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ORIGINAL ARTICLE

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Seasonal changes in temperature and light drive acclimation of photosynthetic physiology and macromolecular content in *Lobaria pulmonaria*

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Abstract *Lobaria pulmonaria* (L.) Hoffm. is an epiphytic lichen common to temperate deciduous forests where it copes with large changes in temperature and light levels through repeated annual cycles. Samples of *L. pulmonaria* were taken from a deciduous forest in southeastern Canada at 35-day intervals from February 1999 to February 2000 and also from a rare population in an evergreen forest in March and August 1999. At field-ambient temperatures and light levels, the realised photosystem II (PSII) electron transport was low both in the summer and winter, with transient peaks in the spring and autumn. In contrast, the seasonal pattern of potential electron transport measured at a fixed 20°C peaked in winter, showing the importance of temperature in driving photosynthesis to low levels in the winter despite an acclimation of electron-transport potential to exploit the high ambient light. Realised gross CO_2 uptake was correlated with PSII electron transport at mechanistically plausible rates at all sampling sites in the summer but not in the winter, indicating electron diversion away from CO_2 fixation in the winter. Chlorophyll content was highest in the dark summer months. The amount of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) large subunit (LSU) was highest in spring. Changes in the level of this hyperabundant protein and in the activity of PSII maintained a relatively constant rate of maximum CO_2 uptake per RuBisCO LSU from April through November, despite great changes in the seasonal light and temperature. *L. pulmonaria* acclimates between light and temperature stress in the winter months to light-limitation in the dark summer months. Transition intervals in the spring and autumn, with warm, bright and wet conditions, are likely the most amenable times for growth.

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Abbreviations ETR: electron-transport rate through PSII, · LSU: large subunit of RuBisCO, · NPQ: non-photochemical chlorophyll *a* fluorescence quenching, · PPFD: photosynthetic photon flux density, · q_P: photochemical chlorophyll *a* fluorescence quenching, · RuBisCO: ribulose-1,5-bisphosphate carboxylase-oxygenase

Introduction

Lichens are unusual in that they are long lived and persist through many seasonal cycles of environmental change, but they are only intermittently active during these changes because they are poikilohydric. The photosynthesis and growth of lichens are influenced by seasonal environmental change in the ambient light, temperature and moisture. Acclimation of their photosynthetic physiology could help lichens to maintain carbon reduction in these changing environments. Alternatively, lichens might wait to exploit only those interludes of conditions that match a more fixed physiological acclimation state.

Lobaria pulmonaria is a tripartite lichen association with a minor nitrogen-fixing cyanobacterial partner and a primary green-algal photobiont (Jordan 1973), whose photosynthesis provides organic carbon to sustain the association (Honegger 1991). It is a foliose, epiphytic lichen with a circumpolar distribution and is common in undisturbed deciduous woodlands as the most conspicuous member of the lobarion alliance of epiphyte species (Rose 1988). Several studies have used this lichen as an old-forest indicator species because it is sensitive to deforestation (Gauslaa and Solhaug 1996, 1999) and SO_2 air pollution (Farmer et al. 1991). It is typically an epiphyte on maple (*Acer* spp.) trees but more rarely it will

grow on spruce (*Picea* spp.). In deciduous woods there is a strong seasonal change in the light environment associated with canopy closure in spring and subsequent defoliation in autumn. Lichens in these forests are subjected to greater maximum solar radiation during the cold winter months because of the open canopy, while those in coniferous forests experience a lower and relatively consistent light environment throughout the year.

Several other lichens have been shown to acclimate to seasonal changes in both light and temperature. Periods of photosynthetic activity measured by chlorophyll fluorescence vary seasonally in *Peltigera* (Lange et al. 1999). Seasonal changes in photosynthetic CO_2 uptake can be driven by light and temperature (Coxson and-Kershaw 1984; Kershaw 1984) or the combination of both (Larson and Kershaw 1975). Chlorophyll content can also change seasonally (MacFarlane et al. 1983; Kershaw and Webber 1984). *L. pulmonaria* is sensitive to high light and particularly to seasonal changes in light (Gauslaa and Solhaug 1999).

Photosynthetic light-response curves are a means to quantitatively assess the light acclimation status of a photosynthetic organism by determining maximal rates and efficiencies of photosynthetic processes. We therefore measured light response curves of photosystem II (PSII) electron transport and CO_2 exchange in populations of *L. pulmonaria* from both deciduous and evergreen forests to detect seasonal changes in photosynthetic performance and acclimation status. Changes in chlorophyll and ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) concentrations and in the level of transcription of the *rbcL* gene encoding the large subunit (LSU) of RuBisCO were also tracked through the year. RuBisCO is the hyperabundant enzyme responsible for primary CO_2 fixation. The concentration of this enzyme is one limit on the maximum rate of photosynthetic CO_2 uptake. These measurements helped explain the molecular basis for the large changes observed in light capture, light energy allocation and CO_2-fixation capacity as *L. pulmonaria* moved from a light-limited acclimation state in the summer to a light-saturated acclimation state in the winter.

Materials and methods

Sampling sites and thallus collection

Populations of *L. pulmonaria* (L.) Hoffm. were collected from four forested sites in southeastern New Brunswick and northern Nova Scotia, Canada. Three of the sites (Walker Road, N.B., 45° 56.65′ N, 64° 24.56′ W; Fenwick Park, N.S., 45° 44.22′ N, 64° 10.01′ W; and Sugarloaf Brook, N.S., 45° 34.23′ N, 63° 49.07′ W) were in maple (*Acer* sp.)-dominated hardwood forests, while the remaining site (Economy Mountain, N.S., 45° 24.33′ N, 64° 01.79 W) was in a dense white spruce (*Picea glauca*) coastal forest. The *L. pulmonaria* population at Walker Road was sampled between 7 February 1999 and 6 April 2000 at approximately 35-day intervals. The remaining sampling sites were sampled in March 1999 and August 1999. Mean daily maximum temperature and integrated precipitation over the 13 days preceding each site visit to Walker Road was calculated from measurements at the nearby Environment Canada Sackville station, and field-ambient photosynthetic photon flux density (PPFD) was measured at the time and site of thallus collection at all sampling sites (Fig. 1).

