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Biomonitoring with lichens on twigs

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Abstract: Two surveys of the lichen and bryophyte flora growing on oak twigs from a Welsh and a Danish locality were compared with additional data on bark pH and % nitrogen in thalli of *Hypogymnia physodes*. Despite differences in climate and lichen flora, both sites showed a shift in the lichen communities from nitrogen sensitive (nitrophobe) to nitrogen tolerant (nitrophile) species, which was correlated with both increasing bark pH and an increase in total nitrogen in thalli of *H. physodes*. The floristic survey from Wales was a repetition of a study eight years earlier (Wolseley & Pryor 1999) now showing a loss of nitrophobes in all sites and the appearance of nitrophiles in pasture sites in 2003. This study demonstrates that lichens on twigs can be used as an early warning system to detect a response to changes in land management and nitrogen deposition.

Key words: bark pH, biomonitor, indicator species, nitrogen, nitrophobe, nitrophile, oak, twig

Introduction

There has been a net increase in nitrogen deposition in the form of ammonia and ammonium across the European Union largely as a result of the introduction of agricultural subsidies through the Common Agricultural Policy (CAP), which has favoured intensification of livestock and crop farming (Asman *et al.* 1997; Fowler *et al.* 2001). At the same time SO_2 emissions have decreased since the late 1970s due to successful environmental amelioration (Heidam 2000; Fowler *et al.* 2001). Although the effect of wet deposited ammonium on sensitive lichen taxa has been shown to occur many hundreds of kilometres from the source (van Herk *et al.* 2003), the major part of nitrogen deposition occurs within 200m from the source (Asman 1998; Fowler *et al.* 2001), where it can cause considerable impact on natural vegetation (Bak *et al.* 1999), particularly lichens.

Lichens form a major component of species that are sensitive to changes in atmospheric nutrient conditions (Barkman 1958; Sutton *et al.* 2004*a*; van Dobben & ter Braak 1998; van Herk 1999, 2001) and have long been used as bioindicators of pollution, such as sulphur dioxide (Hawksworth & Rose 1970) and more recently of ammonia (van Herk 1999, 2001; van Herk *et al.* 2003; Seaward & Coppins 2004; Sutton *et al.* 2004*b*; Wolseley *et al.* 2005, 2006; Frati *et al.* 2007; Sparrius 2007; Berthelsen *et al.* 2008).

While some lichen species are tolerant of increasing atmospheric nitrogen, others are highly sensitive and these include many species of high conservation value that are also indicators of ecological continuity (Rose 1992; Coppins & Coppins 2002). Recent work in the Netherlands (van Herk 1999, 2001) and the UK (Sutton *et al.* 2004*a*; Wolseley *et al.* 2005; Wolseley *et al.* 2006) has shown a correlation between increasing ammonia concentration and loss of nitrogen sensitive species (nitrophobes) and increase in nitrogen tolerant species (nitrophiles) on trunks. These were combined to

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form a lichen index where negative values are correlated with increasing ammonia and increasing nitrophiles, and positive values with increasing nitrophobes (Sutton *et al.* 2008; Wolseley *et al.* 2008). Investigations of total nitrogen in thalli of the nitrophile *Xanthoria parietina* and the nitrophobe *Evernia prunastri* have shown that *X. parietina* accumulates nitrogen in the thallus while *E. prunastri* does not, making it intolerant of high nitrogen (Gaio-Oliveira *et al.* 2005).

Bark pH varies with tree species, age of tree and with local conditions. Trees with a low bark pH, such as oak and pine, support a predominantly nitrophobe, low nutrient lichen flora, while trees with a higher bark pH such as ash, elm and poplar support more nitrophilic species (Wolseley *et al.* 2005). A loss of nitrophobes has been observed in situations, where an increase in bark pH is occurring due to an increase in atmospheric ammonia concentrations (van Herk 1999; Sutton *et al.* 2004*a*; Wolseley *et al.* 2008), this is accompanied by an increase in nitrophiles. The shift from nitrophobes to nitrophiles is more clearly seen on trees with a lower bark pH than on trees with a higher bark pH such as ash (van Herk 1999; Wolseley *et al.* 2005).

Twigs offer an annually renewed bark substratum in a microclimate that is exposed to extreme atmospheric conditions. Therefore lichen colonization and succession on twigs are much influenced by local environmental conditions (Degelius 1978; Esseen & Renhorn 1998; Wolseley & Pryor 1999; Hilmo & Holien 2002; Hilmo *et al.* 2005; Wolseley *et al.* 2005, 2006). With increasing age, trunk bark provides many microniches due to variation in exposure, inclination, bark structure, etc. (James *et al.* 1977). Stone (1989) found that competition had no influence on macrolichen and bryophyte communities on oak twigs within the first 15 years of growth, whereas she considered that atmospheric conditions are more important. The UK survey of trunks and twigs showed that lichens on twigs responded at lower concentrations of atmospheric ammonia than those on trunks (Sutton *et al.* 2004*a*, 2008; Wolseley *et al.* 2005*a*, 2008), suggesting that the lichen communities on the new substratum on twigs better reflect current environmental conditions, while the older trunks may support relict communities reflecting previous conditions of the tree and its environment.

