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Research

Responses of epiphytic lichens to an experimental whole-tree nitrogen-deposition gradient

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Summary

- Here, we examined the responses of the epiphytic lichens *Alectoria sarmentosa* and *Platismatia glauca* to increased atmospheric nitrogen (N) deposition in an old-growth boreal spruce forest, to assess the sensitivity of these species to N and define their critical N load.
- Nitrogen deposition was simulated by irrigating 15 trees over a 3 yr period with water and isotopically labeled NH_4NO_3, providing N loads ranging from ambient to 50 kg N ha^{-1} yr^{-1}.
- Thallus N concentration increased in both species with increasing N load, and uptake rates of both NH_4^+ and NO_3^- were similar. Photobiont concentration increased linearly with increased N in both species, saturating in *A. sarmentosa* in the third year at the highest N loads (25 and 50 kg ha^{-1} yr^{-1}). The simulated N deposition decreased the phosphorus (P) concentration in *A. sarmentosa*, and increased the N : P ratio in both species.
- Significant responses in lichen chemistry were detected to inputs of 12.5 kg N ha^{-1} yr^{-1} or higher, suggesting that resources other than N limit lichens at higher N loads. However, the data also suggest that N saturation may be cumulative over time, even at low N.

Introduction

Lichens are poikilohydric organisms that lack roots to absorb water and nutrients from their substrate. Thus, they are restricted to assimilating elements directly from atmospheric wet or dry deposition, or from stem flow (Nieboer *et al.*, 1978). Deposition of nitrogen (N), in both oxidized (NO_x) and reduced (NH_y) forms, is increasing globally as a result of human activities (Galloway *et al.*, 2008). For lichens, as for higher plants, N is an important nutrient that is involved in many processes for both the photobiont and the mycobiont. However, N can be a stressor if supplied in excess, and many studies have shown that N-sensitive lichens are replaced with more tolerant species in areas with high atmospheric N deposition (Söchting, 1995; Van Dobben & Ter Braak, 1998; Van Herk, 1999; Larsen Vilsholm *et al.*, 2009). In addition to atmospheric N deposition, forest N fertilization represents a potential threat to lichens, as highlighted by the disappearance of the pendulous lichen *Alectoria sarmentosa* following 7–8 yr of fertilization in a Norway spruce forest (Hesselman, 1937).

It has been hypothesized that the sensitivity of lichens to high N deposition may depend on whether or not they can maintain a balanced carbon (C) to N stoichiometry between the symbiont partners when N is supplied in excess (Palmqvist, 2000; Palmqvist *et al.*, 2008). In support of this hypothesis, the N-sensitive lichen *Evernia prunastri* had reduced mycobiont C-pools and mycobiont concentrations after 2 months of weekly N exposure in a study by Gaio-Oliveira *et al.* (2004). Other effects of increased N that might be related to an imbalance in resource investment include distorted coupling between thallus (hyphal) expansion and weight gain, as observed in N-fertilized *Nephroma arcticum* (Sundberg *et al.*, 2001; Dahlman *et al.*, 2002). In addition, the morphology and function of the lichen thallus might constrain how much N can be invested in algal cells in relation to fungal tissue. For example, the algal layer must be loosely packed to allow air circulation and reduce CO_2 diffusion resistance (Honegger, 1991), and avoid self-shading (Valladares *et al.*, 1996). Tolerant lichens are clearly more capable of balancing responses to increases in N loads than sensitive lichens. For instance, in an

experiment in which *Platismatia glauca* was fertilized daily for 3 months, a fivefold increase in thallus N was associated with a four- to fivefold increase in photobiont concentration, accompanied by increases in C-gain capacity, the soluble C pools and growth rate (Palmqvist & Dahlman, 2006). This is probably the reason why *P. glauca* has been found to be capable of surviving 15 yr of heavy forest fertilization (Dahlman *et al.*, 2003).

Another factor that may contribute to the variations in sensitivity among lichens to increases in N availability is variation in their mechanisms for avoiding excessive uptake. Many lichens have evolved in N-poor ecosystems and thus have highly efficient N-uptake mechanisms, but may not have the ability to down-regulate them. In remote areas, the deposition consists of roughly equal proportions of NH_4^+ and NO_3^-. Lichens are able to take up both forms, although NH_4^+ generally appears to be the preferred form (Dahlman *et al.*, 2004; Palmqvist & Dahlman, 2006). Since NH_4^+ may be more physiologically harmful than NO_3^- (Britto & Kronzucker, 2002), differences among species with respect to their N preference may also contribute to the differences in their N sensitivity. The difference in affinity between N forms has led to a considerable discussion, resulting in the idea that both the total quantity of deposited N and chemical form are important determinants for the 'critical load' (Bobbink *et al.*, 2010) – defined as 'a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge' (Nilsson & Grennfelt, 1998). A higher affinity for NH_4^+ could thus explain why NH_4^+ may often be more damaging to lichens than NO_3, as mentioned earlier (and hence its critical load lower). Empirical data suggest that the critical load for epiphytic lichens in boreal forests is *c.* 10–15 kg N ha^{-1} yr^{-1} (Bobbink *et al.*, 2003), although lower critical loads (as low as 3 kg ha^{-1} yr^{-1}) have been suggested (Fenn *et al.*, 2008).

Although many studies have shown that lichen growth appears to be co-limited by N and light once they are wet and metabolically active (Crittenden *et al.*, 1994; Sundberg *et al.*, 2001; Palmqvist *et al.*, 2008), recent studies have shown that lichens can also be limited by phosphorus (P) (Benner & Vitousek, 2007; McCune & Caldwell, 2009; Hogan *et al.*, 2010a,b). These findings suggest that P or other elements may become limiting when N is supplied in excess and that this might constrain the ability of lichens to avoid toxic N concentrations through increased growth.