Intact, air-dry, individual thalli were removed from their substrate trees and sealed in plastic bags for transport back to the laboratory in the dark at < 5°C. On 9 March 1999, 8 August 1999 and 17 October 1999 it was impossible to select dry individuals. In these cases, thalli were air-dried for up to 3 h at 25°C in the dark, immediately upon return to the laboratory. This drying wasdone as rapidly as possible at room temperature and in the dark to minimise excitation pressure within the desiccating photosynthetic apparatus and to minimise acclimation that might be driven by ambient light. We have tested (one-way ANOVA) for significant effects of hydration status at the time of sampling on subsequent physiological measures and found no meaningful effect. We have thus discounted any strong connection between hydration status immediately prior to sample harvesting and the physiological and macromolecular status of the lichens. All thalli were stored dry and in darkness at –20 °C until required for physiological

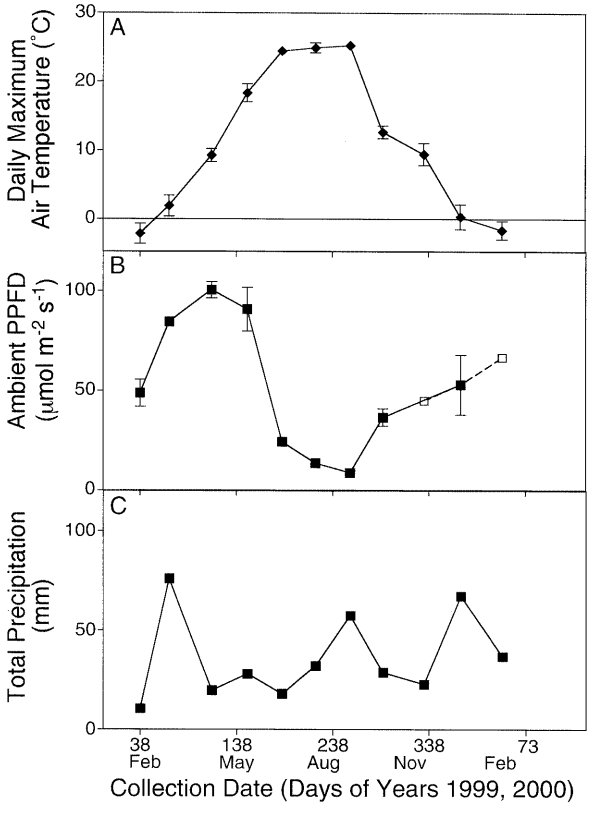

Fig. 1 Mean daily maximum temperature measured at the Environment Canada Sackville station (**A**), field-ambient PPFD measured at the time and site of thallus collection (**B**) and total precipitation at Walker Road (**C**) from 7 February 1999 to 18 February 2000. Mean daily maximum temperature values are means ± SE for the 13 days up to and including the date of sampling. Sample PPFD was determined once for each thallus at the point of collection and averaged to give the ambient PPFD. *Open squares* in **B** indicate data interpolated due to instrument failure; interpolated data were based on recorded values from adjacent sampling dates and were used only as a guide for the realised light levels described in Fig. 4. Total precipitation was normalised to water-column height and is expressed as a total for the 13 days up to and including the date of sampling, rather than mean ± SE because of its discontinuous nature. Precipitation was dominated by snow between mid December and early April, thus liquid water available from precipitation was largely restricted to the spring, summer and autumn months

experimentation, all of which occurred within 15 months of sample collection. *L. pulmonaria* remains photosynthetically active upon re-hydration after many years of dry freezer storage (Feige and Jensen 1987).

Upon their day of use, tissue disks of ca. 1.25 cm^2 were punched from each individual thallus at the first branch node behind the young growing tip of the thallus, a region broad enough to consistently provide full disk samples. The physiological and macromolecular parameters associated with this location in the thallus are similar to those of the marginal 1 cm (data not shown). Immediately prior to use, each disk was allowed to re-hydrate for 90 min between sheets of tissue paper dampened with double-distilled water. This re-hydration period was sufficient to allow full activation of photosynthetic processes. Processing of the lichen samples from collection to physiological examination in the laboratory was planned to minimise the length of metabolically active (hydrated and warm) time for each thallus between collection and measurement, and to minimise the potential for physiological or molecular acclimation away from the field condition at the time of sampling.

Measurement of chlorophyll fluorescence

After re-hydration, tissue disks were blotted of excess water and placed into a cuvette for chlorophyll fluorometry and CO_2-exchange analysis in which the temperature, PPFD, ambient gas and humidity were controlled. For most experiments, measurement temperature was set to match the field-ambient temperature prevailing at the time of sample collection. Cold actinic illumination from a DC-powered incandescent bulb (Phillips) was delivered to the cuvette via one branch of a fibre-optic bundle (Walz) terminating at a glass window just above the lichen surface. The PPFD reaching the lichen surface was continuously monitored within the cuvette by an S1133 silicon photodiode (Hamamatsu), which was periodically calibrated with a LI-250 PAR Meter (Li-Cor). The atmosphere within the cuvette was isolated from the laboratory by a piping system, which drew fresh air from outside the building (which is located near the Walker Road sampling site). The CO_2 concentration of the air flowing into the cuvette was periodically monitored by one of two infrared gas analysers (IRGAs; Analytical Development Corp.; or Qubit Systems).

Chlorophyll fluorescence was measured from the thallus disk samples in the cuvette described above. The samples were kept in the dark in the cuvette for at least 14 min to allow for dark-acclimation, a condition with the algal photosystems maximally able to accept light energyand release electrons to plastoquinone (Schreiber et al. 1994). Chlorophyll fluorescence from the samples was excited by a blue light-emitting diode (LED; peak emission 460 nm) modulated at 1.6 kHz for an average PPFD of ca. 0.02 µmol photons m^{-2} s^{-1}. Fluorescence was measured via a branch of the common fibre-optic bundle by a PAM-101 Chlorophyll fluorometer (Walz). The intensity of the measuring LED was sufficient to excite minimal chlorophyll fluorescence from the sample, but insufficient to drive significant photochemistry. Minimal (F_o) and maximal (F_m) fluorescence were determined from the dark-acclimated samples using a light-saturation flash of ca. 1,800 µmol photons m^{-2} s^{-1} for 0.8 s delivered by a Schott KL 1500 electronic flash lamp (Walz) and controlled by a PAM103 Flash Trigger Control unit (Walz). This light intensity was strong enough to excite maximal fluorescence, but low and brief enough to avoid photoinhibitory damage. Then the sample was illuminated by a range of cold actinic light intensities (PPFDs of 4.4, 5.1, 12, 29, 58, 100, 201 and 298 µmol photons m^{-2} s^{-1}), in increasing order. During illuminated periods, the excitation LED modulation rate was increased to 100 kHz (providing an average PPFD of 1.23 µmol photons m^{-2} s^{-1} in addition to the actinic illumination) to increase the signal/noise ratio of the fluorescence signal. Once the sample reached steady-state fluorescence yield (F_s) at each new light intensity, typically after 100–300 s, a saturation flash was delivered to measure the realised maximal fluorescence (F'_m) at each light intensity, and the sample was then transiently darkened to determine realised minimal fluorescence (F'_o) (fluorescence nomenclature following van Kooten and Snel 1990). In addition to measures at field-ambient temperature, chlorophyll fluorescence was also measured at a fixed 20 °C to distinguish between effects of seasonal changes in temperature and light intensity on PSII function.