Background to survey in Denmark and Tycanol

The sampling of lichen and moss composition on twigs at 4 sites under a range of environmental conditions in West Wales at Tycanol National Nature Reserve (NNR) in 1995 (Wolseley & Pryor 1999) provided the basis for a replicate survey in 2003 using the same methodology. Meanwhile, in 2001 Larsen had used a similar method to sample twigs at Kås Forest in Denmark (Larsen & Søchting 2002*a*). In addition to measuring pH of trunks, pH of twigs and nitrogen content of *Hypogymnia physodes* were investigated in Tycanol Forest, 2003 and Kås Forest, 2001. This provided an opportunity to compare lichen communities on twigs in sites of high conservation value from two geographical regions, together with pH and nitrogen data, and to identify areas where changes in lichen communities were occurring. Tycanol Forest is a grade 1 Site of Special Scientific Interest listed for its rich epiphytic and saxicolous lichen communities. This area of *Quercus petraea* woodland supports 396 taxa of lichens of which 36 are nationally scarce species (Wolseley & Douglass 2008; Woods & Coppins 2003), 34 are Indicators of Ecological Continuity (NIEC) with 6 bonus species (Coppins & Coppins 2002). Many of the nationally scarce species are dependent on low pH substrata supporting epiphytic species such as *Hypotrachyna endochlora, Lecidea doliiformis, Usnea articulata* and *U. wasmuthii* and young bark species *Arthothelium ruanum, Buellia erubescens, Celothelium ischnobelum* and *Graphina ruiziana*. *Lobaria pulmonaria* was noted in 1972 on *Fraxinus* (Rose 1975), but has since disappeared.

Kås Forest is a protected EU habitat area of mixed *Quercus petraea* and *Q. robur* forest situated on a peninsula facing the brackish

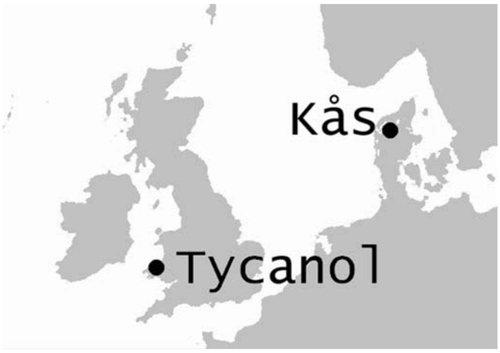

FIG 1. Location of the sites at Kås in Denmark and Tycanol, West Wales, United Kingdom.

water of Limfjorden in Northern Jutland. In addition to having the highest density of *Lobaria pulmonaria* in Denmark, Kås Forest is a sanctuary for many red-listed species such as *Cyphelium sessile, Lecanora confusa, Mycobilimbia pilularis, Opegrapha niveoatra, Pachyphiale carneola, Pertusaria hemisphaerica, P. pupillaris, Porina borreri* (Pedersen 1980), and more species are being added when lichenologists visit the area (Larsen & Søchting 2002*b*; Larsen & Søchting 2003; Alstrup *et al.* 2004; Søchting *et al.* 2007).

Objectives

There were three objectives in the present study. First to compare lichen and bryophyte communities on twigs, nitrogen concentration in thalli of *Hypogymnia physodes*, pH of bark, and the frequency of nitrophobes and nitrophile lichen species at sites of high conservation value in the climatically different regions of West Britain (Tycanol) and West Denmark (Kås) (Fig 1, Table 1).

Second, to assess changes in lichen and bryophyte communities on twigs between 1995 and 2003, using frequency of nitrophile and nitrophobe lichen species and pH of trunks at four different sites within Tycanol.

Third, to trial a method using lichens on twigs as indicators of changes in atmospheric conditions that might be deleterious at sites of high conservation value.

Materials and Methods

Lichens were sampled on twigs following Wolseley and Pryor (1999). Four sites were chosen in Tycanol NNR including a sheltered glade (Gl), a woodland edge facing moorland (Mo), ancient trees in old pasture (P2) and a woodland edge facing improved pasture (P1). All sites were investigated in 1995 and again in 2003. At each site at Tycanol a 20m edge of woodland was selected where twigs were accessible and a 20m tape laid down parallel with the edge. Random numbers were used to select 10 points and at each point the nearest well-illuminated twig above grazing level was selected.

Kås Forest was investigated in 2001. At the edge, 9 well-illuminated twigs above grazing level were selected from trees standing 15 m apart. Nine twigs from different trees in the interior canopy of Kås Forest were destructively collected from 13–18m above ground. Lichens and bryophytes growing on the youngest 15 annual increments were recorded at all sites, using girdle scars as separators. In each increment, species of lichen and bryophyte present were recorded, giving a maximum frequency of 15 for any species on each twig. Most specimens were identified in the field, but critical specimens were collected and identified using microscopes and HPTLC following Arup *et al.* (1993) for Danish material and TLC following Orange *et al.* (2001) for Welsh material. Nomenclature mainly follows Blockeel & Long (1998) for bryophytes and Coppins (2002) for lichens, except *Melanelixia* which follows Søchting & Alstrup (2007). Lichens described as 'nitrophyte' and 'acidophyte' are defined in van Herk (1999). In this paper we are using the terms nitrophile and nitrophobe.