To date, there have been no experimental studies to our knowledge that have examined epiphytic lichen responses to N deposition under natural conditions over more than a few months. Earlier studies of lichen N responses have been either short-term (Loppi & Frati, 2006; Palmqvist & Dahlman, 2006) – sometimes involving unrealistically high N doses (Dahlman *et al.*, 2003) or applications of N to the forest floor instead of directly to the lichens (Hesselman, 1937) – or restricted to studies of mat-forming terricolous lichens (Fremstad *et al.*, 2005). Thus, the paucity of information prompted us to conduct a large-scale, long-term study of the effects of realistically simulating atmospheric N deposition on epiphytic lichens. Here we present the results from a whole-tree fertilization experiment, in which N deposition at loads ranging from 0.6 to 50 kg ha^{-1} yr^{-1} has been simulated by the daily spraying of the trees with low N-concentration solutions during the summer and autumn for 3 yr in an area with low background N deposition (2 kg N ha^{-1} yr^{-1}; Forsum *et al.*, 2006). The specific aim of this study was to follow the initial responses, over 3 yr, of the apparently less N-sensitive foliose lichen *P. glauca*, and the more N-sensitive pendulous lichen, *A. sarmentosa*, when exposed to an N-deposition gradient in their natural low-N environment (a boreal Norway spruce-dominated forest). We examined whether the lichens displayed different N assimilation patterns and subsequent increases in N concentration following the simulated N deposition. We also wanted to determine if changes could be related to different affinities for the two N sources, NH_4^+ and NO_3^-, that could support different critical loads for these two N forms. Previous studies have indicated that when N uptake by *P. glauca* increases, most of the increased supply is allocated to the photobiont, the concentration of which thus increases. We hypothesized that *A. sarmentosa* is less successful in increasing its photobiont concentration because of morphological constraints imposed by its growth form. Therefore, *A. sarmentosa* may be more sensitive to increased N loads than *P. glauca.*

Materials and Methods

Field site and treatment units

A relatively open (basal area 24 m^2 ha^{-1}) old growth forest stand, described in detail by Tiren (1937), dominated by Norway spruce (*Picea abies*) (10–15 m high and 15–30 cm diameter in breast height) at Kulbäcksliden (64°12′N, 19°33′E), was chosen for the experiment. The site (Fig. 1) is part of The Unit for Field-based Forest Research, The Swedish University of Agricultural Sciences (Vindeln, Sweden), and is equipped with electricity (220 V) and a 700-m-long water pipe to the nearest road from where water could be pumped. Sixteen trees taller than 8 m with a rich lichen flora were selected as the treatment units (Figs 1, 2a). Wooden towers (6.5 m high and 4 × 4 m square) made of untreated wood, placed on 1-m-thick concrete blocks partly submerged in the humic soil layer, were built around each tree (Fig. 2a). The towers were each equipped with a circular irrigation-fertilization tube with 10 small sprinklers on top of the construction (Fig. 2b). Each tube was connected to a 100-m-long pipe and a separate 1000 l

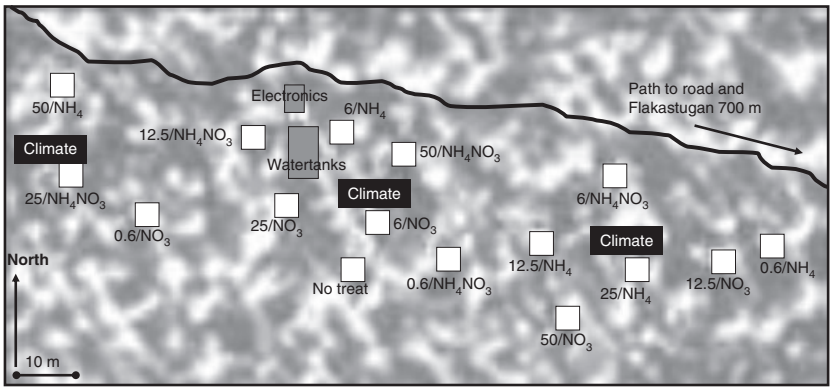

Fig. 1 Map of the experimental site showing positions of the three climate stations and the positions, annual nitrogen (N) load (kg ha^{-1}), and ^{15}N labeling applied to each of the 15 treated trees, and the control tree. The background shows the density of the trees (dark areas) and ground vegetation (lighter areas).

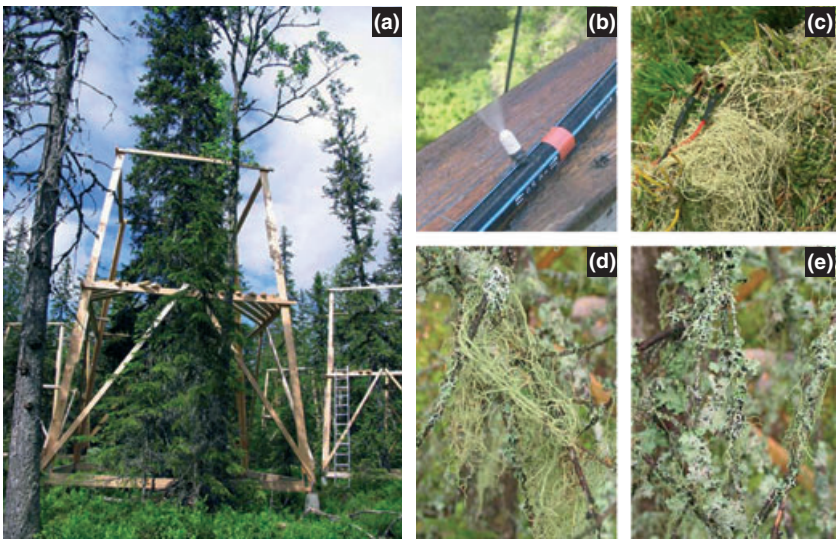

Fig. 2 The experimental set-up showing four of the 16 treatment trees and their wooden towers (a), one of the 10 sprinkler devices for each tree (b), a lichen water content (WC; conductivity) crocodile-clip sensor (c), and typical lichen-covered twigs in the treated trees (d, e).

