Changes in macromolecular allocation in nondividing algal symbionts allow for photosynthetic acclimation in the lichen *Lobaria pulmonaria*

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Summary

• The lichen *Lobaria pulmonaria* survives large seasonal environmental changes through physiological acclimation to ambient conditions.
• We quantitated algal cell population, cell division and key macromolecular levels associated with photosynthesis and nitrogen metabolism in *L. pulmonaria* sampled from four seasons with contrasting environmental conditions in a deciduous forest.
• The algal symbiont population did not vary seasonally and cell division was restricted to the newest thallus margins. Nevertheless the symbiont concentrations of chlorophyll, PsbS, PsbA, and RbcL changed significantly through the seasons in the nondividing algal cells from older thallus regions.
• *L. pulmonaria* reversibly allocated resources toward photochemical electron generation and carbohydrate production through the spring, summer and fall, and towards photoprotective dissipation in the cold, high-light winter. Our study shows that large seasonal molecular acclimation in *L. pulmonaria* occurs within a nearly stable, nondividing algal cell population that maintains photosynthetic capacity through many years of changing environmental cues.

Key words: acclimation, *Dictyochloropsis reticulata*, photobiont, photosynthesis, photoprotection, PsbS, *Lobaria pulmonaria*.

Introduction

A lichen is a symbiosis between a fungus, termed the mycobiont, and either an alga, cyanobacterium or both, termed the photobiont(s). The photobiont transfers carbohydrates derived from photosynthesis, and nitrogen compounds in the case of nitrogen-fixing cyanobacteria such as *Nostoc*, to sustain the heterotrophic metabolism of the mycobiont. The mycobiont passively supplies its photosynthetic partner with water and mineral nutrients (Honegger, 1991). Lichens are poikilohydric with sporadic photosynthesis in the algal symbiont limited to infrequent periods of hydration, producing a discontinuous acclimation history in persistent algal cells and discontinuous growth of the lichen (Palmqvist, 2000).

Lobaria pulmonaria is a tripartite lichen symbiosis which contains a primary green algal photobiont, *Dictyochloropsis reticulata* (formerly *Myrmecia*) and the minor, nitrogen-fixing cyanobacterial partner *Nostoc* sp. (Jordan, 1973). The algal partner occurs in a flat layer under a fungal cortex, while the cyanobacterial partner exists in widely dispersed, discrete specialised structures in the lower layers of the lichen called cephalodia (Brodo *et al.*, 2001). In lichens, algal cell division is generally under the control of the host fungus (Malachowski *et al.*, 1980) and largely restricted to the growing thallus margins (Greenhalgh & Anglesea, 1979). While photosynthetically active for many years, algal cells in the older lichen thallus behind the growing tip can persist with negligible cell division or population turnover (Hill, 1992). The mechanisms supressing algal cell division in lichens may include antimitotic agents produced by the fungus (Sucharita *et al.*, 1983), competition for limited fixed nitrogen between the symbionts or organic carbon export from

the alga to fungus. Such nitrogen or carbon limitation in the alga would favour acclimation via reallocation of existing macromolecular resources, rather than via the division and growth of new cells with altered macromolecular allocations.

L. pulmonaria is a large, relatively fast-growing foliose epiphytic lichen common in eastern Canada in deciduous forests on red maple trees (*Acer rubrum*), and infrequently on spruce (*Picea* spp.) in evergreen forests. In our study site, a swampy, red maple dominated forest in New Brunswick, Canada, the deciduous canopy is open from October to May, which subjects the lichen photobiont to intense light, often in conjunction with freezing temperatures, increasing photoinhibition potential and limiting availability of liquid water. In the summer months, temperature and water availability are more conducive to photosynthesis and growth, but the lichens are light limited because of the closed leaf canopy. Temperature and light changes drive large changes in the photosynthetic physiology of the algal cells through the year (MacKenzie *et al.*, 2001, 2002). The photobiont of *L. pulmonaria* follows the ambient light and temperature levels to maintain a high potential photosynthetic rate in warmer conditions, and a high dissipation capacity during cold and bright periods typical of the winter deciduous forest (MacKenzie *et al.*, 2001).

Evergreens and winter annual plants have different physiological and molecular strategies to cope with low temperature. Evergreen leaves that persist through the winter adopt an energy-dissipation strategy to survive the period less conducive for growth (Ottander *et al.*, 1995). By contrast, winter annuals produce new leaf tissue developed into a cold-acclimation state to sustain productive photosynthesis at low, nonfreezing temperatures (Ivanov *et al.*, 2001; Savitch *et al.*, 2002). Our previous physiological work (MacKenzie *et al.*, 2001, 2002) showed that *L. pulmonaria* thalli displayed strong and sustained down-regulation when winter conditions preempt photosynthetic function. Therefore *L. pulmonaria* and similar lichens present the opportunity to study mechanistic strategies for sustained down-regulation of photosynthesis in the unicellular green algal photobionts coping with the cold and bright conditions of the winter forest.