Fluorescence parameters

With the fluorescence values measured as described above, we calculated PSII electron-transport rate (ETR), photochemical fluorescence quenching (q_p) and non-photochemical fluorescence quenching (NPQ) to track seasonal acclimation of PSII. The photochemical yield of PSII electron transport, ϕ_{PSII}, was calculated as (Genty et al. 1989):

$$\phi_{PSII} = \frac{F'_m - F_s}{F'_m} \quad (1)$$

and electron transport through PSII was estimated as:

$$ETR_{PSII} = \frac{\phi_{PSII} I}{2} \quad (2)$$

where I is the PPFD in µmol photons m^{-2} s^{-1} and ETR is electron transport rate in µmol m^{-2} s^{-1} of thallus tissue. The product of ϕ_{PSII} and I is halved, assuming an equal distribution of absorbed light to PSII and PSI (Gentyet al. 1989). The electron-transport results for the light-intensity series were fitted with a model curve of electron transport through PSII versus PPFD (modified from Ricker 1975):

$$ETR = \alpha I e^{(-\beta I)} \quad (3)$$

where α is the initial slope of electron transport versus PPFD, I is the PPFD and β is a curvature parameter. To assess photosynthetic acclimation, we extracted chlorophyll fluorescence parameters at the PPFD required to reach half-maximum electron-transport rate on the light response curve, measured at field-ambient temperatures (see example in Fig. 2). To assess realised field performance we extracted chlorophyll fluorescence parameters at the field-ambient light level at the time of collection. We also estimated the maximum potential capacities of these parameters by extracting them at the PPFD required for maximal electron transport at a fixed 20 °C. Light response curves of electron transport estimated by chlorophyll fluorescence have been used to assess photosynthetic

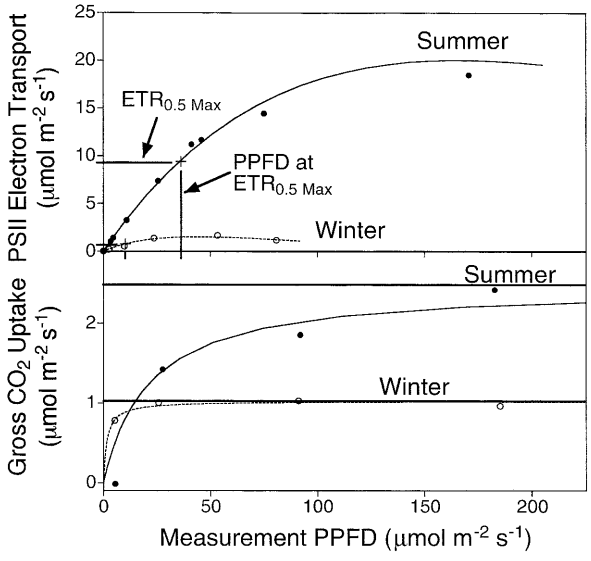

Fig. 2 Light response curves of electron transport, and gross CO_2 uptake light-response curves, showing representative summer (*solid symbols and line*) and winter (*open symbols and dashed line*) samples, measured at field-ambient temperatures from the time of collection of *L. pulmonaria* thallus

acclimation in algae (Masojídek et al. 1999) and other photosynthetic organisms (Schreiber et al. 1994).

The approximate proportion of open PSII centres, q_p, was calculated as (Genty et al. 1989):

$$q_p = \frac{F'_m - F_s}{F'_m - F'_o} \quad (4)$$

Non-photochemical quenching (NPQ), representing the absorbed light energy dissipated as heat, was calculated by (Bilger and Schreiber 1986):

$$NPQ = \frac{F_m - F'_m}{F'_m} \quad (5)$$

Measurement of CO_2 exchange

The gas mixture in the cuvette was sampled for CO_2 analysis in the dark and during the actinic illumination at PPFDs of 5.1, 29, 100, 201 and 298 μmol photons m^{-2} s^{-1}, simultaneously with the chlorophyll fluorescence measurements. The gas was sampled by flushing it into a 20-ml syringe after a measured time of incubation in the static cuvette atmosphere (typically 2–4 min). The gas samples were then injected through a Drierite desiccant cartridge into an IRGA, with responses standardised by known mixtures of 1,020 ppm CO_2 in air and N_2 (BOC Gases). Brown et al. (1983) and Sundberg et al. (1999) have shown that lichen respiration is elevated and variable soon after re-hydration (the respiration-burst phenomenon). Thus all CO_2-exchange data here are expressed as gross CO_2 uptake, correcting net exchange by several measurements of dark respiration rate from each sample.

Chlorophyll fluorescence, CO_2 exchange, actinic light intensity and cuvette temperature were recorded by a Universal Lab Interface and LoggerPro software (Vernier Software), running on a Power Macintosh G3 Model M4787 computer (Apple Computer, Inc.). The CO_2 signal was recorded by a Serial Box Interface and Logger software (Vernier Software), running on a Macintosh IIsi computer (Apple Computer, Inc.).

After physiological analysis, the sample disks were removed from the cuvette and their images were digitised on a UMAX Astra 1220S scanner and analysed with the NIHImage (National Institutes of Health, USA) analysis software package to determine their area. Sample dry weight was measured after baking the samples over a bed of Drierite desiccant at 65 °C for 24 h. The samples were then stored at –20 °C until use for pigment and protein analyses.

Chlorophyll determination

Chlorophyll was extracted from thallus disk samples of ca. 1.25 cm^2 taken from the same point of the thallus at which the physiological measures were recorded. The tissue disks were ground and pigments extracted with 90% acetone in water saturated with $MgCO_3$ as described in Barnes et al. (1992). Tissue pellets were reserved from the acetone extractions for protein extraction. We used acetone extraction rather than the popular dimethyl sulfoxide extraction also described by Barnes et al. (1992) because the latter was incompatible with the following protein extraction protocol. Serial re-extraction of the ground tissue showed we attained >95% yield of chlorophyll using this acetone extraction.