treatment-solution tank. The small sprinklers were adjusted to gently spray an evenly dispersed mist towards the tree crown. Each tower also had two platforms 4 m from the ground to allow sampling of native lichens. One tree was randomly selected as a 'dry' control, which received no irrigation or fertilization, but was still surrounded by a wooden tower. Data from this tree will not be presented in this study. The remaining 15 trees were divided into three groups based on lichen abundance (species and biomass), followed by stratified randomization for five N-concentration treatments within each abundance group (Fig. 1). This created a block design with the trees acting as replicate units.

Irrigation-fertilization

Nitrogen was added in the form of NH_4NO_3 at five concentrations: 0.04, 0.41, 0.81, 1.63 and 3.2 mM. The lowest concentration was the same as that in local rainwater (Forsum *et al.*, 2006); it was not expected to cause any treatment effect and was applied to trace N uptake under normal supply conditions. For tracing, every N solution was spiked with ^{15}N in the form of $^{15}NH_4{}^{14}NO_3$, $^{14}NH_4{}^{15}NO_3$ (0.002 mM ^{15}N) or $^{15}NH_4{}^{15}NO_3$ (0.004 mM ^{15}N) as detailed in Fig. 1. The fertilizer (17–18 l) was administered daily at 06:00 h (GSM + 1) in three 2 min pulses with 10 min intervals (using separate pumps and tubing for each tree) from 7 June to 27 September 2006, 15 June to 21 September 2007, and 19 June to 1 October 2008. The 'growing season' in this part of Sweden starts by the end of May or early June and continues up until late September or early October. All other months can have freezing temperatures and with sub-zero temperatures dominating from November to April. Our 4 month treatment period each year thus coincides with the active growth period for the forest vegetation. For practical reasons, the irrigation could not get started until the road conditions allowed for the heavy transport of water, meaning the first weeks of June, and we had to empty tanks, tubing and pumps before winter conditions set in. The N applications were controlled by an automated system consisting of a PLC205 D2-240 AutomationDirect DirectLOGIC Modular Programmable Logic Controller with Directsoft software programming version 2.0c from Koyo Electronics Industries Company Ltd, Tokyo, Japan. The treatment mimicked a mixture of

dry and wet deposition via condensed fog, so the lichens were less wet following treatment than after rain (Fig. 3). All trees were treated until 1800 l of the solution had been dispersed each year, equivalent to deposition of 0.6, 6, 12.5, 25 and 50 kg N ha^{-1} during the *c.* 4 month treatment period each year. In the first year (2006), the NH_4NO_3 was dissolved in artificial rainwater: 8.8 mg l^{-1} K_2CO_3, 4.6 mg l^{-1} Na_2CO_3, 5 mg l^{-1} $CaCO_3$, 4.4 mg l^{-1} NaH_2PO_4, 0.25 mg l^{-1} $Fe_2SO_47H_2O$, and 0.6 mg l^{-1} $MnSO_4H_2O$ (Tamm, 1953). However, analyses of the local water source revealed that it had similar concentrations of K, Na, Ca, Na, P, Fe, S and Mn, so these nutrients were omitted from the artificial rainwater in the subsequent 2 yr (2007 and 2008). The water originated from the Vindeln community source, which was transported to Kulbäcksliden in clean 1000 l tanks and pumped to 15 tanks (one for each tree) at the site twice each year (in early June and early August). At the site, all tanks were thoroughly rinsed before each treatment year and covered with several layers of black plastic and dark tarpaulins to avoid contamination from algae or cyanobacteria.

Sampling

The trees harbored *c.* 16 species of epiphytic macro lichens (*A. sarmentosa*, *Bryoria capillaris*, *Bryoria fremontii*, *Bryoria fuscescens*, *Bryoria simplicor*, *Cetraria chlorophylla*, *Cetraria pinastri*, *Hypogymnia physodes*, *Hypogymnia tubulosa*, *Mycoblastus sanginarius*, *Parmelia sulcata*, *Parmeliopsis ambigua*, *Parmeliopsis hyperopta*, *Platismatia glauca*, *Usnea fillipendula* and *Usnea subfloridana)* of which we chose *A. sarmentosa* to represent an N-sensitive species (Hesselman, 1937) and *Platismatia glauca* as a model for a less N-sensitive species (Dahlman *et al.*, 2003; Palmqvist & Dahlman, 2006). Both lichen species had relatively high abundance and biomass on all 16 trees (see illustrative twigs, Fig. 2d,e). Native material was sampled for subsequent analyses of chemical traits at the end of each year (treatment period) on the following dates: 18 October 2006, 21 September 2007 and 23 September 2008. For both species (and spruce needles) one thallus was collected from two contrasting aspects at two different heights, *c.* 2 and 4 m from the ground, to cover within-tree variation in treatment responses, although data for all samples of each species from each tree were pooled for statistical analyses. Debris was rinsed from all lichen samples immediately after collection and the samples were stored intact in a deep-freeze (−80°C) until freeze-drying and homogenization with a ball mill. The powder from each sample was weighed and aliquoted for various chemical analyses (described later).