We therefore studied how the lichen photobiont allocates resources to key molecular components of photosynthetic metabolism, and whether molecular and physiological acclimation result through changes or turnover in the algal cell population, or if instead, persisting individual cells reorganise macromolecular resources within a stable population in response to their variable local environment. At four times of the year, typified by contrasting environmental conditions, we followed changes in six macromolecules indicative of key functions in photosynthetic metabolism. In photosynthesis, light is initially absorbed primarily by chlorophyll. Excitation energy from the absorbed photons can drive photochemical electron transport, as mediated by the PsbA subunit of Photosystem II. A variable fraction of the excitation energy can also be dissipated via the photoprotective xanthophyll cycle mediated by the PsbS protein (Li *et al.*, 2000), thereby preempting photochemical electron transport. The electron transport chain requires sinks to accept the electrons generated from water. One major indicator of the electron sink capacity is the level of RbcL, the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Electrons can also flow into nitrogen metabolism, to be used in nitrogen fixation, and nitrate and ammonia assimilation. Nitrogen metabolism is especially important in lichens because of the nitrogen requirements of the mycobiont for cell wall chitin synthesis (Bergman & Rai, 1989; Palmqvist *et al.*, 1998; Sundberg *et al.*, 2001). Therefore we also monitored levels of two key enzymes of nitrogen assimilation in the minor cyanobacterial symbiont: the NifH subunit of the nitrogenase enzyme, and GlnA, the prokaryotic glutamine synthetase enzyme responsible for assimilating ammonia in the nitrogen-fixing cyanobionts.

Materials and Methods

Sample collection

The collection site was in a maple dominated deciduous forest in south-eastern New Brunswick, Canada (45° 44.22′-N, 64° 10.01′-W) where mid-day air temperature varies up to *c.* 50°C through the year and the daily maximum light levels beneath the tree canopy vary nearly 100-fold. Temperature and precipitation data during the sampling period were taken from the Environment Canada Sackville station approximately 5 km from our field site, and are shown in Table 1. More detailed seasonal changes in the field site environment

Table 1 Environmental data from the Environment Canada Sackville station for 13 days up to and including the dates of thallus collection.

Collection date	Mean daily high temperature (°C)	Total precipitation rain (mm)	Total precipitation snow (cm)
29 May 1999	18.3	28.0	0
8 August 1999	25.0	32.0	0
29 November 1999	9.5	22.8	0
18 February 2000	–1.7	17.2	19.6

Temperatures are means of the highest recorded daily temperature during that period, while the precipitation measures are the sum during that period.

were described in MacKenzie *et al.* (2001). *Lobaria pulmonaria* (L.) Hoffm. thalli were sampled from this site on May 29, 1999, August 8, 1999, November 29, 1999, and February 18, 2000, as representative dates through the major seasonal changes in light, temperature and water availability. On each date three thalli, which were air-dry when collected, were removed from their substrate on *Acer rubrum* trunks, and transported back to the laboratory sealed in plastic bags in the dark on ice, and then stored at −20°C until analyses were performed. All of the thalli were growing around breast height on the trunks.

Algal cell population and morphological measurements

Lichen thallus cross-sections were prepared from each replicate thallus from all four sampling dates to determine changes in algal cell population and cell division. The thalli were air dried (to *c.* 0.1 g water (g thallus)$^{-1}$) before fixation and sectioning. The thalli were paraffin embedded (protocol adapted from Jackson, 1989) and several sections were made from three replicates of young thalli taken from near the first branch node, *c.* 30 mm from the thallus lobe tip, which we estimate to be *c.* 10-yr-old at expected growth rates (Muir *et al.*, 1997; Palmqvist, 2000). Old lichen thallus samples, *c.* 25 yr of age, were taken from the oldest distinguishable branch node, usually *c.* 100 mm from the thallus lobe tip. The lichen sections were fixed with 4% formaldehyde in phosphate buffered saline (pH 6.5–7), cut to 5 μm with an American Optical microtome (Leica KM2135, Leica Microsystems Inc., Richmond Hill, Ontario, Canada), viewed under an epifluorescence microscope (AxioSkop 2 plus, Carl Zeiss Ltd., Toronto, Ontario, Canada) and the algal cells identified from their characteristic shape and strong chlorophyll autofluorescence. An image of the epifluorescence microscopic detection of the algal cell population in *L. pulmonaria* is presented in Fig. 1. The three images were taken from three different regions of the same lichen thallus collected on May 29, 1999; an old region *c.* 100 mm from the growing tip (Fig. 1a), a young region *c.* 30 mm from the growing tip (Fig. 1b), and the apical margin itself, *c.* 0.5 mm from the tip (Fig. 1c). Digital images, like the examples in Fig. 1, were analysed to generate algal cell population and morphological

