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Lichen respiration in relation to active time, temperature, nitrogen and ergosterol concentrations

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Summary

1. Respiration in eight lichen species was related to thallus hydration status, external temperature and to total nitrogen, chitin and ergosterol concentrations. Chitin is a nitrogenous and major compound of the fungal cell wall, and ergosterol is a sterol of the plasma membrane in fungi and sometimes in algae.

2. Hydration of previously dry thalli resulted in an initially high rate of respiration. Both the amplitude of this resaturation respiration and the time required to reach steady state varied among species. Generally, peak rates were one to three times higher than steady-state rates, which were reached 3–7 h after hydration.

3. Increases in external temperature also resulted in transient bursts in respiration. Again, both the amplitude of the burst and the time required to reach steady state varied among species. Also depending on species, a temperature increase from 5 to 15 °C resulted in two- to fivefold increases in steady-state respiration.

4. Steady-state respiration, at optimal thallus hydration and a given temperature, varied three- to sixfold among the species, when related to thallus dry mass. This difference correlated best (r^2 = 0·89) with their ergosterol concentration, where a doubling in ergosterol resulted in more than a doubling in respiration. Respiration correlated less well to total nitrogen or chitin.

5. The chitin to ergosterol ratio varied more than one order of magnitude between the species, where species with high nitrogen concentrations had the highest ratio. This implies that species with access to ample amounts of nitrogen can make more fungal cell walls in relation to plasma membrane surface area.

Key-words: Biomass, chitin, microclimate, plasma membrane

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Introduction

Lichens are the symbiotic phenotype of nutritionally specialized fungi that live in symbiosis with algal and/or cyanobacterial photobionts (Honegger 1991). Lichens are often confined to rather dry habitats, such as deserts, arctic tundras, heaths and tree canopies, thus remaining dry and metabolically inactive most of the time (Green & Lange 1995; Hestmark, Schroeter & Kappen 1997; Renhorn *et al*. 1997; Sundberg *et al*. 1997). In addition, a significant part of their metabolically active periods occur at low light intensities or in complete darkness (Smith & Douglas 1987; Sundberg *et al*. 1997). In lichens the photosynthetic partner may constitute as little as 10% of the lichen biomass (Ahmadjian 1993). For these reasons the respiratory load per photosynthetic cell can be major in these symbiotic organisms and, consequently, the rate of respiration will have a large impact on their net carbon gain.

Numerous studies have already documented how environmental factors affect respiration rates in lichens (Kershaw 1985; Green & Lange 1995; Nash 1996). When dry lichen thalli are rehydrated, three distinct stages are apparent. First, there is a large and rapid, non-metabolic release of CO_2, which lasts for a few minutes. This burst is followed by a period of resaturation respiration, which gradually declines to a constant steady-state rate. The duration and amplitude of the two first stages vary among species and seasons (Brown, McFarlane & Kershaw 1983). Respiration also generally increases with increasing water content (WC) up to a WC of 70–200% of the thallus dry mass (DM) (Kershaw 1985). Respiration also increases

with increasing temperature (Kershaw 1985; Nash 1996) where a 10 °C increase in temperature generally results in a two- to threefold increase in respiration (Smith 1962; Farrar 1973). It is thus well documented that respiration, in most lichens, responds similarly to changes in WC and/or temperature. However, in most studies it is not clear whether the measured respiration reflected long-term steady-state rates or transiently high rates achieved only for shorter periods.

In absolute terms, steady-state respiration rates vary significantly both among and within species (Smith 1962; Kershaw 1985; Lange, Kilian & Ziegler 1986). In higher plants, this variation may be positively correlated to both nitrogen and protein concentrations (Lambers 1985; Chapin *et al*. 1987). High nitrogen concentrations may coincide with rapid growth and/or a high protein turnover thus resulting in high respiration rates. However, it is not known whether this can explain the large variation in respiration rates among lichens because few have addressed this question (Green & Lange 1995).

