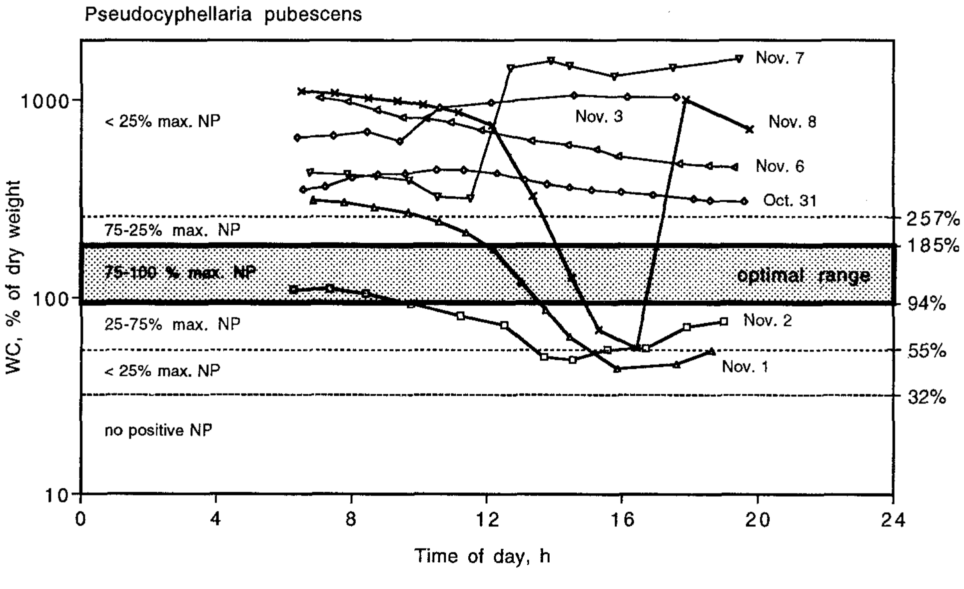

Fig. 5. Natural time courses of thallus water content *(WC)* of *Pseudocyphellaria pubescens* during the daylight hours of 7 measuring days. *Abscissa:* time of day. *Ordinate:* log of dry-weight related WC. According to the WC-dependent rates of net photosynthesis at saturating light (see Fig. 2D) the WC ranges are indicated which would allow optimal NP at saturating light (between 75 and 100% of maximum NP at 94–185% WC), or reduced rates of NP when the thalli are too wet or too dry. The moisture compensation point is at 32% WC

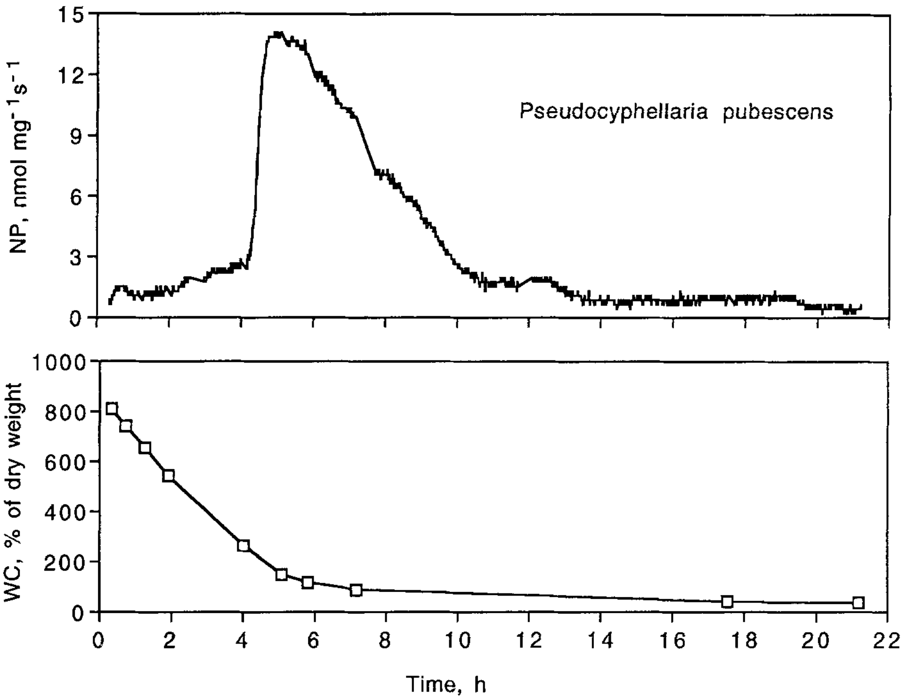

Fig. 6. Time course of chlorophyll-related net photosynthesis *(NP, above)* and water content *(WC, below)* of *Pseudocyphellaria pubescens.* The experiment began at high natural water content of the lichen, and the thalli dried out subsequently (see text for further description)