Trunk pH in Tycanol was measured with a Jencon flathead electrode on site several minutes after moistening the trunk with 10% KCl solution. At least 3 measurements were taken on each trunk (Wolseley & Pryor 1999). Twig pH was measured according to Kermit & Gauslaa (2001) where straight twigs, *c.* 10 cm long and *c.* 6 mm in diameter were collected and dried in paper bags. In the laboratory a 6 cm long piece was cut and sealed at both ends with paraffin wax. The sample was placed in a test tube with 10 ml of 10% KCl and shaken regularly for an hour prior to measuring the pH of the supernatant liquid using the flathead electrode.

Total nitrogen was measured in thalli of *Hypogymnia physodes*. Samples with developed soralia and no sign of necrosis were collected from horizontal twigs and dried in paper bags. Bark and fauna were removed, and samples of 0·045–0·145 g of thallus were weighed and analysed in a Leco FP-428 nitrogen determinator system, which incinerates the lichen thalli, and measures the concentration of nitrogen in the combustion air (Leco 2007). Nitrogen is expressed as percentage of total dry weight.

Multivariate analysis of lichen and bryophyte species data was carried out using PRIMER_6. Within this, Principal Component Analysis (PCA), Non-Metric Multi Dimensional Scaling (MDS), and Cluster Analysis (CA) were used to indicate similarities and differences between sites and species components. For MDS and Cluster Analysis, site similarity was estimated using

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TABLE 1. *Site location and data for Tycanol, west Wales, and Kås, Denmark, including sulphur and nitrogen deposition*

Forest	Tycanol	Kås
Location	Wales, UK	Jutland, Denmark
Conservation status	Grade 1 Site of Special Scientific Interest, National Nature Reserve	EU habitat area (92/43/EEC)
Latitude	52° N	56° 37′ N
Longitude	4°47′W	08° 41′ E
Metres above sea level	303–785	0–10
Rainfall (mm y^{-1})	1400	791
Prevailing wind direction	South-west	West
Height of twigs above ground	1–3 m	1–3m and 13–18m
Year of investigation	1995 and 2003	2001
Known lichen species in forest	396 including saxicolous spp.	147 epiphytes
NIEC (Coppins & Coppins 2002)	38 (Wolseley & James 1994)	41(Larsen 2001)

the Bray Curtis coefficient, calculated from the frequency of species at each site. Individual species contributions to the groupings indicated were elucidated using the SIMPER (SIMilarity PERcentage contribution) routine in PRIMER 6 (Primer 6 2007).

Results

Lichen and bryophyte flora, nitrophile and nitrophobe lichen frequency, nitrogen concentration in *Hypogymnia physodes* and bark pH

The total number of lichen species recorded on twigs at both sites was 67 of which 60 were recorded at Tycanol and 22 at Kås. The nitrophobe lichens observed in this study were *Evernia prunastri, Hypogymnia physodes, H. tubulosa, Lepraria incana, Parmelia saxatilis, Platismatia glauca, Usnea cornuta, U. flammea, U. florida* and *U. subfloridana*, and the nitrophile lichens found were *Candelariella reflexa, Physcia tenella, Xanthoria parietina* and *X. polycarpa*. (grouped respectively as acidophyte and nitrophyte lichens in van Herk 1999, 2001). Out of 40 species occurring with >1% frequency only 15 species occurred in both sites, and only one species occurred at Kås that was not found at Tycanol. Tycanol also supported a higher number of nitrogen sensitive species as well as a higher diversity on twigs. Furthermore, 7 species of bryophytes were found at Tycanol, and none on twigs at Kås. The dendrogram of site similarity (Fig. 2) shows that there was only *c.* 28% similarity between the twig floras at Kås and Tycanol. The major differences between the twig lichen communities at the two sites were that Tycanol has a high frequency of nitrophobes and a low frequency of nitrophiles, while at Kås there is a low frequency of nitrophobes and a high frequency of nitrophiles (Table 2 & Fig. 6).

Within Kås Forest, the dissimilarities between the two sites expressed in Fig. 2 are mainly caused by fewer and less abundant lichen species on the twigs from the interior site compared to the edge (Table 2).

The percentage nitrogen content in *Hypogymnia physodes* from all sites is positively correlated with nitrophile frequency and negatively correlated with nitrophobe frequency (Fig. 3A). The correlation is stronger when using a combined score of nitrophobes (positive) and nitrophiles (negative) (Fig. 3B and 3D), hereafter named 'lichen index of nitrogen'. The loss of nitrophobes is already taking place at pH 4·8 on twigs prior to the increase in nitrophiles on the twigs above pH 5 (Fig. 3C). The percentage nitrogen content in *H. physodes* from Kås and Tycanol is highly correlated with bark pH of twigs (Fig. 4), but not with pH of trunks at Tycanol (data not shown).