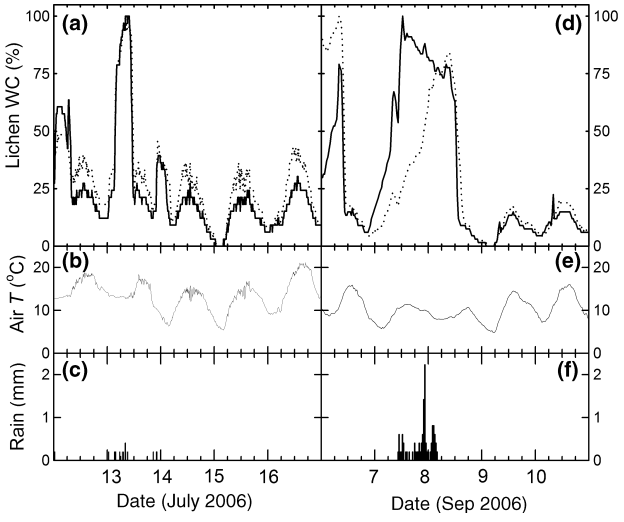

Fig. 3 Typical fluctuations in lichen water content (WC) (*Alectoria sarmentosa*, solid line; *Platismatia glauca*, dotted line), air temperature (*T*), and precipitation over 5 d in July (a–c) and September (d–f) 2006. The fertilization-irrigation treatment was initiated at *c.* 06:00 h (GSM + 1) and performed as described in the text.

Climate measurements

Three 'climate stations' were mounted at the site to cover variation in air temperature and humidity across the site (Fig. 1), and thallus water content in all trees. Variation in lichen water content (WC) was measured using an impedance technique (Coxson, 1991; Jonsson *et al.*, 2008), employing native thalli from the trees (Fig. 2c). This was primarily conducted to check the function of the automated irrigation system and to monitor the effect of the treatment on the lichen activity periods. Precipitation was recorded by a tipping-bucket rain gauge set up in the most open area in the middle of the site. Air temperature and humidity were measured with Rotronic probes (Hygroclip S3; Rotronic AG, Bassersdorf, Switzerland). Data from all sensors were read every minute and summed or averaged over 10 or 30 min periods, and recorded and stored with data-loggers (CR 10 or CR 10X) and a Relay Multiplexer (AM 416 or AM 16/32; Campbell Scientific Ltd, Logan Utah, USA). The impedance/conductivity data were rescaled to calculate lichen water content on a relative scale between 0 and 100% of the maximal signal for a wet thallus.

Chemical analyses

Chlorophyll pigments were quantified after extracting 10 (± 0.01) mg portions of homogenized lichen powder in 1.5 ml $MgCO_3$-saturated dimethyl sulfoxide (DMSO) at 60°C for 40 min (Palmqvist & Sundberg, 2001). The total N concentration and the isotopic ratio of ^{15}N : ^{14}N were analyzed by a certified laboratory (Colorado Plateau Stable Isotope Laboratory, CPSIL, Northern Arizona University, AZ, USA) using an elemental analyzer-isotopic ratio mass spectrometer (EA-IRMS). N uptake was calculated using the following equation:

$$\text{N uptake} = [(\text{At}\%_s - \text{At}\%_c) \times \text{Total N}_s]/\text{fraction}^{15}\text{N} \quad \text{Eqn 1}$$

where $\text{At}\%_s$ is the atomic percentage of ^{15}N in the sample, $\text{At}\%_c$ is the mean atomic percentage of ^{15}N, total N_s is the total N concentration of the sample (g DW), and fraction ^{15}N corrects for the amount of labeled N in the fertilizer, as defined earlier in the text.

The P concentration was also analyzed by CPSIL. The samples were extracted using a microwave digest in nitric acid under controlled temperature and pressure conditions, and analyzed using a Lachat Instruments QuikChem 8000 Series FIA+ equipped with a XYZ autosampler.

Statistical analyses

Differences in N and P concentrations and N : P ratios in response to the treatments and accumulated treatment time (year) were analyzed using ANOVA. As previously indicated, data from all individual thalli sampled from the same treatment unit (tree) were pooled before statistical analyses. To identify treatments that had significantly different effects from the background treatment (0.6 kg N ha^{-1} yr^{-1}) within each year, we used a separate ANOVA followed by an orthogonal contrast to compare, separately, effects of the lowest dose with those of all other doses. For all data $n = 3$, except for the N uptake of NH_4^+, NO_3^- or both N-forms where $n = 1$. For technical reasons only one tree per N treatment could be labeled with either one of the two N forms or both N forms. Differences in N uptake between the two N forms were therefore tested using ANCOVA with N form as a categorical factor and N dose as a continuous factor. The Chl*a* concentration data were fitted to total N load by both an asymptotic exponential function and linear regression, and then the best model was identified using the Akaike Information Criterion (AIC). All statistical analyses were performed using the statistical package R (R Development Core Team, 2009).

Results

The first summer (2006) was significantly drier than the following two summers, with respect to both the total precipitation and the number of rainy days (Fig. 4). In addition to the natural precipitation, which varied from 210 (2006) to 341 (2008) mm, the irrigation-fertilization corresponded to an additional 110 mm each year, although this was more similar to dew condensation or fog than to actual precipitation (Fig. 3). Monthly mean temperatures for July and August were highest in 2006 and lowest in 2008

