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Responses of the lichen *Xanthoria parietina* (L.) Th. Fr. to varying thallus nitrogen concentrations

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Abstract: The responses of the nitrophytic green algal lichen *Xanthoria parietina* to varying nitrogen (N) concentrations were investigated by collecting 67 *X. parietina* thalli from clay roof tiles from 13 sites in Portugal with different exposures to N. Concentrations of total N, chlorophyll *a* (a marker for the photobiont), ergosterol (a marker for the mycobiont), and thallus specific weight (TSW; thallus dry weight in relation to surface area) were quantified for each thallus to see how biont investments were related to thallus N concentrations. Thallus N ranged from 11 to 43 mg g^{-1} DW revealing a wider N concentration range in this lichen compared to other green algal lichen species. Both chlorophyll *a* and ergosterol concentrations increased with increasing thallus N, with a steeper increase of the photobiont marker. TSW was similar in all thalli without any significant effect of thallus N concentration, suggesting that thallus developmental patterns are similar in low and high thallus N concentrations. The relatively higher resource allocation to the photobiont in relation to the mycobiont with increasing thallus N concentrations is an indication of the capacity of *X. parietina* to meet the C demands associated with N assimilation. This result is also in agreement with the inter-specific resource allocation pattern for green algal lichens across the same N concentration range.

Key words: chlorophyll *a*, ergosterol, green algal lichens, intra-specific N concentration, symbionts, thallus nitrogen, thallus specific weight (TSW), *Xanthoria parietina*.

Introduction

The ubiquity of the green algal lichen *Xanthoria parietina* (L.) Th. Fr. is related to its tolerance to several factors, such as high irradiance (Solhaug & Gauslaa 1996, 2004), seawater elements (Hinds 1995) and air pollution (Silberstein *et al.* 1996; Gaio-Oliveira *et al.* 1999; Nimis *et al.* 2000; Scerbo *et al.* 2002). This lichen is able to tolerate high levels of nitrogen (N) deposition in nature (Brown *et al.* 1994; Crittenden *et al.* 1994; Gaio-Oliveira *et al.* 2004), increasing its density in areas where atmospheric N levels are increased (De Bakker 1989; Ruoss 1999; van Herk 2001). The high tolerance of *X. parietina* with respect to varying N levels is furthermore confirmed by its wide range of thallus N concentrations (Rai 1988; Gaio-Oliveira *et al.* 2001, 2004), because thallus N status reflects N deposition in the recent past (Boonpragob *et al.* 1989; Søchting 1995; Poikolainen *et al.* 1998).

The tolerance of *X. parietina* to high N deposition may be related to its lower cation exchange capacity, when compared to lichens considered sensitive or indifferent towards high N levels (Gaio-Oliveira *et al.* 2001), and to an ability to assimilate the surplus N (Gaio-Oliveira *et al.* 2005). Although toxic when present in high concentrations, N is also an essential macronutrient (Turpin 1991). In lichens, N must be allocated to achieve a balance between the carbon(C)-acquiring photobiont cells

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and the C-expending mycobiont hyphae (Palmqvist 2000; Palmqvist *et al.* 2002). This emphasizes the fact that, as in vascular plants (Turpin 1991), the C and N economies must also be integrally coupled in lichens (Palmqvist 2000). Once taken up, N must be assimilated into nitrogenous non-toxic compounds, requiring both C skeletons and energy (Turpin 1991), and be allocated between the bionts in an optimal way. Therefore, it might not only be N toxicity *per se* that affects lichens when exposed to high N concentrations (Brown & Tomlinson 1993; Hyvärinen *et al.* 2003; Gaio-Oliveira *et al.* 2004). Supra-optimal N assimilation may also lead to an energy deficit of the symbiosis, if the photosynthetic capacity is not increased to the same extent as the energy demand following an enhanced N assimilation (Turpin 1991). One of the lichen symbionts may also be better at using the deposited N, leading to a change in the balance between the lichen partners, ultimately being detrimental for lichen survival (Gries 1996). Therefore, in order to tolerate environmental resource changes, lichens must be able to adjust their resource allocations within the thallus in such a way that equilibrium between the two partners can be maintained (Palmqvist 2000). A functional C to N equilibrium model (Brouwer 1962) was recently applied to lichens, based on a broad scale survey of lichen species with different N optima, stating that if the C to N ratio is too low, N resources should be allocated to the photobiont, otherwise N should be invested into the mycobiont (Palmqvist *et al.* 2002). It has also been recently shown for *X. parietina* that the synthesis of the pigment parietin in the mycobiont was directly regulated by photosynthate (Solhaug & Gauslaa 2004), indirectly supporting the equilibrium model. According to this model, the green algal lichens *Hypogymnia physodes* and *Platismatia glauca* responded to N fertilization by increasing their N investments into the photobiont, thereby obtaining a new C to N equilibrium (Dahlman *et al.* 2003). However, even though lichens may be able to maintain a balanced C to N equilibrium, there might be other disturbances, such as in their coordination of growth. This was observed for the tripartite lichen *Nephroma arcticum* displaying a distorted growth pattern in response to N manipulations, whereby its thallus specific weight (TSW) was increased (Sundberg *et al.* 2001; Dahlman *et al.* 2002).