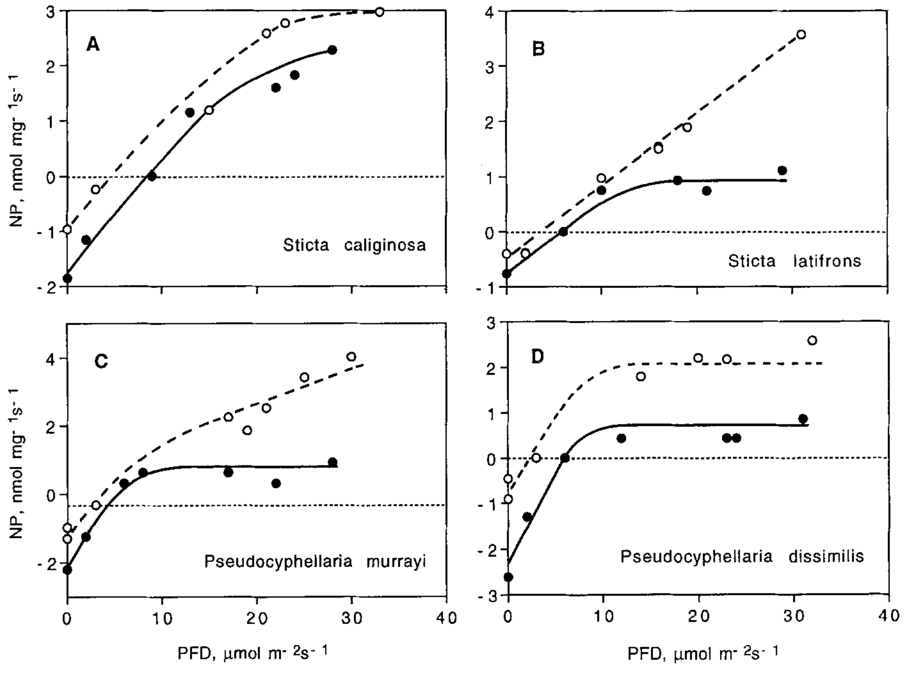

Fig. 7A–D. Chlorophyll-related net photosynthesis *(NP)* in relation to incident photon flux density *(PFD)* under natural conditions at low (before rain, *open symbols*) and high (after onset of rain, *filled symbols*) water content (7 November 1991). The four diagrams represent species which belong to the four photosynthesis versus WC response types (see Fig. 2): **A**: *Sticta caliginosa;* **B**: *Sticta latifrons;* **C**: *Pseudocyphellaria murrayi;* **D**: *Pseudocyphellaria dissimilis*

pared before and after midday, i.e. before the rain (at low thallus water content), and after the rain (at high water content). *Sticta caliginosa* (type A response, Fig. 2, with no depression of photosynthesis even at maximal water content) had almost identical PFD response curves over the range 0–35 μmol m^{-2} s^{-1} , which was the highest PFD reached during this day (Fig. 7A). In contrast, representatives of all of the three other types, namely *Sticta latifrons* (B), *Pseudocyphellaria murrayi* (C) and *P. dissimilis* (D) show saturation of photosynthesis at lower PFD in the afternoon although response at low PFD remained the same. In the afternoon, light saturation was reached at a very low PFD of 10 μmol m^{-2} s^{-1}. The high water content of the thalli allowed only small rates of net photosynthesis in the afternoon compared to the rates under otherwise indentical photosynthetic conditions in the morning. All lichens showed an increased respiration rate after the rainfall which, in itself, would tend to slightly lower net photosynthesis.