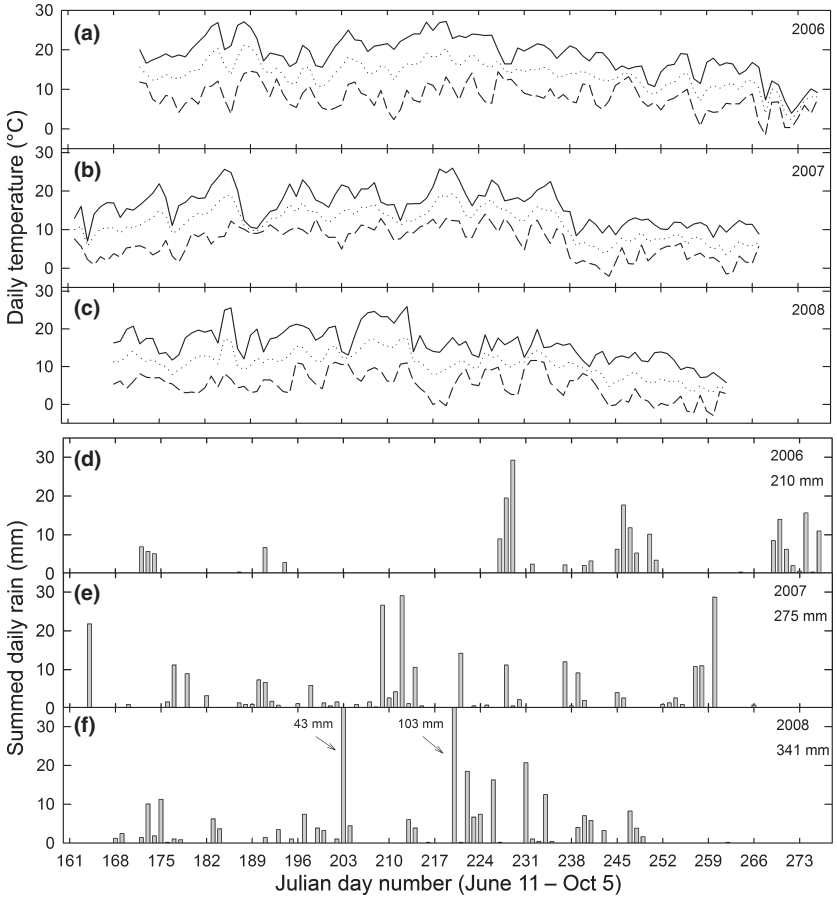

Fig. 4 Daily maximum, mean and minimum temperatures (°C) (a–c), and precipitation (rain) (d–f) at the experimental site during the three treatment years (2006, 2007 and 2008). The values to the right in each panel are summed precipitation (mm) during the treatment period in the respective years. There were 2 d when precipitation exceeded 40 mm during 2008 as indicated in (f). Dates when the treatment began and samples were harvested each year are given in the text.

(Fig. 4), with 2007 having close to 'normal' temperatures for these two months (based on data collected between 1961 and 1990; Swedish Meteorological and Hydrological Institute).

The thallus N concentration increased significantly in both lichen species with increasing N deposition (Fig. 5a,b), and the differences in thallus N concentration between the treatment (T) levels increased over time (Y) (there was a significant T × Y interaction; Table 1). The thallus N concentration varied between 5 and 6 mg g^{-1} DW for both species after the first year at the lowest N deposition (Fig. 5a,b), while after the third year, the N concentration had increased to 10–12 mg g^{-1} DW in *A. sarmentosa* and to *c.* 14 mg g^{-1} DW in *P. glauca* at the highest N deposition

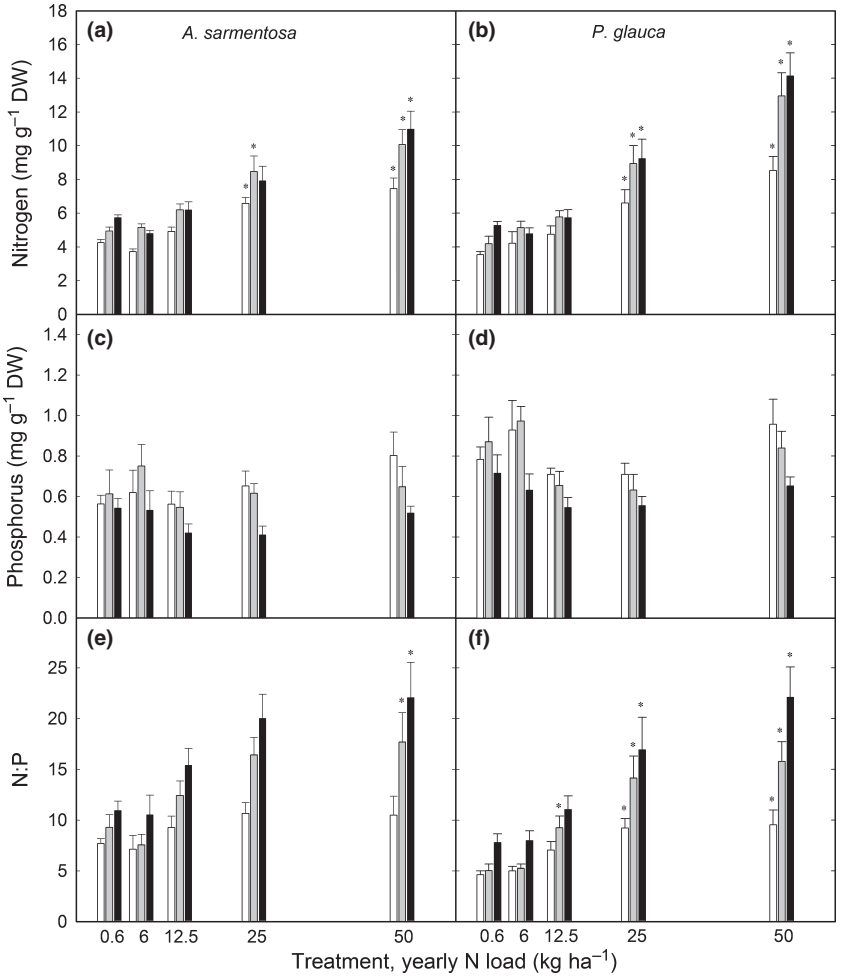

Fig. 5 Mean nitrogen (N) (a, b) and phosphorus (P) (c, d) concentrations (mg g^{-1} DW), and the resulting N : P ratios (e, f) in *Alectoria sarmentosa* (a, c, e) and *Platismatia glauca* (b, d, f) at harvest after one (2006, white bar), two (2007, gray bar) and three treatment periods (2008, black bar) in relation to yearly N deposition. Data represent mean ± 1 SE values for three trees subjected to the same N treatment. Data for the lichens sampled from each tree were pooled from prior statistical (N concentration) or experimental (P concentration) analysis as described in the text. Asterisks indicate treatments that had significantly different effects from the control treatment.