In this work, we aimed at getting a better understanding of factors causing variations in respiration among lichens. This was carried out by first characterizing rehydration- and temperature-response curves for five lichen species. For these, and three additional species, steady-state respiration rates, at optimal WC and a given temperature, were related to thallus concentrations of nitrogen, chitin and ergosterol. These compounds were of interest for the following reasons: (1) few comparisons have been made between respiration rates and nitrogen for lichens (cf. Green & Lange 1995); (2) chitin is a polymer of *N*-acetyl-D-glucosamine that may account for 3–12% of the DM of the mycobiont cell wall (Honegger & Bartnicki-Garcia 1991); (3) ergosterol is the major sterol of the fungal plasma membrane (Weete 1980) and is used as an indicator of metabolically active fungal cells in ectomycorrhizal roots (Ekblad, Wallander & Näsholm 1998). Ergosterol is also a major sterol of the plasma membrane in some free living green algae as well as in cyanobacteria and has been observed in, for instance, the lichen photobiont *Trebouxia* sp. (Goodwin 1974; Kohlhase & Pohl 1988).

In mycorrhizal roots, the ratio of chitin to ergosterol has been used to estimate the proportion of total to living fungal biomass (Ekblad *et al*. 1998). If applicable to lichens as well, this would provide a measure of the relative proportion of metabolically inactive fungal cells to active fungal, or fungal and photobiont, cells in lichens.

All lichens used in this work were of the order Lecanorales. The primary photobionts were from the genera *Coccomyxa*, *Dictyochloropsis*, *Nostoc* and *Trebouxia*. Some of the lichens were tripartite associations. These had green algal primary photobionts and N_2-fixing cyanobacteria in cephalodia.

Table 1. List of investigated lichens, their primary and, where applicable, secondary photobiont (in cephalodia): C, cyanobacterium; G, green alga

Lichen species	Primary photobiont	Secondary photobiont
BIPARTITE GREEN ALGAL LICHENS		
Cetraria islandica (L.) Ach	*Trebouxia* sp. (G)	
Hypogymnia physodes (L.) Nyl.	*Trebouxia* sp. (G)	
Lasallia pustulata (L.) Hoffm.	*Trebouxia* sp. (G)	
Platismatia glauca (L.) W. Culb.	*Trebouxia* sp. (G)	
TRIPARTITE GREEN ALGAL LICHENS		
Lobaria pulmonaria (Scop.) DC	*Dictyochloropsis reticulata* (G)	*Nostoc* sp. (C)
Nephroma arcticum (L.) Hoffm.	*Coccomyxa* sp. (G)	*Nostoc* sp. (C)
Peltigera aphthosa (L.) Willd.	*Coccomyxa* sp. (G)	*Nostoc* sp. (C)
BIPARTITE CYANOBACTERIAL LICHEN		
Peltigera canina (L.) Willd.	*Nostoc* sp. (C)	

Materials and methods

LICHENS AND THEIR PHOTOBIONTS

The investigated lichens and their photobionts are listed in Table 1. All lichens were collected from their natural habitats in the county of Västerbotten, Sweden, in August 1995, except for *Platismatia glauca* (L.) W. Culb., which was collected in September 1993. After harvest the thalli were air dried at 15 °C and subsequently stored in darkness at – 18 °C. Gas-exchange measurements were made during the spring of 1996. Reactivation of the thalli was as in Palmqvist (1993) unless otherwise stated.

GAS-EXCHANGE MEASUREMENTS

CO_2 release was measured in darkness with a flow-through gas-exchange system (Compact Minicuvette System 400, gas mixing unit GMA1 and cuvette GK-022; H. Waltz, Effeltrich, Germany). The temperature in the cuvette was regulated by a built-in Peltier element. The temperature outside the cuvette was held at ± 3 °C of the cuvette temperature by placing the cuvette in a temperature-regulated cabinet. The CO_2 concentration (350 µl CO_2 litre^{-1}) was obtained and controlled as in Palmqvist (1993). Two types of experiments were performed. In the first series of experiments, dark respiration rates were followed as a function of time after thallus rehydration, at 5 °C. In the second series the effect of temperature changes was studied. Here, the respiratory release of CO_2 was monitored for 2–3 h at 0, 5, 10 and 15 °C. In all measurements, five to 10 reactivated thalli were pooled to a large sample. These corresponded to 0·5–1·0 g lichen dry mass (DM). Maximal respiratory activity was ensured by keeping the thalli at an optimal and constant water content, monitored by impedance measurements as detailed in Sundberg *et al*. (1997). The temperature coefficient Q_{10} was used for a quantitative expression of the change in respiratory rate with increasing temperature.