Light response of net photosynthesis in relation to thallus water content

Water content dependencies of CO_2 fixation (see Fig. 2) were established in routine experiments at an incident

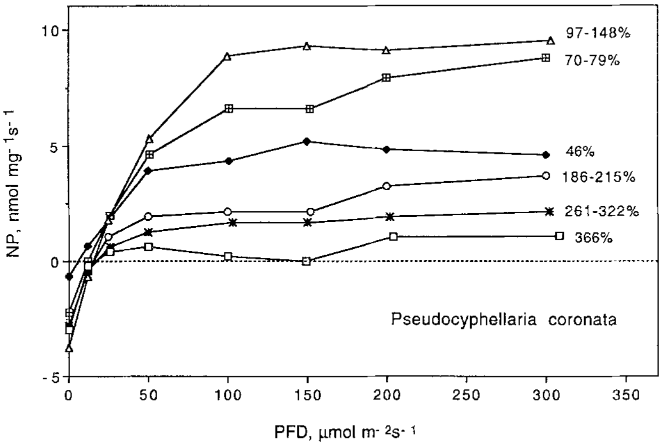

Fig. 8. Chlorophyll-related net photosynthesis *(NP)* of *Pseudocyphellaria coronata,* in relation to incident photon flux density *(PFD)* at the indicated thallus water contents (%WC, dry weight basis)

PFD of 150 µmol m^{-2} s^{-1}. At this PFD, photosynthesis was light-saturated, at least for the shade species from the interior of the rainforest. This is clearly shown in Fig. 8 in which light response curves for *Pseudocyphellaria coronata* at six different water contents are depicted. Highest rates of photosynthesis were attained at water contents between 147 and 96% and, under these conditions, light saturation took place at about 100 µmol m^{-2} s^{-1}. Both lower and higher water contents decreased the rates of net photosynthesis and, above a WC of 300%, CO_2 uptake was small and almost undetectable. Furthermore, there was a tendency for both the apparent quantum use (the initial slope of the light response curve) and the moisture compensation point to decrease with water contents above and below the optimum value. Similar response patterns were also found for other rainforest species. These results explain lichen performance at changing water contents under their natural field conditions, as illustrated for 1 day in Fig. 7.

Discussion

The studies reported here are the first of this kind on rainforest lichens in the field. They cover only a relatively short period of time, and extrapolation from the results must, therefore, be a matter for caution. Although some days were exceptionally wet, even by rainforest standards, suprasaturation of lichens was not confined to those days, but was frequent, and optimal thallus water contents occurred only for short periods. It is considered that the results are most probably of general applicability for these forests.

An astonishing range of maximum water content was found between species. Maximal dry-weight related thallus water content, or the water content which allows optimal net photosynthesis, is more than 10 times larger for the homoiomerous *Collema* species than it is for some of the thin, foliose *Pseudocyphellaria* species. These differences were retained when water content was related to carbon content of the thalli, to chlorophyll content, or to projected thallus area, suggesting that they represent fundamental characteristics of water economy. The worry that water contents often used in laboratory studies were unnaturally high can certainly be laid to rest, at least for this type of species, since these values were almost always exceeded in the field.

It has long been known that the structure of lichens can lead to depressed CO_2 fixation at high thallus water contents (Stocker 1927) due to increased thallus CO_2 diffusion resistances (Lange and Tenhunen 1981). In our investigation this depression did not always occur and a wide range of responses of net photosynthesis to thallus water content was found. Some species (categorised as types A and B; see Fig. 2) apparently do not suffer greatly from high diffusion resistances at maximal water content. Their net photosynthesis is not, or only slightly, depressed even when they are thoroughly soaked. The majority of species, however, are strongly affected by supraoptimal water contents. Apparently very frequent (40% of the species studied) is response type D, in which the WC-dependent NP curves, after a strong, initial fall as water content increases, show an inflection point, and no CO_2 release was observed even though net photosynthesis could be very low, and almost at compensation. It seems that an external water film develops and covers the lichen thallus, allowing only a very reduced rate of CO_2 transport. Changes in the thickness of this water film, once established, probably have only a small effect on total diffusion resistances (Green and Snelgar 1982). The time course of CO_2 exchange during drying supports this explanation with the steep rise in CO_2 uptake occurring over a brief period of time and a change in thallus water content, explicable as the point in which the water film becomes discontinuous (see Fig. 6). This phenomenon has been previously reported for *Sticta latifrons* thalli which had been soaked and then selectively dried on either the upper or lower surface (Green et al. 1982). In that case the difference between the two water content response curves was almost as great as the difference between types C and D reported here, further emphasising the importance of water location to CO_2 exchange by lichens. Interestingly, although for most of the species the classification into one of the response types was unequivocal, and replication of experiments on the same species led to the same results, some transients and changes in response did occur and these were normally between types C and D. From the results of Kershaw (1977) *Peltigera canina* would be classified as type C, but behaved as type D for Lange and Matthes (1981). Similar drying responses, with an increase of net photosynthesis to an optimum as the lichen dries, are already known from laboratory experiments with many other species, such as *Ramalina maciformis* (Lange and Tenhunen 1981) and *Umbilicaria aprina* (Kappen and Breuer 1991).