The aim of this study was to examine partitioning between bionts and other thallus characteristics, in relation to total N concentration of untreated thalli of *X. parietina* collected from natural sites with varying N exposures. The intra-specific N response pattern of the untreated *X. parietina* thalli was also compared with the inter-specific pattern for a broader range of green algal lichens (Palmqvist *et al.* 2002).

Material and Methods

Lichen collection

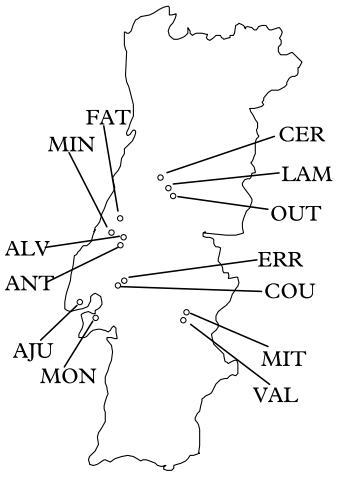
Xanthoria parietina (L.) Th. Fr. is a foliose lichen with the green algal photobiont *Trebouxia arboricola* Puymaly. A total of 67 thalli were collected on dry sunny days between the last week of August and the first week of September 2001 from clay roof tiles from 13 sites in Portugal (Fig. 1). There are no available data on regional N deposition levels in Portugal, but a qualitative characterization concerning the main activities that can influence N deposition at each collection site is presented in Fig. 1.

Morphological measurements

In the laboratory, thalli was cleaned of debris and their dry weight and surface area measured. The weight was determined after the thalli had been air-dried at room temperature, under controlled conditions, for 3 days. Each thallus surface area was traced onto transparent film when wet and fully expanded, after which the drawings were computer scanned, painted with Adobe Photoshop 5.0 (Adobe Systems Inc., USA) and analysed using WinRhizzo software (Régent Instruments Inc., Quebec, Canada). Thallus specific weight (TSW) was determined from the dry weight and surface area measurements. Following this, each thallus was sub-sampled for total N and biont marker analyses.

Total N analysis

Samples were dried for 24 hours at 60 °C, after which they were milled to a powder and analysed for total N concentrations in an automatic Elemental Analyser (EuroVector, Milan, Italy) following standard methods at the Stable Isotopes Laboratory (LIE, ICAT, Sciences Faculty, Lisbon).



Collection sites	UTM coordinates	Characterization	Sampled thalli (n)
AJU	29SNC8384	City	6
ALV	29SND1977	Country-side; agriculture	9
ANT	29SND2373	Country-side; cattle	8
CER	29TNE5470	Country-side; cattle	3
COU	29SND3608	Country-side, agriculture	4
ERR	29SND3608	Country-side, agriculture	5
FAT	29SND2985	Country-side, agriculture	5
LAM	29TNE5169	Country-side, agriculture	7
MIN	29SND2774	City	3
MIT	29SNC8465	Country-side, cattle	5
MON	29SNC0284	City	4
OUT	29TNE5061	City	3
VAL	29SNC8465	City	5

FIG. 1. The location in Portugal of each collection site and its main characterization.