CARBON AND NITROGEN ANALYSES

After gas-exchange measurements, the thalli were freeze-dried (Ice condenser section; Modulyo 4K Freeze Dryer, Edwards High Vacuum International, West Sussex RH10 2LW, UK, and External vacuum pump; Trivac-B D4B) and milled to a powder (Retsch-Mühle, Haan, Germany). At least 2 g DM (*c.* 100–150 cm^2) of each species was used for carbon and nitrogen analyses, carried out by an authorized laboratory (SLU, Rödbäcksdalen, Umeå, Sweden) using the LECO–CHN1000 method.

CHITIN AND ERGOSTEROL ANALYSES

Free ergosterol was extracted from 10 mg of freeze-dried and milled lichen material dissolved in 1 ml ethanol. The sample was intensively shaken on a vortex for 2 min and then centrifuged (11 400 **g** for 30 min), 50 µl of the resulting supernatant was injected and analysed in an HPLC system. Ergosterol was separated from other compounds on a reversed-phase column (ODS-hypersil, 250 × 4·6 I.D., 5 µm) using isocratic elution with 100% methanol as the mobile phase and a flow rate of 1·5 ml min^{-1}. In this system, the retention of ergosterol was *c.* 9 min. The ergosterol peak was detected with a UV detector at 280 nm. The ergosterol values were corrected for a recovery of 81%, as described in Nylund & Wallander (1992).

Chitin was measured on the residue after the ergosterol extraction, according to Ekblad & Näsholm (1996). Briefly, the sample was treated with 0·2 M NaOH to remove proteins and amino acids that could interfere with the HPLC analysis of glucosamine. Acid hydrolysis (6 M HCl) was used to release the glucosamine residues, which were converted to fluorescent derivatives by treatment with 9-fluorenylmethylchloroformate. The derivatives were subsequently analysed by RP-HPLC, using an isocratic elution. Background contamination was reduced by using norvalin instead of homocysteic acid that was used in Ekblad & Näsholm (1996), as the internal standard. The glucosamine residues were separated as three peaks in the HPLC and in this study, Peak 3 instead of Peak 1 was used for the quantification, because this peak was better resolved (cf. Ekblad & Näsholm 1996; Ekblad *et al*. 1998). Chitin values were corrected for a chitin loss of 29% during ergosterol extraction.

CHLOROPHYLL ANALYSIS

Chlorophyll was extracted from freeze-dried and milled lichen samples in $MgCO_3$-saturated dimethylsulphoxide (DMSO) (60 °C for 40 min) and measured with a spectrophotometer. Calculations were made according to Ronen & Galun (1984).

Results

DARK RESPIRATION RATES IN RELATION TO REHYDRATION TIME

Figure 1 shows the time-dependent change in respiration rate after re-wetting of dry thalli. The CO_2 burst during the first minute has been excluded from these data because this burst is not related to metabolic activity (Brown *et al*. 1983). For all species, the initial respiration rate was always one-and-a-half to three-times higher than the final steady-state rate. The time required to reach steady state differed depending on the species. *Cetraria islandica* (L.) Ach and *Hypogymnia physodes* (L.) Nyl. reached steady state within 3 h after soaking, *P. glauca* was close to steady state within 7 h, while respiration in *Peltigera aphthosa* (L.) Willd. and *Lobaria pulmonaria* (Scop.) DC still decreased slightly after 5–7 h of reactivation.