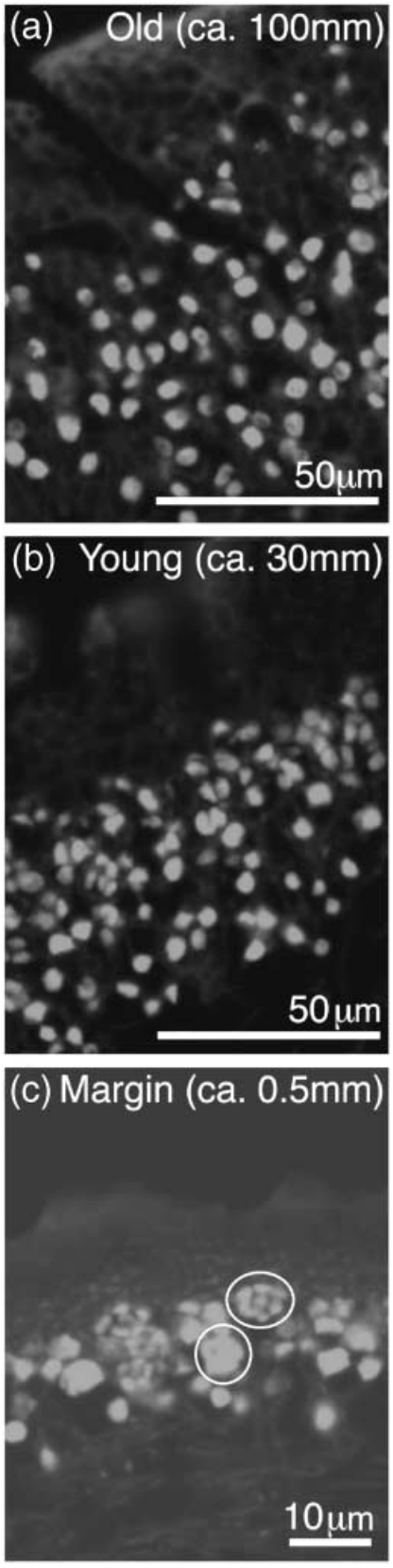

Fig. 1 Epifluorescence images of the autofluorescence from the algal cell population in three age regions from a representative thallus of the lichen *Lobaria pulmonaria*. (a) A region fixed from old thallus 100 mm from the tip (b) young thallus 30 mm from the growing tip (c) and new thallus margin 1.5 mm from the apical tip were imaged from a May 29, 1999 sample. Note the clusters of dividing cells evident in the marginal thallus designated by the white circles. In all panels, sections through the thallus are viewed laterally with the upper cortex toward the top and the lower cortex well below the base of each image. The dimly fluorescent linear structure in the upper left of (a) is due to a brownish fungal pigment described by Gauslaa & Solhaug (2001) at the surface of the upper thallus cortex.

data. Data were collected from eight digital images of each date-age-replicate combination for analysis with NIH Image (National Institute of Health, USA) analysis package and data from these eight images per replicate were averaged to minimise small-scale cell density variations. An image was also made of a stage micrometer for size calibration. Images were analysed for algal cell population density per section, which was scaled to algal cells per square metre of lichen thallus. The cross-sectional area of each algal cell was also measured with NIH Image and used to estimate a spherical volume per cell, as a conservative measure allowing us to detect possible changes in mean algal cell volume over the season or with thallus age.

Several reports suggest algal cell division is limited to a narrow region within 2 mm of the apical margin in lichens (Armstrong & Smith, 1998). To confirm this for *L. pulmonaria* we fixed additional lichen sections from the apical margins of the thallus lobes 0.5, 1.5 and 2.5 mm from the tip. Dividing algal cells can be distinguished from nondividing cells because they form distinctive clusters of 4, 8 (and rarely 16) cells. The number of dividing algal clusters was expressed as a percent of the total number of algal cells detected. Representative images of marginal, young and old thallus regions are shown in Fig. 1. The epifluorescent images were also used to investigate seasonal and age-dependent differences in the algal cell layer thickness (mm) and the upper fungal cortex thickness (mm). We also investigated the dry mass of lichen thallus per thallus area (g m^{-2}) in the marginal, young and old thallus regions over the seasons.

Chlorophyll determination

Chlorophyll concentration was determined according to the standard DMSO extraction technique described by Barnes *et al.* (1992). Lichen thallus disks of *c.* 1.2 cm^2 were taken from young thallus regions (*c.* 30 mm from the growing tip), and old thallus regions (*c.* 100 mm from the growing tip). The surface area of each thallus sample was measured using a Umax Astra 1220 s scanner with the NIH Image analysis package (Umax Technologies, Dallas, TX, USA; National Institutes of Health, Bctnesda, MO, USA). The samples were washed with 90% acetone in water saturated with $MgCO_3$ to remove any secondary lichen substances, and allowed to dry for 30 min. Chlorophyll was extracted for 40 min at 65°C in 2.5 mg ml^{-1} polyvinyl pyrrolidone in DMSO. Thallus samples were extracted three times sequentially and the extracts combined for spectorphotometric analysis on a UV-vis spectrophotometer (Spectronic Unicam, distributed by VMR International, Mississauga, Ontario, Canada).

Protein quantification

Protein measurements were made for the young and old regions described above for each thallus date for age and seasonal comparisons. Samples were taken by punching disks with a large cork bore (*c.* 4 cm^2) at the outermost 20–30 mm from a growing lobe tip, while old thallus sample disks were taken near the oldest branch node of the thallus. Sample surface area was measured for the protein samples as it was for the chlorophyll determination samples.