TABLE 1. *Thallus minimum, maximum and mean N concentrations (mg g^{-1} DW), and mean thallus specific weight (TSW) in* X. parietina *collected from different sites.*

Collection sites	Thallus total N (mg g^{-1} DW)			TSW (g m^{-2})
	Min.	Max.	Mean ± SE*	Mean ± SE*
AJU	18	26	22 ± 1^{bc}	263 ± 31^{ab}
ALV	11	31	23 ± 2^{bc}	169 ± 11^{b}
ANT	12	36	28 ± 3^{ab}	199 ± 12^{ab}
CER	23	34	28 ± 4^{ab}	274 ± 93^{a}
COU	16	27	21 ± 2^{bc}	217 ± 42^{ab}
ERR	16	27	22 ± 2^{bc}	215 ± 38^{ab}
FAT	18	28	23 ± 2^{bc}	204 ± 22^{ab}
LAM	13	29	23 ± 2^{bc}	162 ± 50^{b}
MIN	23	29	25 ± 2^{bc}	223 ± 15^{ab}
MIT	12	30	17 ± 3^{c}	233 ± 25^{ab}
MON	32	43	36 ± 3^{a}	286 ± 20^{a}
OUT	24	27	26 ± 1^{bc}	218 ± 43^{ab}
VAL	18	28	25 ± 1^{bc}	261 ± 46^{ab}

*For the mean concentrations, values with different letters are significantly different, at $P<0{\cdot}05$, after a one-way ANOVA was performed (thallus total N: df=12; MS=92·84; $F=3{\cdot}11$; $P=0{\cdot}0019$. TSW: df=12; MS=8461·89; $F=2{\cdot}11$; $P=0{\cdot}0306$).

Biont markers analysis

Samples were freeze-dried and milled to a powder. Chlorophyll *a* was used as a marker for photosynthetic capacity (Palmqvist *et al.* 1998, 2002) and was quantified after extraction in $MgCO_3$ saturated dimethyl sulphoxide (DMSO), at 60 °C for 40 min (Palmqvist & Sundberg 2001). Ergosterol, the main sterol of fungal plasma membranes, was used as an indirect marker for active fungal tissue (Palmqvist *et al.* 2002; Dahlman *et al.* 2003), and was measured by HPLC after extraction in 99·5% ethanol (Dahlman *et al.* 2001).

Statistical analysis

Linear regressions were made using SigmaPlot version 8.02 (SPSS Inc. Chicago, IL, USA) and one-way ANOVA was performed using Statistix 7 (Analytical Software, Tallahassee, FL, USA). Linear relationships with $P<0{\cdot}05$ were considered significant. Comparisons

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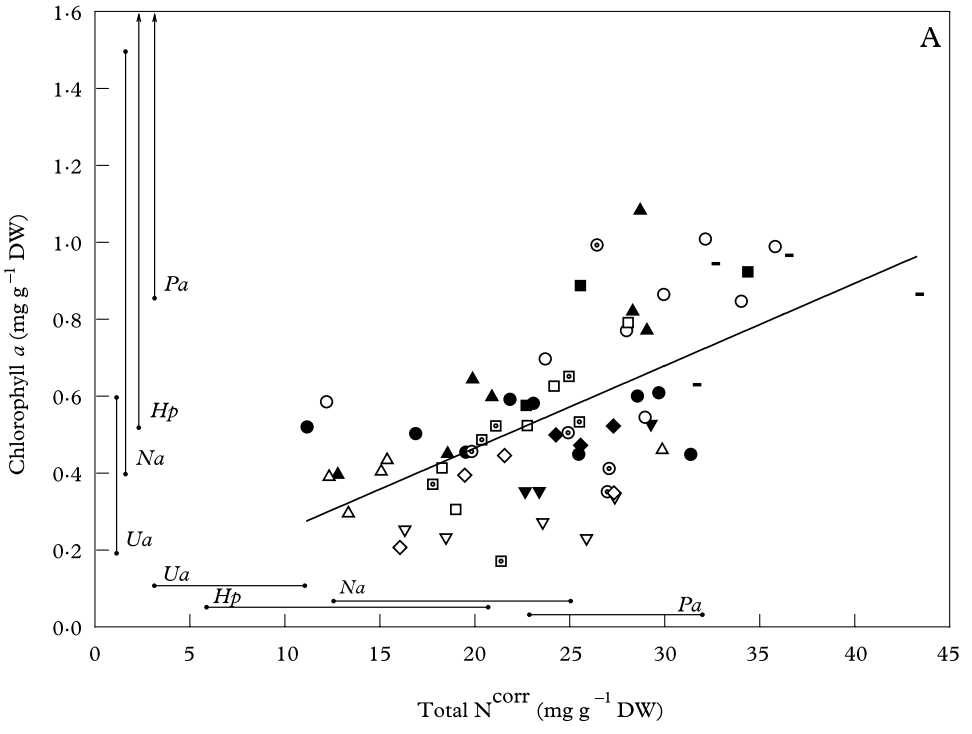