DARK RESPIRATION RATES IN RELATION TO TEMPERATURE

All species showed an initial and rapid increase in respiration during the first 15 min after a 5 °C increase in temperature (Fig. 2). After this initial increase, the rate of respiration gradually decreased. *Lobaria pulmonaria* differed somewhat from this pattern because of a significantly lower rate of decrease. Steady-state respiration, at each temperature, was reached within 1–3 h in all species. *Lobaria pulmonaria* consistently had the highest steady-state respiration rates at all temperatures (1·94–6·13 nmol CO_2 g^{-1} DM s^{-1}) followed by *P. aphthosa* (1·68–3·49 nmol CO_2 g^{-1} DM s^{-1}). *Cetraria islandica* and *H. physodes* had the lowest

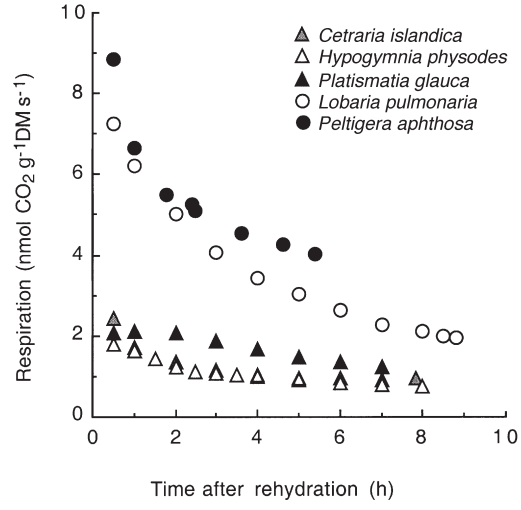

Fig. 1. Dark respiration rates at 5 °C as a function of time after rehydration of dry thalli. Values are mean rates of five to 10 thalli (0·5–1 g DM) of *Cetraria islandica*, *Hypogymnia physodes*, *Platismatia glauca*, *Lobaria pulmonaria* and *Peltigera aphthosa*..

steady-state rates, 0·26–1·90 and 0·0–1·79 nmol CO_2 g^{-1} DM s^{-1}, respectively. *Platismatia glauca* had similar low rates at 5 and 10 °C but increased towards the rates of *P. aphthosa* at 10 and 15 °C (0·0–2·55 mg CO_2 g^{-1} DM s^{-1}). Particularly at the lower temperatures, the lichens were divided into two distinctive groups, where the bipartite green algal lichens all had a lower rate of respiration compared to the of tripartite green algal lichens (Fig. 2). Depending on species, an increase in temperature from 5 to 15 °C caused a two- to fivefold increase in steady-state respiration, as reflected by Q_{10} values between 2·4 and 5·0 (Fig. 2, Table 2).