Few correlations seem obvious between degree of depression, or response type, and known anatomical or morphological structures of the experimental species used here. For instance, the two species which show no depression at high water contents (type A) differ greatly from one another. They are *Sticta caliginosa,* with well-developed cyphellae, and the fruticose multi-branched

Sphaerophorus macrocarpus with a continuous cortex. Both *Usnea* species studied belong to type B with only a small depression, and this may reflect the scattered structure of their photobiont layer and their thin cortex which might avoid formation of high diffusion resistances. But in the same group one also finds the fruticose *Cladia retipora,* and foliose species like *Menegazzia pertransita* and the stipitate *Sticta latifrons.* The genus *Pseudocyphellaria* is well represented in both types C and D. Some consistent differences might be expected between green algal and cyanobacterial lichens since the latter are known to apparently possess a more effective carbon transport system that would work to ameliorate the influence of a high diffusion resistance (Badger et al. 1993). None, however, are obvious, and lichens with both photobionts are found in type A (no depression) and in type C (large depression). This might reflect the dominant influence of the mycobiont; however *Pseudocyphellaria rufovirescens* (green algal) and *P. murrayi* (cyanobacterial) are members of a photosymbiodeme pair with the same mycobiont, but one is type C and the other type D.

The surprisingly wide diversity of reponses found in lichens to high thallus water content has probably underpinned some of the differences and problems in the lichen literature. In particular, there has been a long-running debate over whether or not serious CO_2 exchange depression, sometimes to only respiration, occurs at high thallus water contents. Kershaw (1985; see especially Kershaw 1977) found such responses regularly in his experiments. These results were not confirmed by Lange and Matthes (1981) who claimed that such CO_2 release at high water content could only be understood as a consequence of metabolic reactions which were influenced by the degree of hydration. They found it difficult to understand how the biochemical mechanism of photosynthesis could be affected by changes in the amount of water in the thallus, when such changes occurred under conditions where water potential would not alter, being almost zero. Although this argument may still be applicable to dark respiration, in which Kershaw (1985) often found a continuous, increasing response to water content, even in the supraoptimal range, it no longer seems to be sustainable for changes in net photosynthesis. Cowan et al. (1992) have calculated the distribution of CO_2 diffusion resistances within the cortex and the medulla of the lichen thallus for different degrees of hydration in relation to the carboxylation resistances of the photobionts. They showed that a water content is reached at which some of the respiratory CO_2 of the mycobiont is not reassimilated even if the internal CO_2 partial pressure around the photobiont is very low. This CO_2 then diffuses to the outside because diffusive resistances in that direction are smaller than towards the photobiont. This satisfactorily explains the experimental results which are summarised by Kershaw (1985) and which are shown by some of the species here, those with response type C.

Morphological explanations for the variability in maximum water content and for the different responses at high water content are not yet available, and a large and important field for functional-anatomical studies remains. The interaction between thallus structure, water content and CO_2 exchange is an area of considerable interest. Several *Pseudocyphellaria* and *Sticta* species have been investigated by Green et al. (1985), with the emphasis on clarifying diffusion pathways. Pseudocyphellae and cyphellae, which are mainly found on the lower side of the thalli and which characterise these lichens of the Stictaceae family, proved to be major CO_2 exchange pathways (Green et al. 1982) and were interpreted as special anatomical adaptations to maintain CO_2 flux in the hydrated thallus. Water loss, on the other hand occurs over the whole thallus surface of these foliose lichens (Green and Snelgar 1982). Inside the medulla, protected CO_2 diffusion pathways, which might be waterproofed by lichen substances (see Honegger 1991), are considered to maintain CO_2 supply to the photobionts. All of these mechanisms are apparently of limited success, because depression of net photosynthesis or even total loss of net carbon fixation may still occur at high water contents.