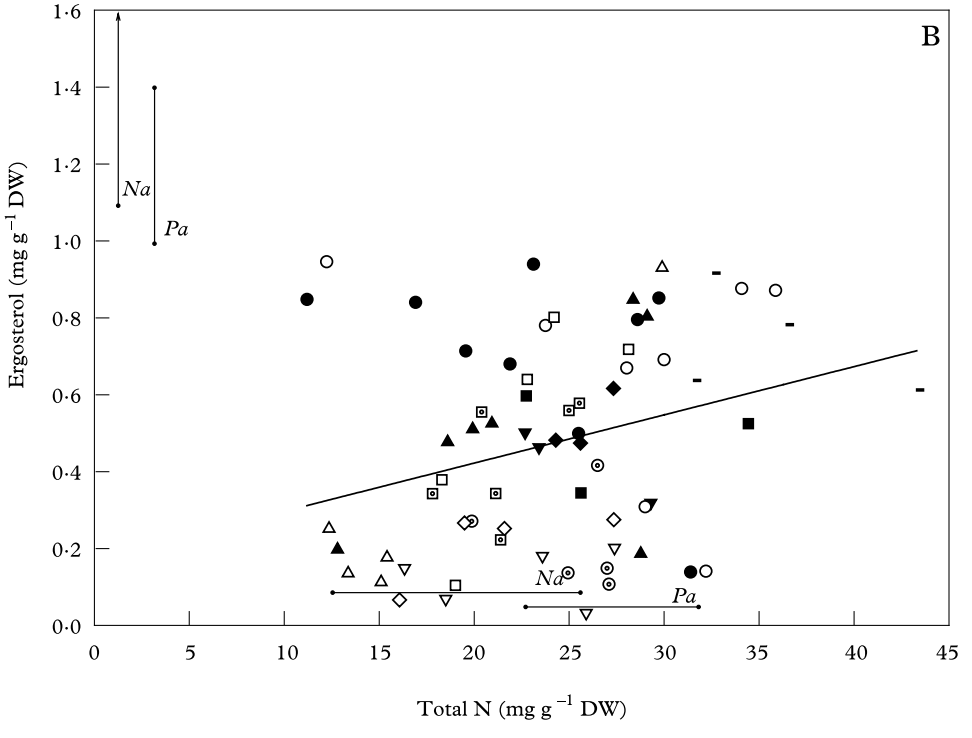

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of means were performed using a Tukey's (HSD) test.

Results

The total N concentration in *X. parietina* in the present study was wide, varying from 11 to 43 mg g^{-1} DW (Table 1). There was marked variation within sites (Table 1) whereas mean values for sites were similar with the exception of that at one site (MON) where thalli had the highest mean N concentration (Table 1).

The chlorophyll *a* concentration ranged from 0·2 to 1·1 mg g^{-1} DW (Fig. 2A) and was positively related to thallus N concentration (Fig. 2A) according to the following linear regression equation: thallus chlorophyll $a = 0{\cdot}02 \times$ thallus N + 0·04 (adj $r^2 = 0{\cdot}38$; $P < 0{\cdot}0001$). Although irradiance levels at each site were not measured, the variation in chlorophyll concentration among thalli is unlikely to be associated with their light environment, as all thalli were collected from roof tiles, directly exposed to sunlight with no shading from trees or other houses. Ergosterol concentration ranged from 0·03 to 0·9 mg g^{-1} DW (Fig. 2B), displaying a weak, although statistically significant, positive relation to thallus N concentration (Fig. 2B) according to the following linear equation: thallus ergosterol $= 0{\cdot}01 \times$ thallus N + 0·17 (adj $r^2 = 0{\cdot}07$; $P = 0{\cdot}0155$).