Changes occurring between 1995 and 2003 at Tycanol

The dendrogram (Fig. 2.) shows that there have been major changes in the lichen flora

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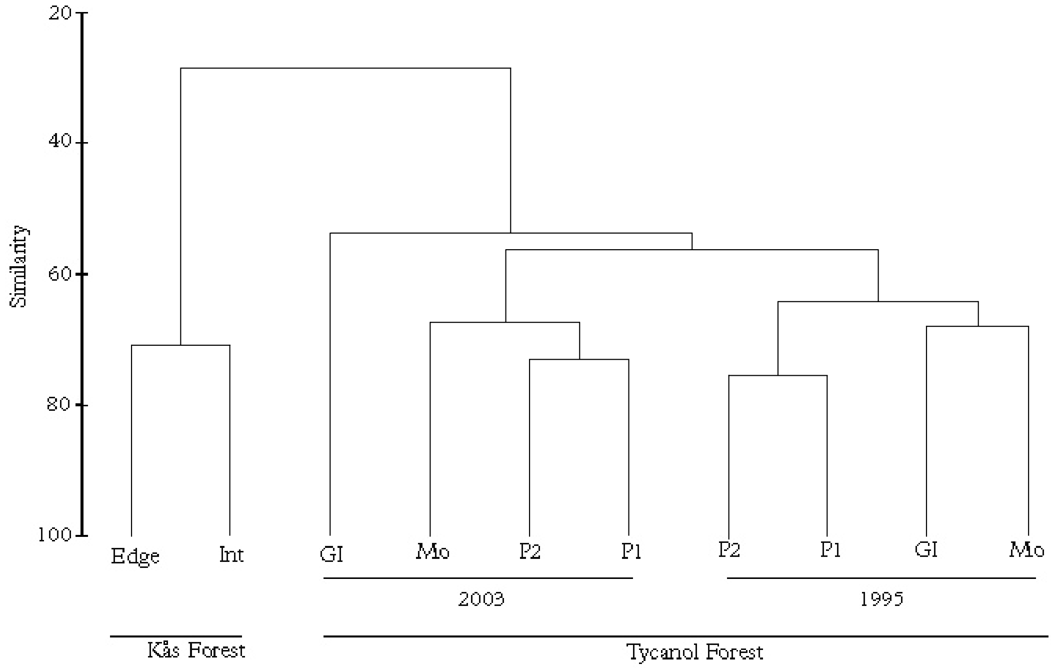

FIG. 2. Dendrogram of site similarity based on epiphytic lichen and bryophyte species found on oak twigs at four sites at Tycanol National Nature Reserve, Wales, in 1995 and in 2003 and at 2 sites in Kås Forest, Denmark, in 2001. Tycanol sites include Gl – glade, Mo – moorland, P1 – pasture 1, P2 – pasture; Kås sites include twigs in the forest edge (ed) and interior (int.).

between 1995 and 2003 at Tycanol. At the same time there has been an increase in bark pH on the trunks (Fig. 5). The 4 sites at Tycanol show a greater similarity to the year of sampling than to each other (Fig. 2), except the woodland glade site which is dissimilar to all sites in 2003, due to an increase in bryophyte cover associated with increased shade from canopy increase and bracken cover (Table 2). There was little change in species diversity at Tycanol between 1995 and 2003 (Table 2), but there was a conspicuous loss in the frequency of nitrophobes at all sites, for example, *Evernia prunastri*, *Hypogymnia* spp., *Usnea* spp. (except *U. cornuta*) and *Parmelia saxatilis*, and an increase in nitrophiles at the pasture sites (Fig. 6, Table 3). The most remarkable changes in lichen frequency were in *Physcia tenella* which were increased from 2% to 45% at Pasture 1 and from 1% to 11% at pasture 2, and *Xanthoria polycarpa* is observed for the first time at Tycanol in Pasture 1 (Table 2).

Discussion

Lichen and bryophyte flora, nitrophile and nitrophobe lichen frequency, nitrogen concentration of *Hypogymnia physodes* and bark pH

The forests of Tycanol in Wales and Kås in Denmark both support elements of the *Lobarion* community, including species that are indicators of long ecological continuity, many of which are also highly sensitive to increases in atmospheric nitrogen (Wolseley *et al.* 2006). Although the composition of the lichen and moss floras in West Wales and Denmark reflect different climatic conditions, particularly of rainfall, both sites show

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TABLE 2. *Frequency of lichen species recorded on twigs at sites at Tycanol NNR, Wales, and Kås Forest, Denmark. Values are the number of branch increments on which the species was recorded (out of 15 expressed as a %). Species with frequency value <% excluded. Nitrophiles in bold and nitrophobes underlined (after van Herk 1999)*