The protein extraction, electrophoresis and immunodetection protocols are detailed in MacKenzie *et al.* (2001). The primary antibodies used in the immunodetections were either Global Antibodies (AgriSera, Sweden, http://www.agrisera.se) raised in chickens against conserved peptide sequence tags diagnostic of the target protein family (PsbA, RbcL, NifH, and GlnA) and used according to the supplier recommendations, or raised in rabbits (spinach RuBisCO, donated from G. Samuelsson, Umeå University, Sweden, and PsbS from Christiane Funk, Umeå University, Sweden). A secondary goat antichicken IgY (or antirabbit IgG) conjugated to horseradish peroxidase (Sigma-Aldrich, St Louis, MO, USA) was incubated with the membrane in a 1 : 5000 dilution with 15 ml of blocking buffer (5% low fat milk powder in TBS-T) at room temperature. Between all steps the PVDF membrane was washed with TBS-T (Tris Buffered Saline: 20 mM Tris base, 500 mM NaCl, 0.05% Tween-20; pH 7.5). The proteins were visualized with the ECL + Plus (Amersham Biosciences, Piscataway, NJ, USA) chemiluminescence detection system according to the manufacturer's protocol. The chemiluminescence reaction was imaged with a Fluor-S Max MultiImager (Bio-Rad, Laboratories Ltd., Mississauga, Ontario, Canada) using the Quantity One (Bio-Rad) software package. Relative quantities of the detected proteins were calculated using the Quantity One software package based on the integrated intensity of the chemiluminescence reaction. All protein quantities were calculated both on the basis of thallus area and algal cell number.

Statistical analysis

Nonparametric, one- and two-factor Friedman ANOVA analyses were performed to assess significance in the date and thallus age comparisons of parameters using MiniTab (MiniTab Inc., State College, PA, USA) software. Post-hoc *t*-tests were performed to determine significance groupings.

Results

Table 2 shows the proportion of dividing algal cell clusters, as a percentage of the total algal cell population, from the different ages and seasons. No dividing algal cells were detected in any of the old thallus samples. In young thalli few or no dividing cells were observed. We did, however, observe large numbers of dividing cells in the thallus margins *c.* 0.5, 1.5 and 2.5 mm from the thallus tip. This algal division in the thallus margins was highest during warm and bright May, with considerably less division observed from the other sampling dates. The majority of cell division occurred within the first 1.5 mm of the apical margin. The finding that the cell division of the

Table 2 Dividing algal cell clusters as a percent of total green algal cell population in *Lobaria pulmonaria* for old, young and marginal thallus collected (approximate distance from the growing margin and estimated age in parentheses) between 29 May 1999 and 18 February 2000

Thallus Region	May	Aug	Nov	Feb
Old (~100 mm; 25 yr)	0	0	0	0
Young (~30 mm; 10 yr)	0.3 (0.3)	0.1 (0.1)	0.1 (0.1)	0
Tip (2.5 mm; 1 yr)	1.3 (0.3)	0.1 (0.1)	0.4 (0.3)	0
Tip (1.5 mm; 1 yr)	10.0 (5.0)	1.3 (0.4)	0.4 (0.3)	0.2 (0.2)
Tip (0.5 mm; 1 yr)	16.7 (4.4)	1.9 (1.5)	3.0 (1.1)	0.3 (0.2)

n = 3 (SE).