Determination of RuBisCO LSU

The reserved tissue pellets from the acetone extractions (see above) were dried to completion in a stream of nitrogen, then proteins were solubilised in 250 μl of 100 mM Tris base, 160 mM sucrose, 1 mM EDTA, 1% sodium dodecyl sulfate, 0.5% dithiothreitol. The samples were frozen in liquid nitrogen, thawed while sonicated by a Sonic 300 Dismembrator (Artek Systems), then heated at 95°C for 5 min in a water bath to extract, denature and solubilise proteins. The samples were centrifuged at 12,000 *g* for 5 min and the supernatants containing the solubilised proteins were stored at –80°C until use. Serial re-extraction of the ground tissue showed we attained an average of 80% yield of RuBisCO using this protocol, with little variation in extraction efficiency between samples.

The proteins were separated on linear 10% SDS-polyacrylamide ReadyGels (BioRad) using a Mini-Protean II (BioRad) cell. Samples were loaded on the basis of equal thallus area, with extract from 8 mm^2 of thallus per sample lane, and with isolated denatured spinach RuBisCO loaded as a quantity standard on all gels. The gels were electrophoresed at 100 V for 100 min in 25 mM Tris base, 192 mM glycine, 0.1% SDS.

The proteins were then transferred from the gel in 49 mM Tris, 39 mM glycine, 0.04% SDS, 20% methanol to polyvinylidenedifluoride (PVDF) membranes in a Semi-Dry Transfer Cell (BioRad) at 13 V for 25 min. The PVDF membrane was then washed in Tris-buffered saline (TBS-T: 20 mM Tris base, 500 mM NaCl, 0.05% Tween-20; pH 7.5) and soaked for 1 h at 25°C in 5% low-fat powdered milk in TBS-T. The membrane was incubated with a primary rabbit anti-spinach RuBisCO LSU antibody (kind gift of Göran Samuelsson, Umeå University, Sweden) diluted 1:8,000 in 10 ml of blocking buffer, for 1 h at 25°C. The membrane was then rinsed with TBS-T and incubated with a secondary goat anti-rabbit IgG antibody conjugated to alkaline phosphatase (Sigma-Aldrich), diluted 1:4,000 in 10 ml blocking buffer, for 1 h at 25°C. The membrane was then incubated in 10 ml 100 mM Tris base, 100 mM glycine, 5 mM $MgCl_2$, 0.028% 4-nitro blue tetrazolium chloride (NBT) and 0.014% 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) for 4–5 min in the dark at 25°C to quantitatively indicate the presence of RuBisCO LSU. The blots were imaged on a UMAX Astra 1220S scanner for later image analysis with the NIHImage analysis package.

Expression of *rbcL*

Expression of *rbcL*, which encodes the protein RuBisCO LSU, was determined qualitatively using reverse transcription polymerase chain reaction (RT-PCR) ina Biometra T-Gradient thermocycler (Whatmann). RNA was extracted from ca. 100-mg samples of *L. pulmonaria* tissue taken from the same thallus region as the samples used for protein measures, using the Trizol reagent and procedure (Life Technologies). A 50-ng sample of extracted RNA was then mixed in 50 μl of DEPC water with Ready-To-Go RT-PCR beads (Amersham Pharmacia) with 20 pmol of each forward (5′-AGGYGTTCCWSCWGAAG-3′) and reverse (5′-CACGRCTACRATCTTTTTC-3′) DNA primer specific to conserved regions of chlorophyte *rbcL*, bracketing a 946-bp segment of the gene. These primers were designed to be specific for chlorophyte *rbcL* to exclude contamination by *rbcL* expressed by cephalodial cyanobacteria. Reverse transcription of *rbcL* transcripts proceeded for 30 min at 42°C, followed by 2 min at 94°C to denature reverse transcriptase. PCR cycling then proceeded directly in the same tube: 94°C for 0.5 min, 48.6°C for 0.5 min, and 72°C for 1 min. The PCR was cycled either 30 or 36 times to gauge the rate of amplification and hence gain a qualitative estimate of initial *rbcL* template concentration. Controls for DNA contamination were prepared for each RT-PCR process by omitting reverse transcriptase in the reaction. In no case did the DNA controls generate amplification products, showing the absence of DNA contamination of the RNA preparations. RT-PCR products from each thallus were run in 1% agarose gels in Tris-borate-EDTA buffer at 70 V for 100 min. Gels were stained with GelStar nucleic acid stain (VWR, Canada). Gel images were captured with NIH Image v1.62 running on a Power Macintosh 7600/120 via a Sony digital video camera.

Results

Photosynthetic optima at field-ambient temperatures

To track photosynthetic performance as a function of the seasonally changing temperature (Fig. 1) we present

physiological parameters measured at field-ambient temperatures. First, we present data measured at the light level required to reach $ETR_{0.5\ Max}$ as an approximation of the optimal light level for the thalli (Fig. 2).

Both the PPFD required to reach $ETR_{0.5\ Max}$ and $ETR_{0.5\ Max}$ rose from low levels in the winter to highs in early summer when measured at the field-ambient temperature in the Walker Road samples (Fig. 3A, B). The seasonal changes in these two parameters were similar, but not as large, in the March and August samples from the other two deciduous forest sites, while the evergreen site with the permanently closed canopy at Economy Mountain showed less change between March and August (Fig. 3A, B). Ambient light was lower than the PPFD required to reach $ETR_{0.5\ Max}$ in the closed-canopy summer months, but ambient light greatly exceeded the PPFD required to reach $ETR_{0.5\ Max}$ in the winter months.