The high lichen biomass indicates that rainforests offer a favourable environment for these organisms; nevertheless lichens seem to be constrained by several factors. Extreme dimness during most of the daylight hours and the danger of photoinhibitory damage by short light flecks with high intensities are limitations often considered in connection with the rainforest habitat (see Demmig-Adams et al. 1990a, b). Surplus of water has now been shown to constitute an additional environmental factor which reduces lichen photosynthetic productivity. High lichen cover in these forest environments is often concentrated in relatively drier places, e.g. the upper canopy, and this may reflect the preferences of lichens for microsites which provide some water loss in order to avoid long-lasting suprasaturation. In the wettest habitats, lichens seem to become outcompeted by bryophytes which, apparently, are better able to handle large amounts of liquid water and maintain a reasonable photosynthetic CO_2 fixation at the same time (see Green and Lange 1993). Thus, the distribution of the different lichen species in the rainforest might, to a considerable extent, be determined by differences in morphological and in functional adaptation to high thallus water contents.

In a following communication we shall report diel courses of CO_2 exchange and water relations of the different rainforest lichens, which will show species-specific peculiarities in response patterns to carbon gain attained under a variety of weather conditions. As a next step of the analysis we plan investigations into the anatomical and morphological structure of the different lichen types with respect to their water-content related photosynthetic performance.

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References

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Arnon DI, McSwain BD, Tsujimoto HY, Wada K (1974) Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin. Biochim Biophys Acta 357:231–245

Badger MR, Pfanz H, Büdel B, Heber U, Lange OL (1993) Evidence for the functioning of photosynthetic CO_2 concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. Planta 191:57–70

Bruns-Strenge S, Lange OL (1991) Photosynthetische Primärproduktion der Flechte *Cladonia portentosa* an einem Dünenstandort auf der Nordseeinsel Baltrum. I. Freilandmessungen von Mikroklima, Wassergehalt und CO_2-Gaswechsel. Flora 185:73–97

Cowan IR, Lange OL, Green TGA (1992) Carbon-dioxide exchange in lichens: determination of transport and carboxylation characteristics. Planta 187:282–294

Demmig-Adams B, Màguas C, Adams III WW, Meyer A, Kilian E, Lange OL (1990a) Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. Planta 180:400–409

Demmig-Adams B, Adams III WW, Green TGA, Czygan F-C, Lange OL (1990b) Differences in the susceptibility to light stress in two lichens forming a phycosymbiodeme, one partner possessing and one lacking the xanthophyll cycle. Oecologia 84:451–456

Galloway DJ (1985) Flora of New Zealand – lichens. P.D. Hasselberg, Government Printer, Wellington

Green TGA, Lange OL (1991) Ecophysiological adaptations of the lichen genera *Pseudocyphellaria* and *Sticta* to south temperate rainforests. Lichenologist 23:267–282

Green TGA, Lange OL (1993) Photosynthesis of poikilohydric plants: Lichens and bryophytes – a comparison. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis (Ecological Studies 100). Springer, Berlin Heidelberg New York (in press)

Green TGA, Snelgar WP (1981a) Carbon dioxide exchange in lichens: relationship between net assimmilation rate and CO_2 concentration. Plant Physiol 68:199–201

Green TGA, Snelgar WP (1981b) Carbon dioxide exchange in lichens: partition of total CO_2 resistances at different thallus water contents into transport and carboxylation components. Physiol Plant 52:411–416

Green TGA, Snelgar WP (1982) Carbon dioxide exchange in lichens: relationship between the diffusive resistance of carbon dioxide and water vapour. Lichenologist 14:255–260

Green TGA, Snelgar WP, Brown DH (1982) Carbon dioxide exchange through the cyphellate lower cortex of *Sticta latifrons* Rich. New Phytol 88:421–426

Green TGA, Snelgar WP, Wilkins AL (1985) Photosynthesis, water relations and thallus structure of Stictaceae lichens. In: Brown DH (ed) Lichen physiology and cell biology. Plenum Press, New York London, pp 57–76