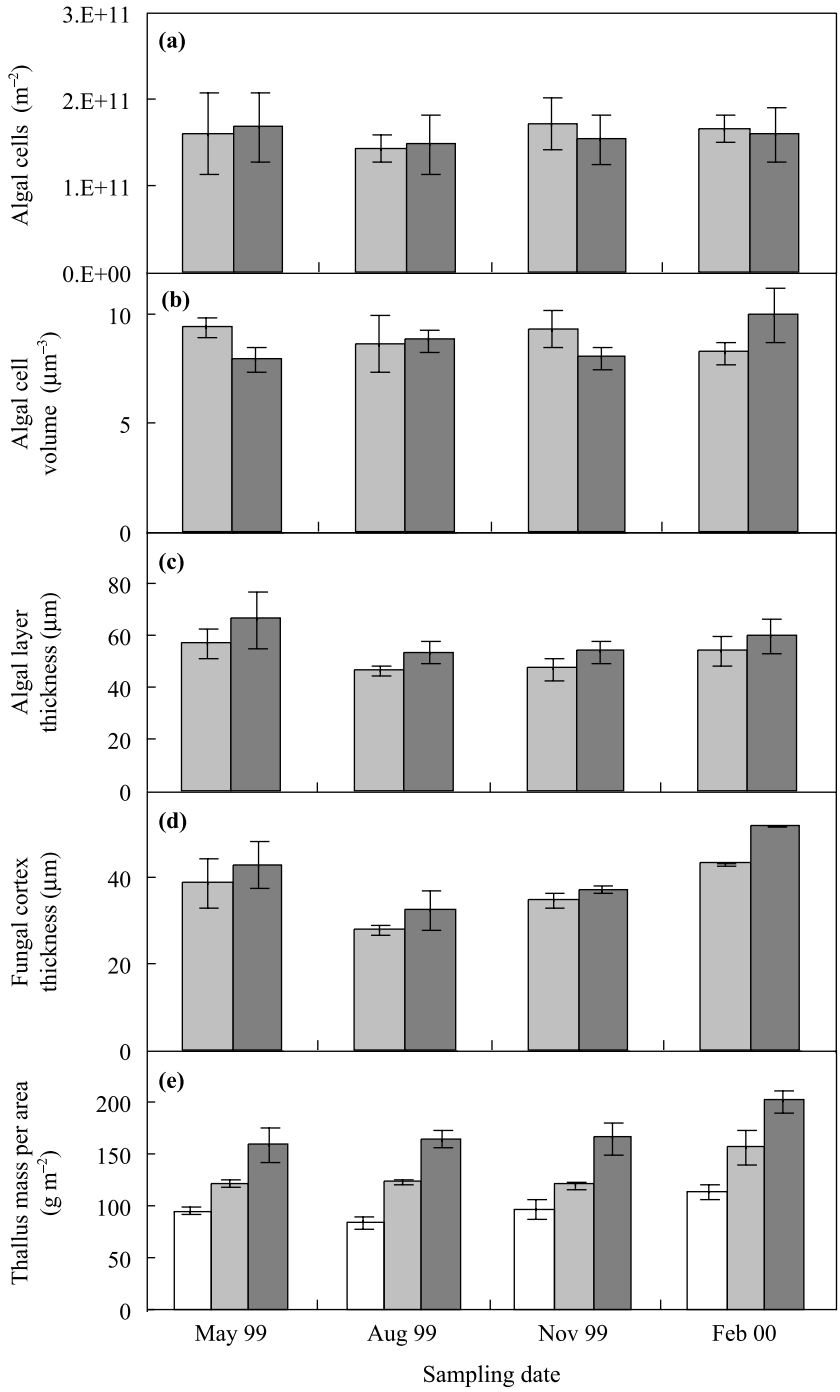

Fig. 2 Algal cell population and other lichen thallus measures from microscopic analyses of *Lobaria pulmonaria* cross-sections. (a) Algal cell population density (b) the estimated spherical volume per algal cell (c) the algal cell layer thickness, and (d) the upper fungal cortex thickness at the different dates. In all cases for panels a–d the young thallus is indicated by light shading and the old thallus by dark shading. (e) The dry weight for the thallus margin (no shading), young thallus (light shading), and the old thallus (dark shading) regions over the same samples. *n* = 3 ± SEM.

green algal symbionts of *L. pulmonaria* is largely restricted to the apical thallus margins is consistent with studies of other lichens (Hill, 1993; Armstrong & Smith, 1998).

Neither the algal cell population density (Fig. 2a) nor the volume per algal cell (Fig. 2b), were significantly different on different dates or between young and old thallus regions. No cephalodia containing *Nostoc* were observed in the numerous lichen thallus cross sections studied, suggesting their dispersal in the thallus is sparse. There were no significant date or age-related changes in the algal cell layer thickness (Fig. 2c), nor in the upper fungal cortex thickness (Fig. 2d). However, the d. wt per square meter of thallus (Fig. 2e) increased with thallus age (two-factor ANOVA $P < 0.001$) and was elevated in the February samples over the other sampling dates (two-factor ANOVA $P = 0.001$). All area and thickness measurements were made on air-dry thallus sections. Slight but variable residual hydration may account for some variation in the measured parameters, since the thalli expand slightly upon hydration. However, this cannot account for the 60% greater cortex thickness in February compared to August thalli, nor the larger age-related differences in thallus mass per area. The data in Fig. 2 clearly show some morphological changes in the mycobiont occurred through the year and across thallus age, while the photobiont population remained nearly static over most of the lichen thallus area, including the young and old regions we used for macromolecular analyses. It must be underlined, however, that the data may under-represent the full extent of variation in these morphological parameters. Lichens from different substrates, tree aspects and other microhabitat differences may not all be represented in our limited sampling.

In marked contrast to the algal population data, the pigment analyses and protein immunodetections showed large changes in the molecular composition of the photobionts throughout the year. These large changes in macromolecular content therefore occurred within the cells of the stable algal population and not via changes or turnover in the cell population. The algal population parameters showed no significant differences between samples from young and old regions of the lichen thallus, nor did our macromolecular determinations. There was, however, variation in the macromolecular determinations between lichen