Table 1 ANOVA results for differences in lichen concentrations of nitrogen (N) and phosphorus (P) and the N : P ratios using N treatment (kg ha^{-1} yr^{-1}) as a continuous variable and year (2006, 2007 and 2008) as a factor. **, $P < 0.01$, ***, $P < 0.001$

	Alectoria sarmentosa			*Platismatia glauca*		
	df	*F*	*P*	df	*F*	*P*
N						
Treatment	1,13	33.62	< 0.001***	1,13	89.13	< 0.001***
Year	2,26	30.58	< 0.001***	2,26	19.32	< 0.001***
T : Y	2,26	5.70	0.009**	2,26	12.34	< 0.001***
P						
Treatment	1,13	0.41	0.534	1,13	0.03	0.868
Year	2,26	17.84	< 0.001***	2,26	12.83	< 0.001***
T : Y	2,26	6.17	0.006**	2,26	0.57	0.572
N : P						
Treatment	1,13	11.26	0.005**	1,13	33.97	< 0.001***
Year	2,26	41.72	< 0.001***	2,26	37.66	< 0.001***
T : Y	2,26	10.57	< 0.001***	2,26	12.84	< 0.001***

(Fig. 5a,b). There were significant differences in N concentration between the control and both of the two highest N doses in all years for both species, except for *A. sarmentosa* in the third year where only the highest doses had a significant effect. By contrast, neither the 6 nor 12.5 kg doses induced significant differences in thallus N concentrations from the control in any of the three years.

The increase in thallus N concentration was attributed to the fertilizer application, and (accordingly) there were strong linear relationships between the N concentration and uptake of both NH_4^+ and NO_3^-, which were independent of year (*A. sarmentosa*, $F = 354$, $P < 0.001$; *P. glauca*, $F = 610$, $P < 0.001$, Fig. 6). There was no significant difference in the uptake of the two N forms for either species. The uptake of N from the fertilizer was low in the spruce needles, < 1 mg g^{-1} DW in the current-year needles for all three years (data not shown) and there was no difference between the NH_4^+ and NO_3^- uptake. The needle N concentration (mean values for each tree and year) varied between 6.3 and 9.8 mg g^{-1}.

The increase in thallus N concentration also resulted in an increased Chl*a* concentration, which is indicative of an increased photobiont concentration in the thallus, and consistent with results of previous N fertilization experiments with lichens. When related to the accumulated N load over the 3 yr, there was a curvilinear relationship between N exposure and Chl*a* concentration in both species (Fig. 7). In *A. sarmentosa* the thallus appears to have been saturated with photobiont cells at a Chl*a* concentration of 1.5 mg g^{-1} DW (Fig. 7a), with no further increase beyond an accumulated N load of 50 kg N ha^{-1}. In *P. glauca*, the Chl*a* concentration increased to 2 mg g^{-1} DW at the highest accumulated N load, that is, 100–150 kg N ha^{-1} (Fig. 7b).

The P concentration was significantly decreased by N fertilization in *A. sarmentosa* (T × Y interaction), while in *P. glauca* the P concentration decreased over time independently of N treatment (Table 1, Fig. 5c,d).

The combined changes in N and P resulted in a significantly increased N : P ratio. for *P. glauca* there was a significant response already at the 12.5 kg N ha^{-1} load during the second year, while for *A. sarmentosa* it was only significant at the 50 kg N ha^{-1} load. The N : P ratio ranged from < 5 after the first year at the lowest N deposition to > 20 at the highest N deposition by the third year in *P. glauca*, and from < 8 to > 20 in *A. sarmentosa* (Fig. 5e,f). It should be noted that while the increase in N concentration slowed between the second and the third year, the N : P ratio continued to increase at the same rate throughout the 3 yr period.

Discussion

Many species of lichen have disappeared from areas that have been subject to high N loads for long periods of time (Hesselman, 1937; Söchting, 1995; Van Dobben & Ter Braak, 1998; Van Herk, 1999; Dahlman *et al.*, 2003; Larsen Vilsholm *et al.*, 2009). By contrast, some lichens have been reported to grow better following high doses of N fertilization in short-term studies (< 5 months) (Palmqvist & Dahlman, 2006). Nitrogen may therefore be