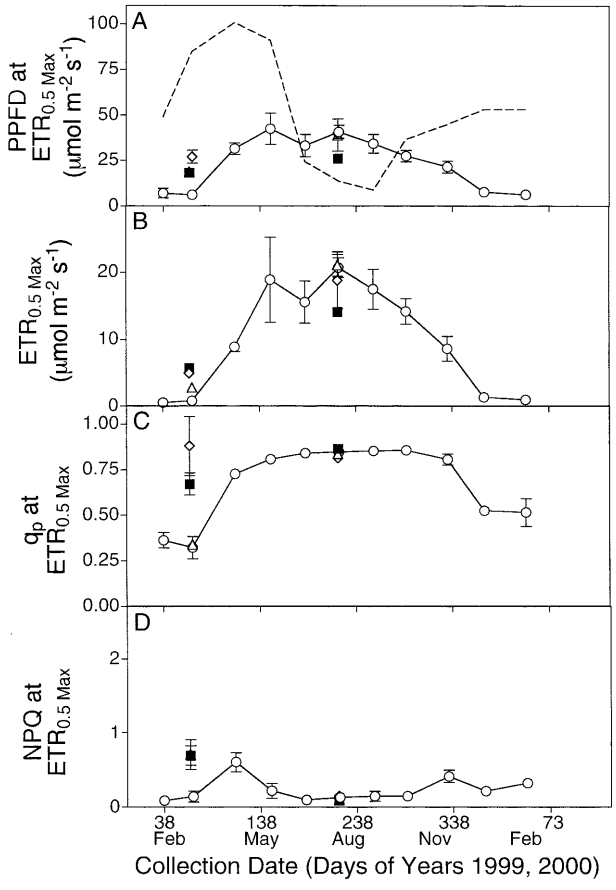

Fig. 3A–D PSII parameters of *L. pulmonaria* thallus measured at field-ambient temperatures and near-optimal PPFD. PPFD at $ETR_{0.5\ Max}$ (*dashed line* shows field-ambient light level at Walker Road) (**A**), $ETR_{0.5\ Max}$ (**B**), q_p at $ETR_{0.5\ Max}$ (**C**) and NPQ at $ETR_{0.5\ Max}$ (**D**) between 7 February 1999 and 18 February 2000 for the Walker Road sampling site (*open circles*), and in March and August 1999 for the Fenwick Park (*open triangles*), Sugarloaf Brook (*open diamonds*) and Economy Mountain (*filled squares*) sampling sites. Electron-transport estimates assume 100% absorbance of photosynthetically active photons by photosynthetic pigments. Values are means ± SE with $n = 3$

q_p, the proportion of open PSII units measured at $ETR_{0.5\ Max}$, was high throughout early spring to lateautumn, but was depressed in the winter at the Walker Road and Fenwick Park deciduous sites. Samples from the evergreen Economy Mountain population and from the deciduous Sugarloaf Brook showed nearly steady q_p in winter and summer (Fig. 3C). The Sugarloaf Brook site is located on a north-facing hillside with low incident light on the site in the winter which may make Sugarloaf Brook more similar to the evergreen Economy Mountain site than the other deciduous forest sites.

The non-photochemical dissipation of light energy (NPQ) at $ETR_{0.5\ Max}$ was low throughout the year except for two transient peaks in the spring and autumn in the Walker Road samples, coinciding with periods when ambient light exceeded the PPFD required for $ETR_{0.5\ Max}$ (Fig. 3D).

Realised photosynthetic parameters at field-ambient light level and temperatures

To approximate field photosynthetic performance we present physiological parameters measured at the field-ambient light level and temperatures.

The electron transport rate at the field-ambient light and temperature level was low in the winter months despite the high light levels at that time. Electron transport was relatively high in spring and autumn, outside the period ofclosed canopy at Walker Road, but dropped in the summer when the leaf canopy was closed and light levels were low (Fig. 4A). This pattern contrasts with the pattern of higher levels of $ETR_{0.5\ Max}$ in the summer than in the spring and autumn (Fig. 3B), and suggests that low temperature limits realised PSII electron transport in spring and autumn while low light limits PSII electron transport in summer. Thus, the other sampling sites, measured only in March and August, showed low and consistent values for both dates.

The seasonal pattern of q_p determined at field-ambient light and field-ambient temperatures (Fig. 4B) was similar to the pattern in q_p at $ETR_{0.5\ Max}$. These patterns were probably driven mainly by temperature.

NPQ determined at field-ambient light was high throughout the winter but dropped in the summer to lows similar to that of the NPQ determined at $ETR_{0.5\ Max}$ (Fig. 4C).

Coupling of gross CO_2 uptake and PSII electron transport

Maximum gross photosynthesis at field-ambient temperatures peaked in the late spring and early summer, and was lowest in the winter (Fig. 5), suggesting that seasonal changes in gross CO_2 uptake were dominated by the changing measurement temperatures. Gross potential CO_2 uptake measured at 20 °C, using a slightly different method, was lower in winter than in summer

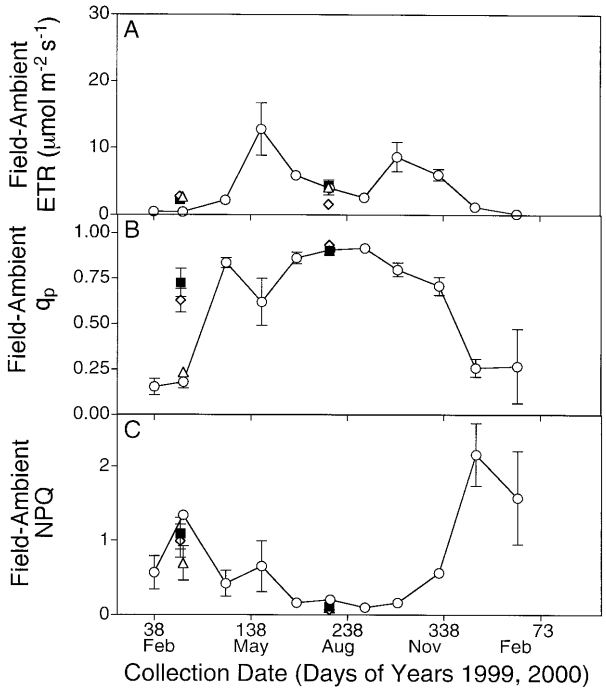

Fig. 4A–C Realised PSII parameters of *L. pulmonaria* thallus measured at field-ambient temperatures and field-ambient light levels. Electron transport through PSII (**A**), q_p (**B**) and NPQ (**C**) between 7 February 1999 and 18 February 2000 for the Walker Road sampling site (*open circles*), and in March and August 1999 for the Fenwick Park (*open triangles*), Sugarloaf Brook (*open diamonds*) and Economy Mountain (*filled squares*) sampling sites. Electron-transport estimate assumes 100% absorbance of photosynthetically active photons by photosynthetic pigments. Values are means ± SE with $n = 3$