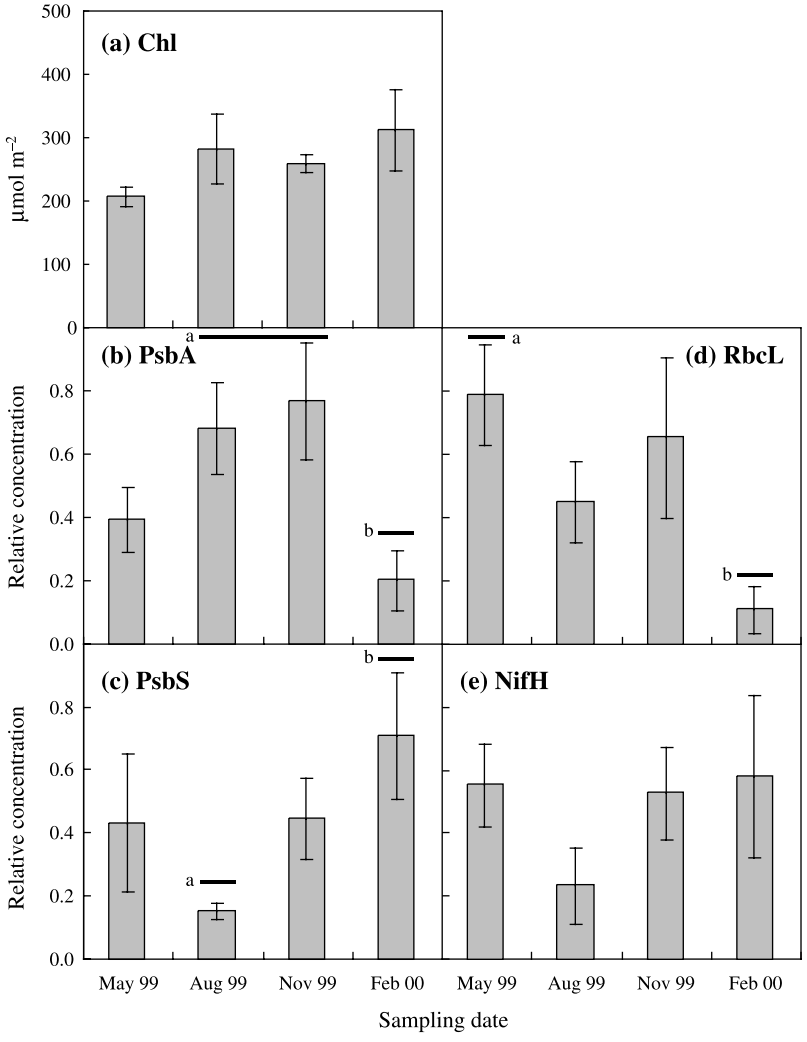

Fig. 3 Levels of key macromolecules of carbon and nitrogen metabolism from *Lobaria pulmonaria* thalli at different sampling dates. (a) absolute total chlorophyll a + b (b) Relative PsbA concentration (c) relative PsbS concentration (d) relative RbcL concentration (e) relative NifH concentration. All macromolecules are expressed on a per thallus area basis with protein concentrations relative and fixed to an arbitrary scale. $n = 6 \pm$ SEM; bars noted 'a' and 'b' indicate significantly differing groups at $\alpha = 0.05$.

thalli samples. For our macromolecular analyses we therefore pooled all samples from both old and young thallus regions, to generate a sample set adequate to test for date-related differences in macromolecular allocations in the photobionts.

Surprisingly there was no significant change in chlorophyll concentration of the samples from the different dates (Fig. 3a) nor of chlorophyll a : b ratio, which could indicate a change in pigment allocation to antenna and reaction center. PsbA, the D1 core protein in PSII that is responsible for the photochemical generation of electrons from water (Fig. 3b), showed significant changes through the year (one-factor ANOVA P = 0.016), being highest in the August and November, somewhat lower in May and lowest in February samples. Conversely, PsbS, an important element in the light excitation dissipation capacity of the photobiont, was highest in February, under the bright and cold winter conditions when it would be most needed (one-factor ANOVA P = 0.038). The August samples, taken from under the shade of the forest leaf canopy, which would least need the dissipatory activity mediated by PsbS, had the lowest PsbS levels (Fig. 3c). RbcL, the CO_2-fixing enzyme that is a key element in the Calvin Cycle sink for photosynthetic electrons, was lowest in February (Fig. 3d) (one-factor ANOVA P = 0.045). This is coincident with the low concentration of the electron-generator PsbA.

NifH, a subunit of the nitrogenase enzyme responsible for nitrogen fixation in the cyanobiont partner, showed no significant trend through the year except for a decrease in August (Fig. 3e). The thallus concentrations of NifH were variable however, and the drop in August was not statistically significant. The variation in NifH determinations among samples may result from the sparse nature of the *Nostoc*-bearing cephalodia in the thallus. Cyanobacterial glutamine synthetase (GlnA), a key enzyme for the incorporation of nitrogen into carbon skeletons within the cyanobacterial cells, was not detectable in the lichens, presumably because of very low levels of this protein in the cephalodial cyanobacteria symbionts. As a positive control, the anti-GlnA antibody did detect GlnA from samples of free-living *Nostoc* and *Synechococcus* cyanobacteria (data not presented here).

Ratios of macromolecules are presented (Fig. 4) for analyses of molecular resource allocation by the lichen photobiont. The light harvesting capacity per PSII reaction center, represented by Chl/PsbA, peaked in February as the chlorophyll concentration remained high at this date but PsbA levels had dropped (Fig. 4a). Therefore, even though the thallus light capture capacity was high in the bright winter samples, the capacity to convert absorbed light energy into photochemical electrons was lower in February than at other dates. In parallel, despite great seasonal variation in the concentrations of PsbS and PsbA, the ratio of PsbS/PsbA, indicative of the potential to dissipate excitation energy per PSII reaction center, was highest in February when there was high light, low temperatures and little available liquid water (Fig. 4b). RbcL/PsbA (Fig. 4c) was highest in May, during the bright, moist and temperate weather favoring high levels of carbon fixation. Although the date-related changes in most individual protein concentrations were statistically significant, the combined errors in the ratio data marginalized their statistical resolution. Thus, date-related changes were only statistically significant in the PsbS per PsbA data (one-factor ANOVA P = 0.038).