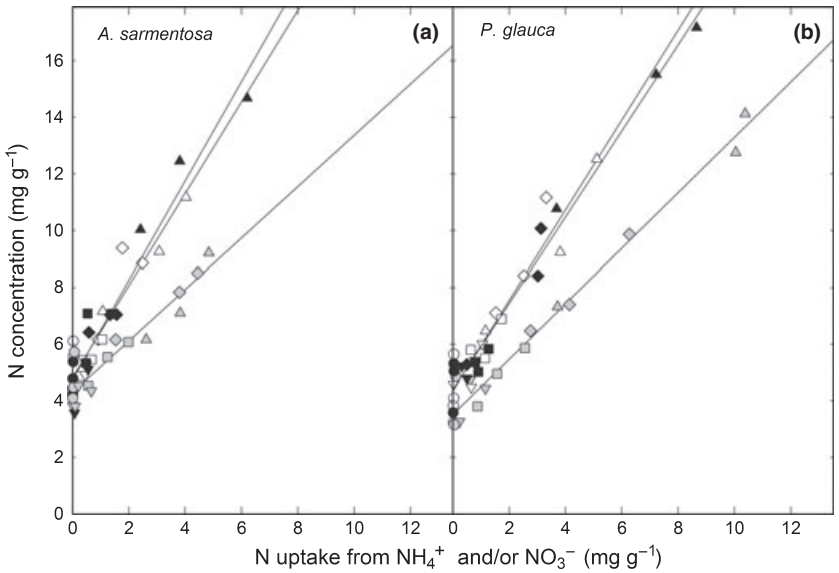

Fig. 6 Thallus nitrogen (N) concentrations (mg g^{-1} DW) of *Alectoria sarmentosa* (a) and *Platismatia glauca* (b) as a function of their accumulated NH_4^+ (black symbols), NO_3^- (white symbols) or NH_4NO_3 (gray symbols) uptake at harvest in 2006, 2007 and 2008. Data represent mean values for thalli harvested from one tree per N-deposition level and year (kg N ha^{-1} yr^{-1}) (circles, 0.6; triangles, apex down, 6; squares, 12.5; diamonds, 25; triangles, apex up, 50) as described in the text. Linear regressions: *P. glauca*, NO_3^- ($y = 1.59x + 4.37$), Adj $r^2 = 0.91$, NH_4^+ ($y = 1.52x + 4.29$), Adj $r^2 = 0.98$, NH_4NO_3 ($0.98x + 3.52$), Adj $r^2 = 0.98$; *A sarmentosa*, NO_3^- ($1.62x + 4.83$), Adj $r^2 = 0.87$, NH_4^+ ($y = 1.75x + 4.76$), Adj $r^2 = 0.93$, NH_4NO_3 ($0.91x + 4.31$), Adj $r^2 = 0.89$.

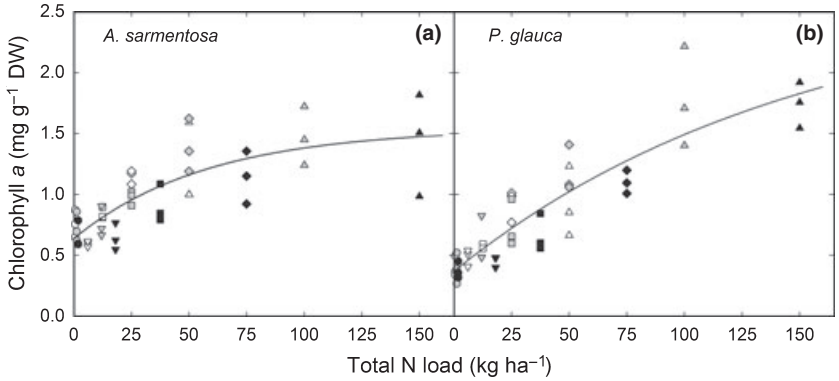

Fig. 7 Chlorophyll *a* concentration as a function of total accumulated nitrogen (N) load over the 3 yr for *Alectoria sarmentosa* (a) and *Platismatia glauca* (b) at harvest each year. Data represent mean values for the thalli harvested from one tree per N-deposition level and year (2006, 2007, 2008) (kg N ha^{-1}) (circles, 0.6, 1.2, 1.8; triangles, apex down, 6, 12, 18; squares, 12.5, 25, 37.5; diamonds, 25, 50, 75; triangles, apex up, 50, 100, 150) as described in the text. Curves were fitted to the data as described in the text, yielding equations with the following forms and constant values: $y = 1.54 - 0.90 \exp(-0.017x)$ (*A. sarmentosa*), $y = 2.70 - 2.33 \exp(-0.0066x)$ (*P. glauca*).

either deleterious for the lichen symbiosis or promote growth, depending on the species and the conditions in the environment where the N is deposited. The mechanisms behind changes in species composition following N deposition thus need to be studied, while considering both of these aspects of lichen ecology. In this study we compared N uptake and allocation of the pendulous N-sensitive lichen *A. sarmentosa* and the foliose, less N-sensitive lichen *P. glauca* during 3 yr of simulated N deposition. The simulated N deposition increased the N concentration in both species, indicating that these epiphytic lichens, like previously studied terricolous lichens, lack mechanisms to down-regulate their N uptake with increasing N load (Hyvärinen & Crittenden, 1998). This is not unexpected since the majority of lichens have evolved in N-poor ecosystems (Palmqvist *et al.*, 2002) and might therefore (in a similar way to plants of such habitats) be adapted to low-resource environments, be nutrient-conservative and take up high amounts of resources whenever available (Chapin, 1980). The thallus N concentration had increased in both species to *c.* 12–14 mg g^{-1} DW after the third year at the highest N loads (Fig. 5), which for *P. glauca* was similar to the N concentration previously reported for this species after 15 yr of intensive fertilization (Dahlman *et al.*, 2003). Both lichens further displayed a smaller increase in N concentration during the last year (2008), compared with the first two years (Fig. 5). This suggests that the lichens in our study may be able to adapt to the increased N supply either through uptake regulation, or through increased growth, as discussed later.