Green TGA, Kilian E, Lange OL (1991) *Pseudocyphellaria dissimilis*, a desiccation sensitive, highly shade adapted lichen from New Zealand. Oecologia 85:498–503

Hahn SC (1991) Photosynthese und Wasserhaushalt von Flechten in der Tundra Alaskas: Gaswechselmessungen unter natürlichen Bedingungen und experimentelle Faktorenanalyse. Dissertation Würzburg

Hahn SC, Speer D, Meyer A, Lange OL (1989) Photosynthetische Primärproduktion von epigäischen Flechten im "Mainfränkischen Trockenrasen". I. Tagesläufe von Mikroklima, Wassergehalt und CO_2-Gaswechsel zu den verschiedenen Jahreszeiten. Flora 182:313–339

Hahn SC, Tenhunen JD, Popp PW, Meyer A, Lange OL (1993) Upland tundra vegetation in the foothills of the Brooks Range, Alaska: diurnal CO_2 exchange patterns of characteristic lichen species. Flora 188:125–143

Honegger R (1991) Functional aspects of the lichen symbiosis. Annu Rev Plant Physiol 42:553–578

Kappen L (1988) Ecophysiological relationships in different climatic regions. in: Galun M (ed) CRC-Handbook of lichenology, Vol II. Boca Raton, Fla, pp 37–100

Kappen L, Breuer M (1991) Ecological and physiological investigations in continental Antarctic cryptogams II. Moisture relations and photosynthesis of lichens near Casey Station, Wilkes Land. Antarct Sci 3:273–278

Kershaw KA (1977) Physiological-environmental interactions in lichens. II. The pattern of net photosynthetic acclimation in *Peltigera canina* (L.) Willd. var. *praetextata* (Floerke in Somm.) Hue and *P. polydactyla* (Neck.) Hoffm. New Phytol 79:377–390

Kershaw KA (1985) Physiological ecology of lichens. Cambridge University Press, Cambridge

Lange OL, Bruns-Strenge S (1991) Photosynthetische Primärproduktion der Flechte *Cladonia portentosa* an einem Dünenstandort auf der Nordseeinsel Baltrum. II. Photosynthesemodell: Entwicklung, Parametrisierung durch Messungen des CO_2-Gaswechsels unter kontrollierten Bedingungen und Test. Flora 185:214–232

Lange OL, Matthes U (1981) Moisture-dependent CO_2 exchange of lichens. Photosynthetica 15:555–574

Lange OL, Tenhunen JD (1981) Moisture content and CO_2 exchange of lichens. II. Depression of net photosynthesis in *Ramalina maciformis* at high water content is caused by increased thallus carbon dioxide diffusion resistance. Oecologia 51:426–429

Lange OL, Tenhunen JD, Harley PC, Walz H (1985) Method for field measurements of CO_2-exchange. The diurnal changes in net photosynthesis and photosynthetic capacity of lichens under mediterranean climatic conditions. In: Brown DH (ed) Lichen physiology and cell biology. Plenum Press, New York London, pp 23–39

Lange OL, Green TGA, Ziegler H (1988) Water status related photosynthesis and carbon isotope discrimination in species of the lichen genus *Pseudocyphellaria* with green and blue-green photobionts and in photosymbiodemes. Oecologia 75:394–411

Lange OL, Meyer A, Ullmann I, Zellner H (1991) Mikroklima, Wassergehalt und Photosynthese von Flechten in der küstennahen Nebelzone der Namib-Wüste: Messungen während der herbstlichen Witterungsperiode. Flora 185:233–266

Ronen R, Galun M (1984) Pigment extraction from lichens with dimethylsulfoxide (DMSO) and estimation of chlorophyll degradation. Env Exp Bot 24:239–245

Snelgar WP, Green TGA, Beltz C (1981a) Carbon dioxide exchange in lichens: Estimation of magnitude and location of internal CO_2 transport resistances. Physiol Plant 52:417–422

Snelgar WP, Green TGA, Wilkins AL (1981b) Carbon dioxide exchange in lichens: Resistances to CO_2 uptake at different thallus water contents. New Phytol 88:353–361

Stocker O (1927) Physiologische und ökologische Untersuchungen an Laub- und Strauchflechten. Flora 121:334–415