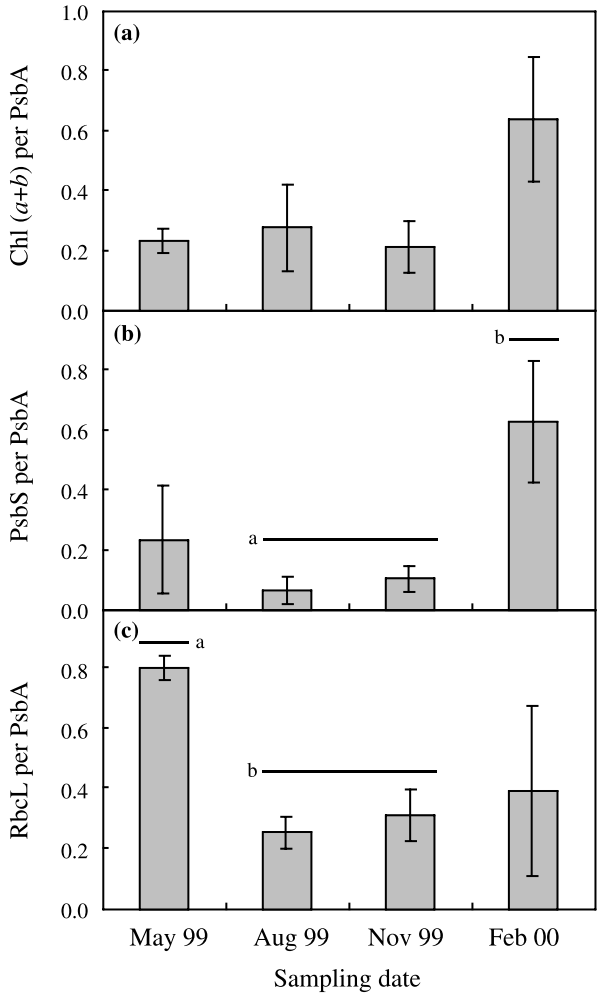

Fig. 4 Key macromolecules in *Lobaria pulmonaria* related to PSII concentration. (a) Total chlorophyll per PsbA (b) PsbS per PsbA, and (c) RbcL per PsbA. All macromolecular ratios are relative and fixed to an arbitrary scale. $n = 6 \pm$ SEM; bars noted 'a' and 'b' indicate significantly differing groups at $\alpha = 0.05$.

Discussion

In *L. pulmonaria* we show that significant molecular acclimation to seasonal environmental change occurs within a persistent and stable algal cell population, and not from turnover of the algal cells. In some lichens algal cell density can fluctuate according to seasonal changes (Greenhalgh & Anglesea, 1979; Galun *et al.*, 1970; Harris, 1971). We, however, did not observe such seasonal population changes in mature *L. pulmonaria* thalli. We saw evidence of algal cell division

only in the marginal tips of the thallus lobe. This agrees with Hill (1992), that cell division in the photobiont is arrested immediately behind the growing thallus margins, leading to oversized, persistent photobiont cells. Most algal cell division occurred in May when the combination of light, water availability and temperature were optimal (MacKenzie *et al.*, 2001), compared to samples from other dates when key factors necessary for growth were limiting, such as light (Aug), temperature (Nov) or liquid water availability (Feb).

L. pulmonaria typically grows up to 4 mm yr^{-1} (Muir *et al.*, 1997), near the maximal rates for foliose lichen species (Palmqvist, 2000). Therefore, the old lichen thallus regions we investigated (*c.* 70–100 mm) were approx. 25 yr old, and the young thalli (from the margin to *c.* 30 mm) were up to about 10 yr old. The nondividing algal cells in these thallus regions therefore remain photosynthetically active through many cycles of environmental change, and perform repeated cycles of acclimation of their macromolecular composition to maintain a balance between photosynthesis and photoprotection under vastly different environmental conditions through the year (MacKenzie *et al.*, 2001, 2002).

The allocation of protein resources to excitation dissipation capacity per PSII reaction center is described by the PsbS per PsbA ratio. In February, after several months of relatively bright, cold and dry conditions not conducive to photosynthesis, PsbA concentration dropped to its lowest levels while PsbS concentration increased, leading to maximal PsbS to PsbA ratios. Needles of the evergreens Scots Pine and Lodgepole Pine, when acclimated to cold temperatures, decreased PsbA concentration coincident with an increase in PsbS (Ottander *et al.*, 1995; Savitch *et al.*, 2002), as occurred in our *L. pulmonaria* samples. By contrast neither PsbA nor PsbS changed in the leaves of the annual winter wheat (Savitch *et al.*, 2002), while PsbS levels were also stable in spinach leaves during wide changes in PSII concentration in response to changing light conditions (Lindahl *et al.*, 1997). Gilmore & Ball (2000) showed that overwintering *Eucalyptus* leaves also undergo pigment rearrangements that facilitate thermal dissipation of excitation, and speculated (Gilmore, 2001) that a complex containing PsbS may mediate this dissipation, as is suspected in pines (Ottander *et al.*, 1995). Like the evergreens, and in contrast to annual or deciduous plants which die or shed their photosynthetic tissues for overwintering, we found large changes in PsbS concentration in the lichen photobiont that likely contributed to seasonal changes in excitation dissipation we have observed in these lichens (MacKenzie *et al.*, 2001, 2002).