Previous studies have shown a preference for NH_4^+ over NO_3^- uptake for a range of lichens (Dahlman *et al.*, 2004; Palmqvist & Dahlman, 2006). This might, however, be related to low light (since there is a higher energy cost when assimilating NO_3^- than when assimilating NH_4^+) and high N doses. At the ecologically relevant N concentrations and doses used in the present study there was no significant difference in uptake of the two N forms by either lichen species, regardless of thallus N concentration and accumulated N load (Fig. 6). This is also in accordance with the results of an Antarctic study showing equal uptake of both NH_4^+ over NO_3^- for *Usnea spacelata* during natural conditions (Crittenden, 1998). Hence, our results indicate that in the case of *P. glauca* and *A. sarmentosa* there is no need to differentiate between NH_4^+ and NO_3^- when determining critical N loads, as there was no support for species-specific differences in the physiological capacity of these lichens to take up different relative amounts of NH_4^+ and NO_3^- that could influence their N sensitivity.

The increase in Chl*a* with increased N load observed in both lichen species (Fig. 7) supports earlier studies both within and across species (Palmqvist *et al.*, 2002, 2008). We have also found a good correlation between Chl*a* and photobiont concentration for *A. sarmentosa* and *P. glauca* (O. Johansson *et al.*, pers. comm.). Chl*a* is also positively correlated with the photosynthetic capacity of lichens (Palmqvist *et al.*, 2002, 2008), and can be used as an indirect measure of photobiont biomass (Raven *et al.*, 1990). Hence, both photobiont biomass and net CO_2 gain may have increased in the two lichens in response to the increased N load. This is consistent with the hypothesis that tolerance to increased N may be coupled with the ability to maintain balanced carbon (C) to N stoichiometry between the symbiont partners when excess N is supplied (Dahlman *et al.*, 2003). Provided that N is the most limiting resource, a balanced C to N stoichiometry would, according to this hypothesis, be maintained by the investment of the extra N into more photobiont cells, thereby increasing net CO_2 gain and potentially lowering the tissue N concentration through increased growth. However, the two species also displayed somewhat different responses with respect to Chl*a* investment. In *P. glauca*, the Chl*a* concentration increased linearly with increasing N load up to an accumulated N load of 150 kg N ha^{-1}, showing little sign of

saturation at a Chl*a* concentration of 1.8–2 mg g^{-1} DW; still well below the 4.6 mg g^{-1} DW found in thalli after 15 yr of N fertilization by Dahlman *et al.* (2003). The Chl*a* concentration also initially increased with N load in *A. sarmentosa*, but was apparently already saturated at *c.* 1.5 mg g^{-1} DW and at accumulated N loads above 50 kg N ha^{-1} (Fig. 7). This apparent saturation for photobiont density in the thallus may be caused by continued thallus expansion or arrestation of photobiont division (Hill, 1993). The latter possibility is supported by the observations that the thallus N concentration was increased in both species at the 25 and 50 kg N ha^{-1} yr^{-1} loads, indicating that at these loads the N deposition and uptake were greater than the lichens could dilute through growth (Fig. 5). However, Chl*a* saturation may also result from morphological and/or developmental constraints. In addition to allowing adequate gas diffusion and reducing self-shading (Honegger, 1991; Valladares *et al.*, 1996), the thallus structure of the filamentous *A. sarmentosa* imposes an additional limitation compared with the foliose structure of *P. glauca*. In the former, the photobiont cells are situated inside the cortex towards the filament cavity, where the cells are normally discontinuous and clumped (Galloe, 1950; Hawksworth, 1972). Hence, the volume of the cavity surrounded by the fungal hyphae sets limits for algal expansion in the absence of accompanying fungal growth along the length axis.

There is also the possibility that other nutrients (e.g. P, magnesium or iron) may have become limiting for continued photobiont investments and/or the thallus growth. A likely candidate is P, concentrations of which decreased in *A. sarmentosa* during the third year of treatment (Fig. 5). Phosphorus limitation has previously been shown for epiphytic lichens in a Hawaiian rainforest, especially for N-fixing species (Benner & Vitousek, 2007; Benner *et al.*, 2007), in *Cladonia portentosa* in heathlands and moorlands in the UK (Hogan *et al.*, 2010a) and in *L. pulmonaria*, growth of which doubled following P additions in a study by McCune & Caldwell (2009).

Although coniferous trees may take up N via the canopy (Gaige *et al.*, 2007), the spruce needles in this study displayed very low N uptake compared with the two lichen species. The low concentrations of ^{15}N label found in needles may originate from canopy uptake or from soil N uptake by the trees. Independent of the route for tree N uptake, it highlights that tree-living lichens can be important in boreal forest N cycling by efficiently absorbing N deposited in the tree canopy and thereby altering precipitation chemistry.

In conclusion, our data indicate that there is a critical N load of 10–15 kg N ha^{-1} yr^{-1} as in Bobbink *et al.* (2003) for the epiphytic lichens also in our study, since the only observed significant response to N deposition of 12.5 kg N ha^{-1} yr^{-1} or less was an increased N : P ratio in *P. glauca*. However, the linear responses of chlorophyll concentrations to accumulated N load that we detected suggest that lower loads may have significant effects over the longer term. A mechanism that could allow lichens to tolerate increases in N concentration, within certain limits, is that the additional N may be invested in photobiont tissue, thereby increasing net C gain and growth. However, the substantial uptake of both NH_4^+ and NO_3^- observed in lichens subjected to all treatments, and the significant increase in the N concentration following the two highest N-deposition treatments, indicate that neither of the two lichen species down-regulated their N uptake in response to the N load. Moreover, the photobiont concentration and the concomitant hyphal growth can only increase linearly with increasing N until limited by morphological constraints, water, light or other nutrients. The filamentous *A. sarmentosa* lichen reached Chl*a* saturation more rapidly than the foliose *P. glauca*, providing a mechanistic reason for the sensitivity of the former species detected in earlier N fertilization studies.

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