Thallus surface area ranged from 10 to 115 cm^2 and thallus dry weight from 0·2 to 2·5 g (Fig. 3). A positive linear relation was found between these two parameters when including all thalli in a regression analysis (Fig. 3): thallus area $= 37{\cdot}5 \times$ thallus dry weight + 5·2 (adj $r^2 = 0{\cdot}75$; $P < 0{\cdot}0001$). Thallus specific weight (TSW) varied 5-fold, from 92 to 456 g m^{-2}, but no significant relation was found between this parameter and thallus N concentration (Fig. 4). Furthermore, no major differences were found when *X. parietina* mean TSW was compared among the collection sites (Table 1). An attempt was made to group the lichen data collected from sites with the same type of N emission source (e.g. agriculture, cattle or city) (Fig. 1) and to screen for differences in the parameters studied depending on N source. However, no significant patterns were found when pooling and analysing the data in this way (not shown).

Discussion

The total N concentration range recorded in *X. parietina* [11 to 43 mg g^{-1} DW (Table 1)], suggests that this lichen is well adapted to handle both low and high levels of atmospheric N deposition in nature, since thallus N concentration reflects deposition in the recent past (Boonpragob *et al.* 1989; Søchting 1995; Poikolainen *et al.* 1998). Although the majority of green algal lichens contain less than 20 mg N g^{-1} DW (Palmqvist *et al.* 2002), some thalli of *X. parietina* had an N status similar to N_2-fixing cyanobacterial and tripartite lichens (Fig. 2) (Palmqvist *et al.* 2002; Dahlman & Palmqvist 2003). This is quite surprising considering that *X. parietina* only intercepts N through wet or dry deposition. The N concentration range of *X. parietina* obtained here was thus wider than for most other green algal lichens (Palmqvist *et al.* 2002), including *Hypogymnia physodes* from a

FIG. 2. Thallus chlorophyll *a* (A) and ergosterol (B) concentrations as a function of thallus total N concentration in *X. parietina* collected at different sites: AJU (⊡), ALV (●), ANT (○), CER (■), COU (◇), ERR (▽), FAT (□), LAM (▲), MIN (▼), MIT (△), MON (—), OUT (◆) and VAL (⊙). The N content of chlorophyll *a* (6·27% of molecular weight) was subtracted from the thallus total N (=total N^{corr}) to avoid autocorrelation. A positive linear relation was found between chlorophyll *a* and thallus N ($P < 0{\cdot}0001$) (A): thallus chlorophyll $a = 0{\cdot}02 \times$ thallus total N + 0·04 (adj $r^2 = 0.38$). A positive linear relation was also found between ergosterol and thallus N ($P = 0{\cdot}0155$) (B): thallus ergosterol $= 0{\cdot}01 \times$ thallus total N + 0·17 (adj $r^2 = 0{\cdot}07$). Horizontal lines represent the range of thallus N concentrations and vertical lines represent the range of thallus chlorophyll *a* or ergosterol concentrations found in the green algal lichens *Hypogymnia physodes* (*Hp*) (Palmqvist *et al.* 2002) and *Usnea aurantiaco-atra* (*Ua*) (Valladares & Sancho 2000) and in the tripartite lichens *Nephroma arcticum* (*Na*) and *Peltigera aphthosa* (*Pa*) (Dahlman & Palmqvist 2003).