Through most of the year, there was a consistent level of chlorophyll per PsbA, except in February, when PsbA dropped substantially while the chlorophyll concentration was maintained or even increased. The low winter stock of PsbA shows that during the winter the potential for photochemical generation of electrons was low while the potential for excitation dissipation was high. The macromolecular balance returned to a low PsbS/PsbA ratio and high RbcL concentration in May, when resources were redeployed into RbcL, a component of the Calvin Cycle enzymes, thereby regenerating a sink for photosynthetic electrons. In two previous studies using similar lichen samples (MacKenzie *et al.*, 2001, 2002), we showed a physiological down-regulation of carbon fixation, with a coincident increase in excitation dissipation capacity (xanthophyll pool size and nonphotochemical quenching capacity) in the winter months. We also noted the most intense brownish pigmentation of the surface of the lichen (Fig. 1a), identified as a fungal melanin screening pigment by Gauslaa & Solhaug (2001), and a thickening of the fungal cortex over photobionts in February (Fig. 2d), possibly contributing to the photoprotective screening of the algae by their mycobiont partner (MacKenzie & Campbell, 2001). We have previously shown that carbon fixation at the thallus scale drops dramatically in the winter months, in part because the rate of carbon fixation per remaining RbcL active sites drops to near negligible levels (MacKenzie *et al.*, 2002). Together with the observed decrease in photosynthetic electron transport and increase in excitation dissipation (MacKenzie *et al.*, 2002) we conclude that there is a strong down-regulation of assimilatory photosynthesis in the bright and cold winter in these lichens, mediated in large part by reallocations of macromolecular pools. These results corroborate our earlier physiological data and show that *L. pulmonaria* allocates resources toward carbohydrate production through the spring, summer and fall, and toward photoprotective energy excitation dissipation in the high-light, cold and dry winter. Green algal lichen photobionts are likely maintained under nitrogen limitation imposed by the mycobiont, even within lichens with cephalodial nitrogen-fixing cyanobacteria. Nitrogen can limit lichen growth (Crittenden *et al.*, 1994), with the mycobiont and photobiont competing for scare resources. Mycobionts also rapidly sequester fixed nitrogen from cephalodial cyanobionts (Rai *et al.*, 1981), and there is no evidence that nitrogenous nutrients can be transferred to the photobionts (Smith, 1993). Thus, photobiont re-allocations of protein resources are in the context of both mitotic arrest and limited capacities for net biosynthesis of new protein. Furthermore there is evidence the mycobiont contributes to the excitation tolerance of the photobionts by altering levels of screening pigments (Gauslaa & Solhaug, 2001; MacKenzie & Campbell, 2001).

We also investigated nitrogenase and glutamine synthetase in *L. pulmonaria*, which, unlike the photosynthetic proteins, did not show significant change through the seasons. There was no significant seasonal change in the average levels of the NifH subunit of the cyanobacterial nitrogen-fixing enzyme nitrogenase, although there was high variation in detected levels between samples. We did not, however, detect any cyanobacterial GlnA, the glutamine synthetase enzyme that incorporates ammonium into glutamine. Previous studies on a similar tripartite lichen, *Peltigera aphthosa*, show that cephalodial *Nostoc* has low glutamine synthetase activity relative to

the isolated free living *Nostoc* (Rai *et al.*, 1981). This may indicate a suppression of glutamine synthetase in the cyanobacterial symbiont that helps effect the export of fixed nitrogen to the mycobiont.

Photosynthetic acclimation in annual and deciduous plants shows limited plasticity within existing tissues. Fully expanded leaves of winter cereals exhibit stress responses with only limited acclimation when subjected to temperature or light changes, and their capacity for electron transport and carbohydrate production is generally inhibited by such shifts (Öquist & Huner, 1991). In these plants the greatest acclimatory response is through development, with new tissue growing into a relatively fixed acclimatory state. In such plants therefore the time of exposure and life history of the leaves are the major factors in the photosynthetic responses to low temperatures (for review see Huner *et al.*, 1993). Acclimation of photosynthetic components, however, has been observed within the lifetime of individual *Dunaliella salina* algal cells (Maxwell *et al.*, 1995). Persistent evergreen plant tissue can also show reversible reallocations of molecular resources during seasonal acclimation to survive through periods less conducive to photosynthesis (Ivanov *et al.*, 2001). In our study, the *L. pulmonaria* photobionts showed large and rapid (MacKenzie *et al.*, 2001) reallocations of their macromolecular contents within a population of nondividing algal cells that persist through many seasonal cycles. Unlike nonevergreen plants therefore the *L. pulmonaria* photobionts regulate a large acclimatory potential through decades of strong seasonal environmental changes, in persistent unicellular algal cells during prolonged mitotic arrest.

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