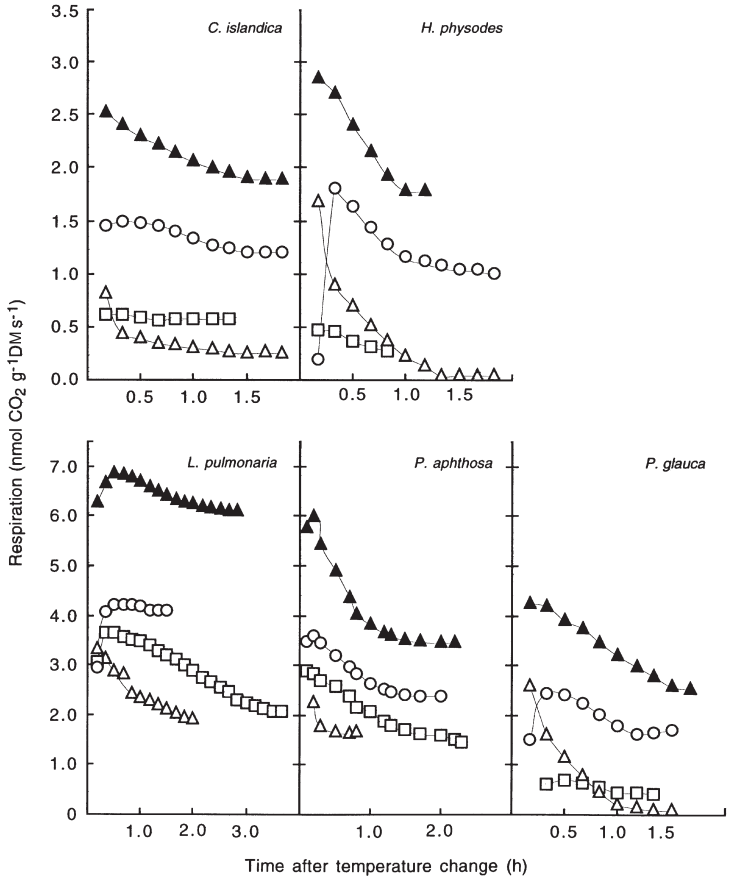

Fig. 2. Dark respiration rates at 0 °C (△), 5 °C (□), 10 °C (○) and 15 °C (▲). Values are mean respiration rates of five to 10 thalli that were pooled to a sample. Reactivated thalli were mounted in the cuvette and CO_2 release was measured at each temperature for 1–3 h, beginning at 0 °C and increasing the temperature stepwise with 5 °C. All measurements were made with water-saturated thalli. SE for the species were; *C. islandica* 15%, *H. physodes* 21%, *P. glauca* 22%, *L. pulmonaria* 15% and *P. aphthosa* 22%.

NITROGEN, CHITIN, ERGOSTEROL AND CHLOROPHYLL CONCENTRATION

Both the chitin and the ergosterol concentration increased with increasing nitrogen concentration, resulting in higher concentrations of both these compounds in the lichens with N_2 fixation [*L. pulmonaria*, *Nephroma arcticum* (L.) Hoffm., *P. aphthosa* and *Peltigera canina* (L.) Willd.]. However, with increasing nitrogen concentration, the chitin concentration increased four times more than the ergosterol concentration (Table 2). As a result, the chitin to ergosterol ratio varied more than one order of magnitude among the species, from 10 in the low nitrogen species *P. glauca* to more than 100 in the two *Peltigera* spp. (Fig. 3, Table 2).

The chlorophyll *a* concentration, which varied two- to threefold among the species, also increased with increasing nitrogen concentration (Table 2).

DARK RESPIRATION RATES IN RELATION TO NITROGEN, CHITIN AND ERGOSTEROL CONCENTRATION

Dark respiration correlated best with ergosterol concentration (r^2 = 89%, $P < 0·01$), where a doubling in ergosterol concentration corresponded to about a doubling in respiration (Fig. 4c). Respiration rates were also positively correlated with nitrogen concentration as well as with chitin concentrations (Fig. 4a,b). However, r^2 was lower in both of these regressions (r^2 = 75%, $P < 0·05$ and 79%, $P < 0·05$, respectively). The slopes of these two regressions were also shallower than the one for ergosterol.

Table 2. Some physiological characteristics of the investigated lichens. C:N ratio is based on mol g^{-1} DM. All values represent the mean of $n \geq 3$

Species	Chlorophyll *a* (mg g^{-1}DM)	Nitrogen (mg g^{-1}DM)	Chitin (mg g^{-1}DM)	Ergosterol (µg g^{-1}DM)	Chitin:ergosterol (g:g)	C:N (mol:mol)	Q_{10}
BIPARTITE GREEN ALGAL LICHENS							
Cetraria islandica	0·38 ± 0·04	2	1·9 ± 0·3	118 ± 7	16	380	3·3
Hypogymnia physodes	0·44 ± 0·02	2	3·7 ± 0·6	82 ± 6	45	380	5·0
Lasallia pustulata	0·75 ± 0·11	18	8·1 ± 2·0	209 ± 24	39	29	
Platismatia glauca	0·43 ± 0·06	4	1·7 ± 0·3	166 ± 23	10	126	4·2
TRIPARTITE GREEN ALGAL LICHENS							
Lobaria pulmonaria	1·05 ± 0·09	20	24·1 ± 4·9	313 ± 11	77	27	2·9
Nephroma arcticum	0·66 ± 0·06	17	24·3 ± 3·5	481 ± 36	51	33	
Peltigera aphthosa	1·12 ± 0·04	28	40·0 ± 3·4	289 ± 15	138	20	2·4
BIPARTITE CYANOBACTERIAL LICHEN							
Peltigera canina	0·87 ± 0·05	37	68·4 ± 5·4	420 ± 52	163	13	