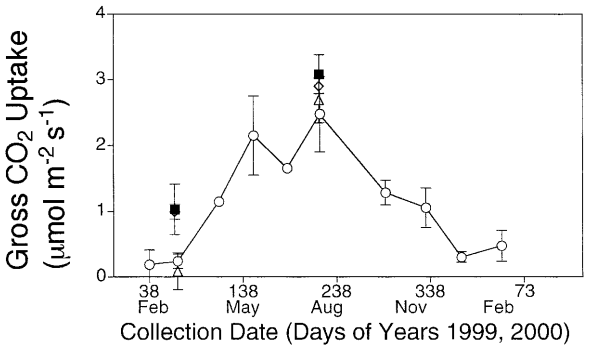

Fig. 5 Maximum CO_2 fluxes of *L. pulmonaria* thallus between 7 February 1999 and 18 February 2000 for the Walker Road sampling site (*open circles*), and in March and August 1999 for the Fenwick Park (*open triangles*), Sugarloaf Brook (*open diamonds*) and Economy Mountain (*filled squares*) sampling sites. Gross photosynthetic CO_2 uptake was measured at field-ambient temperatures. Values are means ± SE with $n = 3$

(data not shown). CO_2 uptake at 10 °C was only 20–35% of that at 20 °C. Only 6% of days between the November and March sampling dates had a daily maximum temperature exceeding 10 °C and on no date was 20 °C reached. The suppression of gross CO_2 uptake at low temperature and the paucity of warm winter days imply little winter CO_2 uptake occurs in this lichen population.

The reliability of PSII electron transport as a predictor of CO_2 uptake in lichens has been questioned elsewhere (Green et al. 1998). The mechanistic minimum rate of electrons transported through PSII per CO_2 fixed is 4 electrons CO_2^{-1} fixed. Our summer data agreed well with this, ranging from 3.82 ± 1.11 to 8.74 ± 1.05 electrons CO_2^{-1} with strong correlations for all sites. This rate dropped to zero or below in the winter, showing that PSII electron transport was only a good predictor of CO_2 uptake in the darker summer months (Table 1), and that few PSII-generated electrons were being used for assimilating CO_2 in the winter. We did not artificially fix the regression equations to transit the origin; thus the slopes were free to follow the trend of the data regardless of background levels of electron transport or CO_2 exchange, or errors in either. This slope shows the coupling between the extreme ends of photosynthetic energy metabolism, and demonstrates that this coupling breaks down at ambient winter temperatures and light intensities. That the slope is actually slightly negative in the March samples is not surprising. In these samples, even under the lowest light level for both electron transport and gross CO_2 uptake (5 μmol m^{-2} s^{-1}), there was considerable light stress and non-photochemical dissipation of excess excitation energy. Several photosynthetic organisms regularly perform non-assimilatory electron flow, and Green et al. (1998) described it as a likely photoprotective strategy explaining the poor overall coupling of electron transport and CO_2 uptake observed in lichens. This or other dissipatory mechanisms would likely increase with increasing light stress, possibly causing the negative correlations observed it the winter samples in this study.

Potential photosynthesis at a constant, moderate temperature

Measured at 20°C, the PPFD for maximum potential electron transport (Fig. 6A) and the potential for

Table 1 Seasonal changes in the coupling of PSII electron transport and gross CO_2 uptake of *L. pulmonaria* thallus measured at field-ambient temperatures over a range of light levels

Site	Month	PSII e–/CO_2	*P*
Walker Road (deciduous)	August	3.82 ± 1.11	0.0064[a]
	March	-1.59 ± 1.48[b]	0.3082
Fenwick Park (deciduous)	August	6.69 ± 1.21	0.0002[a]
	March	-0.69 ± 0.88[b]	0.4491
Sugarloaf Brook (deciduous)	August	8.74 ± 1.05	< 0.0001[a]
	March	2.09 ± 0.91	0.0429[a]
Economy Mountain (evergreen)	August	4.99 ± 0.94	0.0003[a]
	March	-1.91 ± 0.76[b]	0.0309[a]

[a] *P*-values indicate significant correlation at 0.05 probability level
[b] Regression equations were not forced through zero, permitting negative slopes

maximum electron transport through PSII (ETR_{Max}) (Fig. 6B) were higher in the bright winter months than in the dark summer months. Comparing PPFD at ETR_{Max}, ETR_{Max} and the actual field-ambient PPFD show similar seasonal patterns in all three. The other deciduous sites, sampled in March and August, were similar to the Walker Road samples. In contrast, the seasonal amplitude of change in the evergreen Economy Mountain samples was lower. Under saturating light at 20°C, q_p also showed relatively high values in the winter and low values in the summer and autumn, indicating a greater potential to keep PSII open during the bright winter months at Walker Road (Fig. 6C). Under saturating light at the other deciduous sites, q_p also dropped in the summer compared to the winter, but q_p under saturating light was consistent in winter and summer at the evergreen Economy Mountain site. Finally, changes in maximum NPQ at 20°C showed that the samples from the deciduous sites had a higher capacity to dissipate light energy in the bright winter months, while the evergreen forest Economy Mountain samples showed little seasonal change in their low NPQ capacity (Fig. 6D). These results show that seasonal patterns in the potential photosynthetic performance of PSII at 20°C were distinct from the realised patterns achieved at field-ambient temperatures.