	Wales								Denmark	
	Tycanol NNR								Kås Forest	
Site*	Gl		Mo		P1		P2		ed	int
Year of data collection	'95	'03	'95	'03	'95	'03	'95	'03	'01	'01
Arthonia punctiformis	4	2	13	22	17	15	9	5		
A. radiata		3	1	4	1	5	3	3		
Arthopyrenia analepta	6	6	1	11		5	5	2		
A. punctiformis	7	3	5	13	9	7	5	6		
Candelariella reflexa			1							
Cyrtidula quercus	37	7	32	6	21	3	34	9		
Evernia prunastri	65	25	41	17	35	31	30	20	11	6
Flavoparmelia caperata	5	10	3	17	2	12	3	9		
Fuscidea lightfootii	68	40	73	65	36	30	37	31		
Graphis scripta	14	38	5	21		1	6	15		
Hypogymnia initials†	4		6				1			
Hypogymnia physodes	41	29	27	14	4	9	11	5	6	2
H. tubulosa	57	23	41	3	15	2	13	6		1
Hypotrachyna revoluta	6	47	13	49	3	33	2	28		
Lecanora carpinea									29	22
L. chlarotera	3	13	6	24	31	37	28	39	72	67
L. confusa		1	1	1	7	8	31	13		
L. symmicta	1	1	14	1	2	1	9	5		2
Lecidella elaeochroma						3		10	45	12
Lepraria cf. *incana*	5	1	1	1					1	1
Melanelixia fuliginosa		1	1				3	4	13	3
M. subaurifera	46	60	45	37	53	53	40	24		
Melanohalea exasperata	11	1	6			7	9	9		

Table 2. *continued.*

	Wales								Denmark	
	Tycanol NNR								Kås Forest	
Site*	Gl		Mo		P1		P2		ed	int
Year of data collection	'95	'03	'95	'03	'95	'03	'95	'03	'01	'01
Parmelia initials	15	1	21		10		4			
<u>*Parmelia saxatilis*</u>	3		1							
P. sulcata	63	51	47	30	51	46	39	28	32	22
Parmotrema perlatum	4	3	1	1		3	1	14		
Phaeographis smithii	2							10		
Physcia aipolia				2	15	22	1	11	10	
P. tenella		1		4	2	45	1	11	40	3
<u>*Platismatia glauca*</u>	1	2	3	3						
Punctelia subrudecta	5	35	9	9	5	17	3	11		
Ramalina farinacea		3		5	11	15	12	15		
R. fastigiata				1		4	1	3	8	
Rinodina sophodes	1		1		7		1		7	21
Scoliciosporum chlorococcum	64		79	6	73	35	81	9	26	41
<u>*Usnea cornuta*</u>	1	15	1	1		1		2		
<u>*U. flammea*</u>	1		1							
<u>*U. florida*</u>	2	4								
<u>*Usnea* initials</u>	51	35	40	13	21	14	30	5		
<u>*U. subfloridana*</u>	54	29	11		1	2	9	4		
Xanthoria parietina									1	
X. polycarpa						3			49	50

*Tycanol: Gl = glade, Mo = moorland, Pa 1 = pasture 1, Pa 2 = pasture 2; Kås: ed = forest edge, int. = interior.
†*Hypogymnia* initials were only registered when specimens were too small to identify.
Presence of species on each annual increment (between girdle scars) is recorded for 15 years growth on each twig and 10 twigs at each site.