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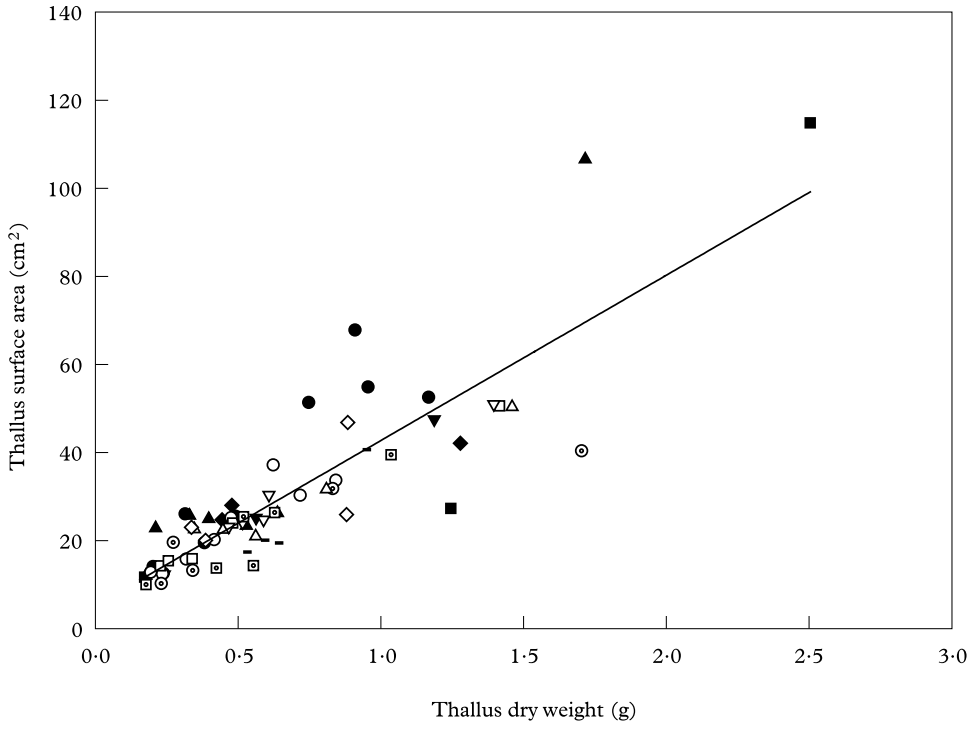

FIG. 3. Thallus surface area as a function of thallus dry weight in *X. parietina* collected at different sites: AJU (⊡), ALV (●), ANT (○), CER (■), COU (◇), ERR (▽), FAT (□), LAM (▲), MIN (▼), MIT (△), MON (—), OUT (◆) and VAL (⊙). A positive linear relation was found between the two parameters ($P<0{\cdot}0001$): thallus area = 37·5 × thallus weight + 5·2 (adj $r^2=0.75$).

heavily N-fertilized location, which had 16 to 21 mg N g^{-1} DW (Fig. 2A; Dahlman *et al.* 2003). The green algal lichen *Usnea aurantiaco-atra* from an N-fertilized penguin colony also displayed a lower N range, compared to *X. parietina*, with thallus N concentrations between 3 to 10 mg g^{-1} DW (Fig. 2A; Valladares & Sancho 2000). Taken together, one could infer that *X. parietina* is a highly tolerant species towards atmospheric N, being able to cope with both a low and a high environmental N supply.

The large variation in thallus N concentration at each collection site was quite surprising (Table 1). This could be an indication that several factors, such as their orientation and distance from the N sources, can influence N uptake by lichen thalli within a particular species growing at a specific location (Brown *et al.* 1994; Søchting 1995). Moreover, the dominant N form can vary from site to site, as it is known that in industrial and urban areas N oxides are the main N sources, whereas in rural areas the main N sources are ammonia and ammonium ions, from fertilizers and cattle manure (Bytnerowicz & Fenn 1996; Gaio-Oliveira *et al.* 2001; Krupa 2003). It is interesting to see that the three locations with the least variation in N concentration (MIN, MON and OUT) (Table 1) were all classified as 'city' in terms of potential N sources. A difference between city and rural sites might relate to a difference in the dominant form of N emission. NO_x generated from vehicular traffic and industrial activities in cities has a low deposition velocity and might deposit more homogeneously compared to ammonia which might be the predominant N form in rural areas; ammonia has a high deposition velocity and deposition might vary markedley with prox-