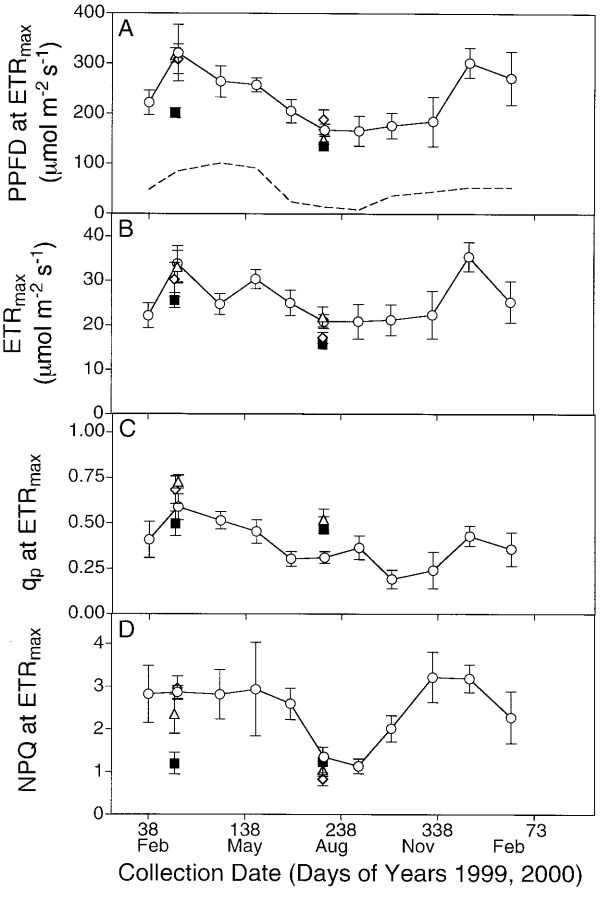

Fig. 6A–D PSII parameters of *L. pulmonaria* thallus measured at a fixed 20°C. PPFD at ETR_{Max} (*dashed line* shows field-ambient light level) (**A**), ETR_{Max} (**B**), maximum q_p (**C**) and maximum NPQ (**D**) between 7 February 1999 and 18 February 2000 for the Walker Road sampling site (*open circles*), and in March and August 1999 for the Fenwick Park (*open triangles*), Sugarloaf Brook (*open diamonds*) and Economy Mountain (*filled squares*) sampling sites. Electron-transport estimate assumes 100% absorbance of photosynthetically active photons by photosynthetic pigments. Values are means ± SE with $n = 3$

Seasonal changes in macromolecular composition

Chlorophyll content rose in the Walker Road population (Fig. 7A) from basal levels of 85–130 μmol m^{-2} to a peak of about 180 μmol m^{-2} in the dark, late summer months. The chlorophyll *a*/*b* ratio (Fig. 7B) averaged about 4.9 throughout the year, but was generally higher (> 5.5) under high-light conditions in the spring and fall and showed a sustained low (< 4.5) in the dark, late summer months, suggesting a higher proportion of antenna chlorophyll under the closed canopy.

There were also changes in the concentration of RuBisCO LSU throughout the year in the Walker Road population. RuBisCO LSU remained at about 0.25 μmol m^{-2} from October through April, doubled in May, then decreased over the summer months to a minimum in September (Fig. 8A). There was thus a pattern of rapid accumulation in the late spring, at about +7.7 nmol m^{-2} day^{-1}, followed by a sustained decay of RuBisCO LSU through the closed canopy summer months, at about –3.8 nmol m^{-2} day^{-1}, with slow re-accumulation in the fall after defoliation, at about +1.9 nmol m^{-2} day^{-1}. The concentrations we

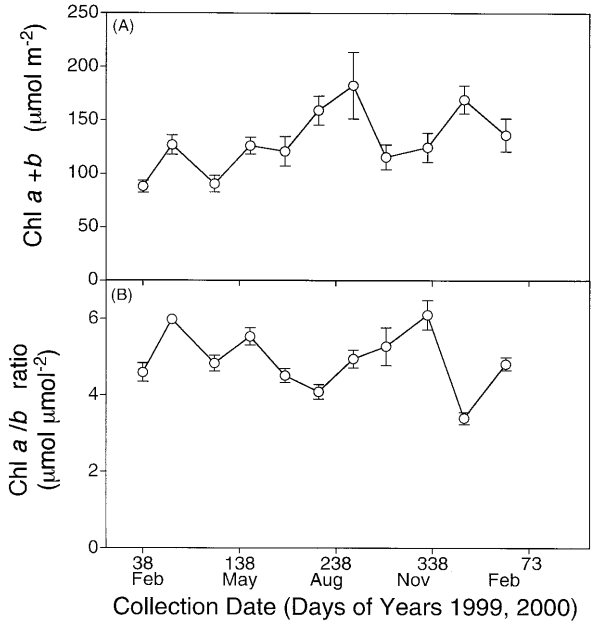

Fig. 7 Chlorophyll content of *L. pulmonaria* thalli estimated by spectrophotometry of acetone extracts of thallus samples (**A**), and chlorophyll *a*/*b* ratios (**B**) from 7 February 1999 to 18 February 2000 for the Walker Road sampling site. Values are means ± SE with $n = 3$

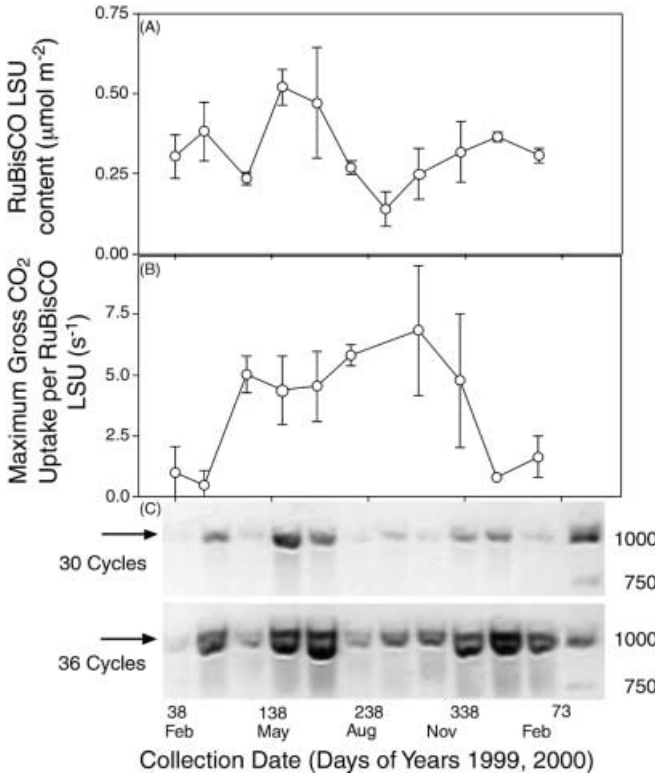

Fig. 8 RuBisCO LSU content of *L. pulmonaria* thalli estimated from immunoblots of tissue extracts (**A**), maximum gross CO_2 uptake at field-ambient temperatures per RuBisCO LSU (**B**) and RT-PCR products from 30- and 36-cycle reactions showing changes in the *rbcL* transcript pool (**C**) from 7 February 1999 to 18 February 2000. RT-PCR bands (marked by *arrows*) correspond to sampling dates in graphs (**A, B**) and band migration was downward. The lane at extreme right is a molecular weight standard showing bands of 1,000 bp and 750 bp, as indicated. RuBisCO LSU content in May is significantly higher than April, August, September, October, January 2000 and February 2000, and CO_2/RuBisCO LSU values in April and July are significantly higher than in February, March, January 2000 and February 2000 at the $\alpha = 0.05$ level (one-way ANOVA). Values in **A** and **B** are means ± SE with $n = 3$

found were similar to values in lichens derived from previously published data (Palmqvist et al. 1998), although rates of accumulation and decay have not previously been calculated in lichens. The rates of CO_2 uptake per RuBisCO LSU were in a narrow range from 4.4 to 6.8 CO_2 RuBisCO LSU^{-1} s^{-1} from April through November (Fig. 8B). CO_2 uptake per RuBisCO LSU was low in the winter because of the depressed rate of CO_2 uptake in the cold. Our qualitative data for *rbcL* transcript levels (Fig. 8C) show a temporal pattern of transcript levels closely matching RuBisCO LSU concentration.