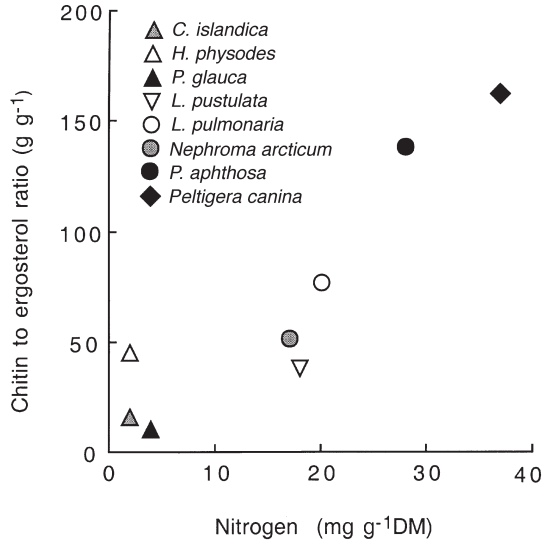

Fig. 3. Chitin to ergosterol ratio as a function of nitrogen content in *C. islandica*, *H. physodes*, *P. glauca*, *Lasallia pustulata*, *L. pulmonaria*, *Nephroma arcticum*, *P. aphthosa* and *Peltigera canina*.

Discussion

DARK RESPIRATION RATES IN RELATION TO REHYDRATION AND TEMPERATURE

Upon rehydration, the species of our investigation showed differences both in amplitude and in time required to reach steady state after an initial burst in respiration (Fig. 1). The underlying biochemichal mechanisms for this so-called resaturation respiration is still an open question. Several suggestions have been put forward, such as an increased energy demand for repair of damaged membranes (Smith & Molesworth 1973), uncoupling of mitochondria (Brown *et al*. 1983) and/or a burst in respirable substrates related to drought damage of membranes (Farrar & Smith 1976). Differences in amplitude of the burst and in time required to reach steady state has been suggested to be an effect of the time a thallus has been metabolically active before drying out and also in the rate of drying, with fast drying resulting in a higher rate of resaturation respiration (Brown *et al*. 1983).

The transient and high respiration rate immediately after each temperature increase (Fig. 2) cannot be mechanistically explained from our data; however, the subject has been discussed by Lambers (1985) and Smith & Molesworth (1973).

The two- to fivefold increase in steady-state respiration with a 10 °C temperature increase (Table 2, Fig. 2) is in the same range as previously reported both for lichens and higher plants (Smith 1962; Farrar 1973; Lambers 1985) and is most likely a consequence of the overall increase in metabolism with increasing temperature (Lambers 1985).

DARK RESPIRATION RATES IN RELATION TO NITROGEN, CHITIN AND ERGOSTEROL CONCENTRATION

The rather large difference in steady-state respiration among species was best correlated with an equally large difference in ergosterol concentration (Fig. 4c). There was also a positive but lower correlation between respiration rates and total thallus (Fig. 4a). In higher plants, high nitrogen concentrations may coincide with rapid growth and/or a high protein turnover requiring high respiration rates. However, the stronger correlation between respiration and ergosterol, compared to nitrogen, of lichens may well be explained by the following: (1) mycobiont hyphae make up most of the lichen biomass and, therefore, a relatively large proportion of the total nitrogen will be bound in metabolically inactive structural elements because chitin, in contrast to proteins, does not turn over; (2) ergosterol might be present in equal concentrations