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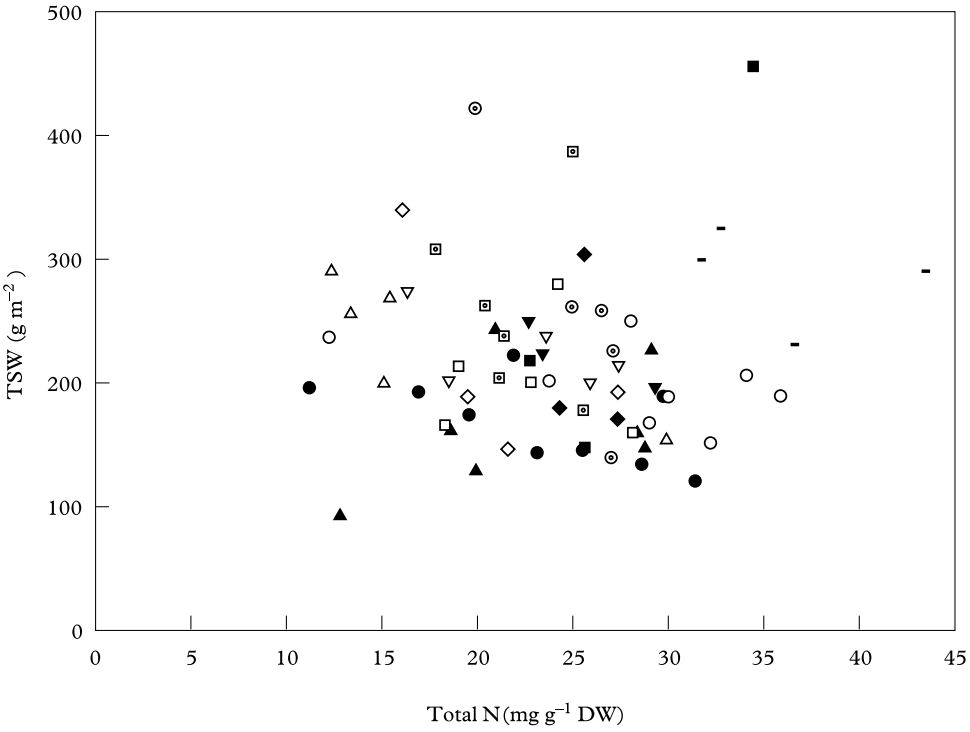

FIG. 4. Thallus specific weight (TSW) as a function of thallus total N concentration in *X. parietina* collected in different sites: AJU (⊡), ALV (●), ANT (○), CER (■), COU (◇), ERR (▽), FAT (□), LAM (▲), MIN (▼), MIT (△), MON (—), OUT (◆) and VAL (⊙).

imity to sources. Nevertheless, these are only hypotheses, as N deposition values were not available and no distinction was made between the different N forms taken up by the lichens. Moreover, the cellular location of N was not determined meaning that part of the quantified N could have been, for example, in the particulate form within the intercellular spaces (Garty *et al.* 1979; Knops *et al.* 1991), thereby not affecting lichen metabolism. Nevertheless, it has previously been shown that *X. parietina* is able to take up and assimilate concentrations of N as high as 86 mM NH_4NO_3 (Gaio-Oliveira *et al.* 2005).

The concentration of the biont markers did not decrease with increasing thallus N (Fig. 2), an additional indication that increased N concentration does not harm *X. parietina*. In contrast, there was a significant increase in both chlorophyll *a* and ergosterol concentrations with increasing thallus N (Fig. 2). Moreover, the N investment was higher in the photobiont than the mycobiont with increasing N status of the thalli, as chlorophyll *a* concentrations increased more than ergosterol concentrations (Fig. 2). This pattern of increased N allocation to the photobiont with increasing N concentration is in agreement with the functional C to N equilibrium model proposed by Palmqvist (2000) and Palmqvist *et al.* (2002) for a range of lichen species. The increased investment in C-acquiring photobiont cells with increasing N status of the thallus would allow the lichen to increase its C pools through photosynthesis (Palmqvist 2000), because an increase in chlorophyll *a* has been correlated to an increase in photosynthetic capacity among lichens (Palmqvist *et al.* 1998; Palmqvist & Sundberg 2000; Palmqvist *et al.* 2002). By this mechanism the C cost of increased N assimilation would be met and the effects of toxicity avoided;

this might partly explain how *X. parietina* might adapt to environments with extreme N deposition loads.

Previous studies of tripartite lichens have shown that both sub-optimal and supra-optimal N supply might distort the area expansion of the thallus (Sundberg *et al.* 2001; Dahlman *et al.* 2002). The similar TSW in the *X. parietina* thalli regardless of N concentration (Table 1; Figs 3 and 4) is then an indication that this species has a co-ordinated developmental pattern between these two growth processes irrespective of N concentration. Again this demonstrates a high tolerance to varying N supply.

To conclude, this study clearly shows the great tolerance of *X. parietina* to varying thallus N concentrations, with a capacity to handle both low and high N levels. The relatively higher resource allocation to the photobiont in relation to the mycobiont with increasing thallus N concentration suggests that this green algal lichen might adjust photosynthetic rate to meet an increasing metabolic cost of N assimilation. This result is also in agreement with the C: N functional equilibrium model and with the inter-specific resource allocation pattern for green algal lichens across the same thallus N concentration range.

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