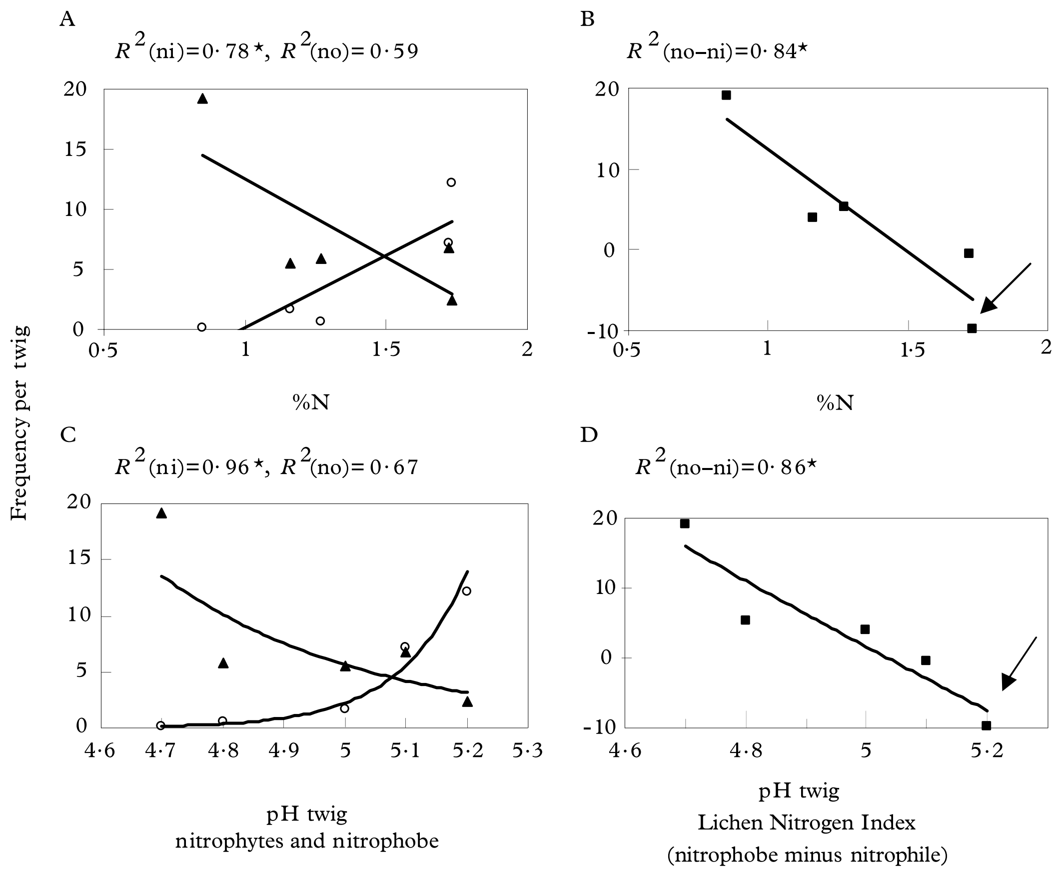

FIG 3. The relationships between nitrophobes (no. ▲), nitrophiles (ni, ○) and the 'Lichen Nitrogen Index' (no–ni, ■) and nitrogen content in *Hypogymnia physodes* and twig bark pH at Tycanol, Wales, and Kås Forest, Demark. A & B, lichen frequency and % nitrogen; C & D, lichen frequency and twig bark pH. R^2 values and linear trendlines are indicated. The significant correlations [Pearson Product Moment $P<0{\cdot}05$, (based on H^+-ion concentration for pH) indicated with *]. Data are based on measurements at the 4 sites at Tycanol in 2003 and the edge site from Denmark in 2001. Danish site is marked with arrows in B&D.

a trend towards an increase in nitrophilous species on twigs, providing an opportunity to test the application of a Lichen Index of Nitrogen in different areas of northern Europe. The strong statistical correlations of nitrogen content in *H. physodes* from this study to acidophytes (nitrophobes) and nitrophytes (nitrophiles) as defined in the Netherlands by van Herk (1999)(Fig. 3B) indicate that the method is applicable in a range of different regions at least including the Netherlands, Great Britain and Denmark. However, regional differences in distribution patterns may affect the definitions of nitrophile and nitrophobe. For example *Physcia aipolia* is not included in the list of nitrophytes in van Herk (1999), although Wirth (1992), Frati *et al.* (2007) and Nimis and Martellos (2008) considered it to be a nitrophyte. In this study, *P. aipolia* has shown an increase in the pasture sites concomitant with *P. tenella* (Table 2). *Physcia aipolia* is rare in the Netherlands, which is probably the reason that it is not included in the list of nitrophytes in van Herk (1999). The variation in sensitivity between species of the same genus has as yet been little investigated, but the results at Tycanol suggest that there are differences in sensitivity between species of *Usnea* in the glade site,

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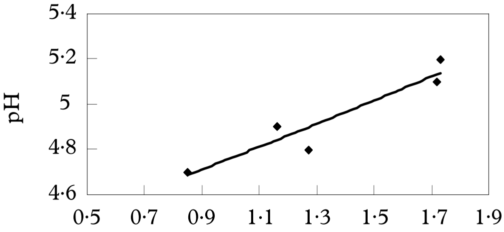

FIG 4. The correlation between percentage total nitrogen in *Hypogymnia physodes* and bark pH of oak twigs (◆) at 4 sites at Tycanol, Wales, and the edge site from Kås Forest, Denmark. The correlation (R^2=0·89, Pearson Product Moment P<0·05) is based on H^+-ion concentration for pH.

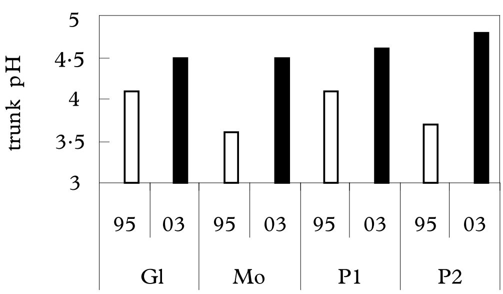

FIG. 5. Bark pH on oak trunks at sites in Tycanol, Wales, in 1995 (white columns) and 2003 (black columns). Gl = glade, Mo = moorland, P1 = pasture 1, P2 = pasture 2.

where *U. cornuta* increased whereas all other species of *Usnea* decreased.