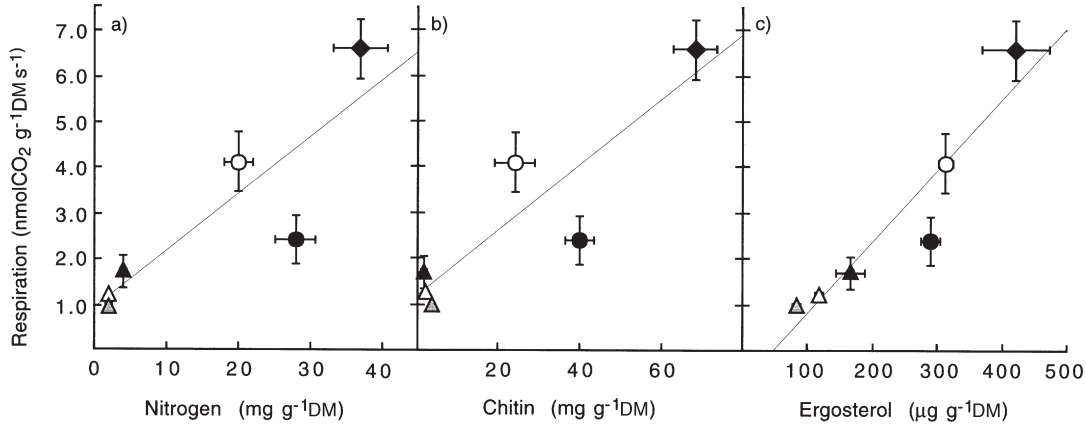

Fig. 4. Dark respiration rates in relation to (a) nitrogen, (b) chitin and (c) ergosterol concentrations. Values are mean steady-state respiration rates of *C. islandica* (▲), *H. physodes* (△), *P. glauca* (▲), *L. pulmonaria* (○), *P. aphthosa* (●) and *P. canina* (◆) at 10 °C. Respiration was measured for water-saturated thalli. Equations for the regression lines were (a) $y = 0{\cdot}89 + 0{\cdot}12x$, $r^2 = 0{\cdot}75$, $P < 0{\cdot}05$, (b) $y = 1{\cdot}16 + 0{\cdot}07x$, $r^2 = 0{\cdot}79$, $P < 0{\cdot}05$ and (c) $y = -0{\cdot}8 + 0{\cdot}016x$, $r^2 = 0{\cdot}89$, $P < 0{\cdot}01$, respectively.

both in the photobiont and in the mycobiont plasma membranes. The ergosterol concentration may thus reflect the amount of metabolically active cells in the mycobiont as well as in the photobiont. However, as data on ergosterol concentrations of lichen photobionts are lacking, this remains an open question. Evidently, further studies are needed to address this in a more comprehensive way.

THE RATIO BETWEEN METABOLICALLY ACTIVE AND INACTIVE LICHEN BIOMASS

It has previously been shown that chitin may serve as a marker of total fungal biomass in, for instance, mycorrhizas (Nylund & Wallander 1992; Ekblad *et al*. 1998), while ergosterol correlates to the amount of metabolically active fungal cells. In mycorrhizal roots, the chitin to ergosterol ratio has been used as a relative measure of metabolically inactive to active fungal biomass, where a low ratio indicates a higher proportion of metabolically active biomass (Ekblad *et al*. 1998). The ratios of the investigated lichens were in the same range as those previously found in ectomycorrhizal roots (Ekblad *et al*. 1998). In lichens, however, this ratio may rather reflect the ratio between metabolically inactive mycobiont biomass in relation to active mycobiont and photobiont cells.

There was a marked effect of increased nitrogen concentration on the chitin to ergosterol ratio in the lichens (Fig. 3). Thus, from our data it appears that the species with high nitrogen contents (*P. canina* and *P. aphthosa*) have invested a larger part of their nitrogen into metabolically inactive biomass thereby increasing their mass without increasing their respiratory load. This may well be of competitive advantage because a larger mass would increase the water-holding capacity of lichens, thereby prolonging periods with metabolic activity. An increased water-holding capacity would also protect a thallus from fast dehydration and the subsequent carbon losses associated with resaturation respiration.

Allocation of extra nitrogen into inactive biomass, however, is probably only possible for species adapted to both a high and reliable nitrogen supply. Species adapted to a more restricted supply of nitrogen, only covering the basal needs of structural elements and photosynthetic units, might have developed other partitioning patterns (Palmqvist *et al*. 1998).

In conclusion, our data show that both upon hydration and increases in temperature lichens display transiently high respiration rates. Furthermore, up to 7 h may be required to reach the lower steady-state respiration rates. It is hence important to emphasize this behaviour when monitoring respiration rates of lichens in conjunction with microclimatic changes, otherwise the measurements might lead to overestimation of the respiration under normal conditions.

The sixfold difference in respiration rate among the species correlated best with their ergosterol concentrations. This indicates that ergosterol is a suitable marker of metabolically active (respiring) cells in lichens. Whether or not this marker is exclusive for the fungal cells or includes also the photobiont cells remains, however, an open question.

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