Discussion

In the deciduous forest populations of *L. pulmonaria*, the physiological and macromolecular data suggest there was a strategy shift in light harvesting and electron transport from energy dissipation in the open-canopy cold months to light-limitation in the closed-canopy summer months. In the open-canopy months chlorophyll content and realised electron transport at ambient temperatures were low despite abundant ambient light. Realised electron transport peaked in spring and autumn when high light and moderate temperatures coincided. In the closed-canopy warm months, when available light was at a minimum the chlorophyll content rose but realised electron transport again fell. Ambient light was in excess of the PPFD to drive $ETR_{0.5\ Max}$ for most of the year, except in the dark summer months. Kershaw and Webber (1984) have previously shown chlorophyll content and CO_2-exchange changes in *Peltigera* that were interpreted as causing higher light-harvesting efficiency in closed-canopy populations.

The photochemical performance of the photosystems and associated electron-transport chains are sensitive to their redox status, which in turn responds to the integrated light and temperature environment (Huner et al. 1998). We separated seasonal light and temperature effects on electron transport and energy dissipation by holding measurement temperature constant at 20°C. These data clearly showed that the potential for both electron transport and excitation-dissipation capacity were high in the bright winter months in the deciduous forest populations, and lower in the darker summer months. Furthermore, the seasonal patterns of ambient PPFD, PPFD for potential ETR_{Max} and potential ETR_{Max} at 20°C were all similar, showing that the lichens actively tracked ambient PPFD by acclimating their potential for electron transport, even though low temperature depressed their realised electron transport in winter. The dependence of these seasonal patterns in potential photosynthetic performance on the light environment was clear because only small seasonal changes were observed in samples from the permanently closed-canopy site at Economy Mountain. The reversal of the seasonal patterns of realised electron transport measured at the field-ambient temperature, however, showed the greater and overwhelming importance of temperature in determining the realised performance.

Muir et al. (1998) have shown greatest growth rates of *L. pulmonaria* during the winter months in western Oregon USA. Unlike southeastern Canada (this study), Oregon has wet, mild winters and relatively dry summers, and daily maximum temperatures are rarely below 0°C. Thus, the lichens were exposed to more liquid water and metabolically active temperatures in winter in the Muir et al. (1998) study than our lichen population, which is rarely exposed to drought conditions at metabolically permissive temperatures. In summer they exist in the dark and moist understorey of a swampy maple forest. In winter they are deprived of liquid water for periods, but only in the extreme cold of dry arctic air masses, when they are not metabolically active due to low temperature. We contend that in this population the seasonal changes in water availability are minor

compared to changes in light and temperature and the latter's strong effects on metabolic rates.

The CO_2-exchange data supported the hypothesis of a seasonal strategy shift in photosynthesis. Green et al. (1998) suggested lichens have a capacity to divert excess electrons from PSII away from CO_2 fixation. In the bright, cold March samples there was no meaningful correlation between PSII electron transport and CO_2 uptake at any site,suggesting extensive diversion and dissipation of electrons from PSII. In the warm, light-limited August samples, however, the correlation between PSII electron transport and CO_2 uptake was strong, and mechanistically plausible, indicating that electrons from PSII were channelled efficiently to CO_2. Sundberg et al. (1997), however, have shown that thallus growth in *L. pulmonaria* is not correlated with common productivity indices such as net CO_2 uptake or chlorophyll content. Overwhelming and variable fungal respiration, especially the high respiration rates of *L. pulmonaria* (Sundberg et al. 1999), may affect thallus growth rate more than does algal productivity (Sundberg et al. 1997).

The seasonal changes in CO_2 uptake in terms of RuBisCO content also showed a strong difference between winter and summer months. Gross CO_2 uptake at field-ambient temperatures per RuBisCO LSU was near zero in winter but then remained high and fairly constant from late April to late November, through a 4-fold seasonal change in RuBisCO content and through great changes in the light and temperature environment. Furthermore, the calculated rates of gross CO_2 uptake per RuBisCO LSU in the period between April and November are near maximum published turnover rates for this enzyme (Badger and Andrews 1987), suggesting that the RuBisCO pool was nearly fully activated. Examination of the RuBisCO LSU and *rbcL* transcript patterns shows there was a variable allocation of resources to RuBisCO that maintained and maximised the rate of CO_2 fixed per RuBisCO LSU. Through the spring, there was abundant light, warming temperatures and increasing liquid water availability as snow melted and drier winter air gave way to spring, leading to a great potential for CO_2 fixation supported by accumulation of *rbcL* transcript and rapid accumulation of RuBisCO LSU. After canopy closure, the *rbcL* transcript concentration dropped, and RuBisCO LSU fell steadily through the summer. After the leaf canopy opened again in the autumn, *rbcL* transcript and RuBisCO LSU re-accumulated to a second peak in the early winter. This suggests that RuBisCO LSU content is dynamically modulated in part by transcript concentration and in response to seasonal environmental changes.

The large seasonal shifts in photosynthetic physiology, macromolecular composition and *rbcL* transcription in the photobiont of *L. pulmonaria* occurred despite the discontinuous nature of lichen physiology, which interacts with the environment only during hydration episodes of variable duration and frequency. Although temperature caused great changes in measured photosynthetic rates, changes in the light environment also drove striking changes in the photosynthetic potential throughout the seasons in these lichens. The photosynthetic apparatus responds to the interaction of light and temperature, however, so measurements of realised acclimation must account for both variables. Measurement at the field-ambient temperature and light is one means to simplify this interaction and is a physiologically relevant approach, given support by the good correlations found between gross photosynthesis and electron transport. This approach is also faster and less laborious than the matrix-response approach extolled by Kershaw (1984).

This study encompassed one annual environmental cycle while these lichens cope with both open- and closed-canopy environments by profoundly switching photosyntheticstrategy throughout the year over many years of life. This active acclimation allows them to maintain photosynthetic performance under a wide range of conditions.

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