The nitrogen content in *Hypogymnia physodes* has been widely used as a measure of nitrogen deposition (e.g. Bruteig 1993; Søchting 1995). The present results show a strong positive correlation between the Lichen Nitrogen Index (nitrophobe minus nitrophile frequency) and both percentage nitrogen in the thallus of *H. physodes* (Fig. 3B) and bark pH (Fig. 3D). The nitrogen content in *H. physodes* is highest at Kås and results in the lowest Lichen Nitrogen Index (arrows to Danish site in Fig. 3B & D) suggesting that at this site atmospheric nitrogen has had an effect on the lichen community over a considerable period of time, whereas at Tycanol the effect can be shown over a period of eight years. The results of lichen surveys in Holland (van Herk 1999) and of macrolichens across the UK in sites adjacent to ammonia monitoring stations showed that the loss of nitrophobes and increase in nitrophiles is occurring over a wide geographical range (Wolseley *et al.* 2005, 2007; Sutton *et al.* 2007) and is correlated with increased ammonia concentration and with an increase in bark pH. Percent nitrogen in *H. physodes* also correlates with twig bark pH in this study (Fig. 4). However, bark pH varies with the species and age of the tree whereas the shift in lichen communities is occurring on all tree species subjected to atmospheric ammonia (Wolseley *et al.* 2008). At rural sites livestock farming is the major source of reduced nitrogen, but in urban areas oxidized nitrogen from heating and traffic plays a major role affecting the lichen flora. Gombert *et al.* (2003) showed that percentage nitrogen in *Physcia adscendens* was better correlated to traffic density in areas around Grenoble than to percentage nitrogen in *Hypogymnia physodes*. Likewise, the number of nitrophile and nitrophobe lichens living on oak trunks in the parks of London correlated with traffic related NO_x concentration (Larsen *et al.* 2007; Davies *et al.* 2007), suggesting that lichens are affected by a variety of sources of nitrogen.

Changes occurring between 1995 and 2003 at Tycanol

The increase in trunk bark pH at all sites at Tycanol from 1995 to 2003 indicates that there is an ongoing change in the atmospheric environment. This may reflect the general trend of increased nitrogen deposition across Europe. Tycanol is on the Atlantic coast of Wales where long distance pollution is assumed to be very rare, but where local changes in adjacent land management may have an effect on lichens. The frequency of nitrophobes and nitrophiles on twigs in all sites in 1995 and 2003 shows a loss of nitrophobes at all sites at Tycanol, whereas the increase in nitrophiles is occurring mainly in the most exposed sites; Pastures 1 and 2 (Fig. 6). Our results suggest that loss of nitrophobes occurred prior to the increase in

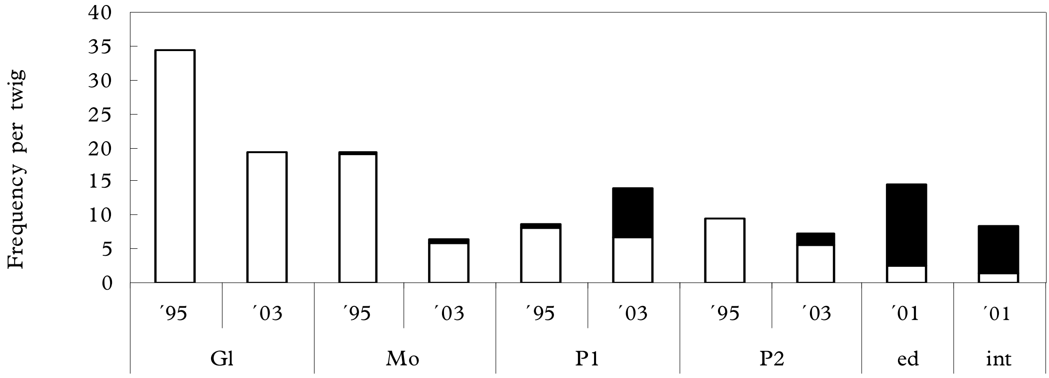

FIG. 6. The average frequency per twig of nitrophiles (black) and nitrophobes (white) on twigs at four sites at Tycanol NNR, Wales, in 1995 and 2003 and Kås Forest, Denmark, in 2001. Sites at Tycanol: Gl = glade, Mo = moorland, P1 = pasture 1, P2 = pasture 2; sites at Kås: ed = forest edge and int = interior.

TABLE 3. *Frequency of lichens and bryophytes at all sites at Tycanol, Wales, and Kås Forest, Denmark*

	Number of species		Average number per twig*.				pH twig	%N
Site†	Lichen	Bryophyte	Lichen	Bryophyte	Nitrophobe	Nitrophile		
Gl 95	36	5	43·7	1·4	19·0	–	n.m.	n.m.
Gl 03	37	5	34·0	7·9	10·9	0·1	4·7	0·85
Mo 95	34	1	37·4	0·2	11·5	0·1	n.m.	n.m.
Mo 03	34	1	26·2	0·1	3·5	0·3	4·8	1·27
Pa1 95	27	0	29·0	0	5·1	0·1	n.m.	n.m.
Pa1 03	30	2	31·4	0·1	3·9	3·2	5·1	1·72
Pa2 95	32	1	31·5	0·1	6·3	0·1	n.m.	n.m.
Pa2 03	35	4	25·1	1·3	2·8	0·7	5	1·16
DK ed 01	19	0	24·1	–	1·2	6·0	5·2	1·73
DK int 01	17	0	17·3	–	0·7	3·5	n.m.	n.m.

n.m.= not measured
*note that average number of bryophytes, nitrophobes and nitrophiles per twig can exceed the number of increments (15), as there are several species included in each group.
†see Table 2 for explanation of abbreviations.

nitrophiles (Fig. 3A & C), which is in accordance with other observations (Sutton *et al.* 2004*a*, 2008; Wolseley *et al.* 2005, 2006).

The present study shows that these changes are correlated with an increase in bark pH at all sites (Fig. 5), the lowest increase being in the Glade site and the highest increase in Pasture 2, adjacent to improved pasture and more intensive farming. The most exposed site adjacent to Moorland also shows an increase in bark pH, suggesting that the affect is not restricted to trees adjacent to pasture. At the sheltered Glade site with the lowest recorded percentage nitrogen in *Hypogymnia physodes* (Table 3) the loss of nitrophobes (mainly *Hypogymnia* species and *Usnea subfloridana*) together with the appearance of the nitrophile *Physcia tenella* shows that this area is also affected. There is also a marked increase in *Hypotrachyna revoluta* and *Punctelia subrudecta* s. lat. from 1995 to 2003 (Table 2), indicating that these species are tolerant of increasing nitrogen deposition, although not designated as nitrophiles. This is supported by Sparrius (2007) who has categorized *H. revoluta* and *P. subrudecta* s. lat. as neutrophytes together with *Melanelixia* *subaurifera*. Furthermore, the tremendous increase of *Phaeographis smithii* at Pasture 2 (Table 2), suggests that this species is tolerant of increasing atmospheric nitrogen. In sites in the west of Britain this species has been increasing rapidly (P. A. Wolseley, unpublished). Other factors that may have caused changes in conditions at Tycanol include increasing shade from canopy encroachment into glades. The woodland glade site is situated on the edge of prehistoric fortifications within the woodland. Since the first survey this long-established open glade has become increasingly shaded due to canopy and bracken encroachment resulting in a shift from photophilous lichens of the *Parmelion* to shade-tolerant bryophytes. Differences in light exposure can introduce bias, so that we recommend studying only well-exposed twig environments. However, the loss of nitrophobic species of *Usnea* and of *Cetrelia* and the appearance of *Physcia tenella* suggests that there is an ongoing change in atmospheric conditions in the Glade site. This suggests that small conservation sites containing nitrophobic species may be affected to a greater extent than larger sites.

Lichen flora of twigs as a biomonitoring method

Wolseley *et al.* (2005) investigated the lichen flora of trunks and twigs, bark pH and ammonia concentrations at two sites in Devon and Norfolk, UK, with different pollution histories. They demonstrated a good correlation between bark pH and ammonia concentrations with twig flora, and a poor correlation with trunk flora. The trunk flora continued to support nitrophobes whereas the twig flora was dominated by nitrophiles. Lichens are often relatively long-lived and nitrophobes may continue to survive on an older substratum despite moderately increasing ammonia concentrations. Furthermore, there is a hysteresis effect (resilience) in the bark properties following changes in the environment.

Canopy twigs are not useful for this biomonitoring method as conditions on the twigs of the interior are very different to those on the accessible twigs of the lower branches. The difference between the twig floras from the two Danish sites (edge and interior) is mainly due to the low frequency of lichens on the twigs from the forest interior canopy (Table 3). The low lichen diversity of the twigs from the interior forest may be caused by drought and exposure due to increased air turbulence at canopy height, rather than to differences in nitrogen deposition. Furthermore, canopy twigs are not only difficult to access, but also difficult to monitor without destructive collection of twigs.

The method used at Tycanol is relevant at sites where branches are accessible. In the Netherlands the surveys were made on wayside trees, where branches have been removed up to 3–4 meters from the ground, providing a well-lit substratum for lichen colonization. At sites where it has been possible to compare the trunk and twig floras, the twig flora has responded earlier to changes in atmospheric conditions, than that of the trunk (Wolseley *et al.* 2006, 2008). This may be especially important at sites of high conservation value where the rarer lichen species are associated with veteran trees. In this situation a change in frequency on the twigs of nitrophobe and nitrophile lichen species can act as an early warning system for long term changes associated with nitrogen or acidifying deposition.

Conclusions

The frequency of nitrophobe and nitrophile lichen species on twigs is combined in a Lichen Nitrogen Index which correlates well at both sites with twig bark pH and with percentage nitrogen in the thallus of *Hypogymnia physodes*, suggesting that the index is associated primarily with nitrogen deposition and possibly secondarily with decreasing SO_2 deposition. This applies to both sites, despite their different climatic conditions.

The lichen flora on twigs can change according to current air conditions within a period of 8 years.

The method described is suitable for use in areas where branches are accessible from the ground, for example, along forest and glade edges or in parkland sites. It is reproducible, reflects current atmospheric conditions, and can be used to monitor changes over time.

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