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Detecting changes in epiphytic lichen communities at sites affected by atmospheric ammonia from agricultural sources

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Abstract: Lichens were recorded on *Quercus petraea* trunks and twigs near ammonia recording stations in 'continental' Norfolk, and 'oceanic' Devon in order to test indicator values developed for epiphytic lichens in areas of high atmospheric ammonia in the Netherlands. Lichens on trunks in Norfolk showed a similar correlation of nitrophyte indices with ammonia concentration and bark pH as those in Holland, whereas in Devon there was no correlation with nitrophyte indices on trunks and a negative correlation with acidophyte indices. Results on twigs in both sites suggest that lichens on twigs respond more rapidly to recent changes in ammonia concentrations while trunks may maintain relict lichen communities due to either a legacy of previous acidification or ecological continuity. The results suggest that loss of acidophytes is taking place prior to the establishment of nitrophytes indicating the importance of establishing levels of ammonia at which sensitive communities are at risk.

Key words: acidophytes, ammonia, lichens, nitrophytes, oak trees

Introduction

Over a relatively short period of time rapid changes in atmospheric pollutant concentrations across Europe have occurred following a reduction in SO_2 emissions. In Britain annual emissions of sulphur dioxide fell from *c.* 3259 kt-S in 1970 to 594 kt-S by 1999 and a further decline is predicted to *c.* 312 kt-S by 2010 (NEGTAP 2001). Nitrogen emissions have also changed during the same time period, but these are made up of several compounds produced from a variety of sources whose effects may vary with the compound and its source. The main sources of reactive nitrogen in the atmosphere are nitrogen oxides (NO_x) and ammonia (NH_3). Road traffic contributes a major part of the NO_x along with other high temperature combustion processes, whereas NH_3 is largely a product of intensive farming.

Although there has also been a substantial decline in total NO_x emissions since 1990, NH_3 emissions have only decreased slightly (<10%) and are still high especially in agricultural areas (NEGTAP 2001; Sutton *et al.* 2001*a*; Sutton & Fowler 2002). While nitrogen oxides and their reaction products may be carried over long distances in the atmosphere in a number of forms, ammonia is deposited as NH_3 along very local gradients of up to 300m so that it is often difficult to detect on a grid scale of 5 × 5 km (Fowler *et al.* 1998; Dragosits *et al.* 2002; Sutton *et al.* 1998, 2001*a*; Sutton & Fowler 2002). However in agricultural areas with a high cattle density many local sources may add up to high background levels so that deposition gradients may be considerably affected.

Epiphytic lichens have long been known to be highly sensitive to atmospheric pollutants and have been used to map the effect of sulphur dioxide deposition in and around

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industrial and urban centres in many parts of Europe (e.g. Barkman 1969; Batic 2002; Hawksworth & Rose 1970; Nimis *et al.* 1990; Seaward 1993; van Haluwyn & Lerond 1988). Following a reduction in SO_2 deposition in these areas lichens are now reappearing (e.g. Hawksworth & McManus 1989; Kricke & Feige 2004; Seaward 1997). In Holland, elevated atmospheric NH_3 was recognized as a serious threat to vegetation by van der Knaap (1984) and van Dobben (1986) leading to the testing of epiphytic lichen communities as bioindicators of atmospheric ammonia (van Herk 1999). From a data set of all lichens on ten oak trunks at 100 sites in the Netherlands, van Herk (*loc. cit.*) used Canonical Correspondence Analysis to identify species associated with abiotic factors such as mean annual concentrations of NH_3, NH_4^+ and SO_2 together with measurements of bark pH, NH_4^+, and SO_4^{2-}. Species associated with high ammonia levels were termed 'nitrophytes' and those associated with naturally acid bark and low N levels termed 'acidophytes'. In order to quantify these components he used a weighted scoring system based on cover of nitrophyte and acidophyte species to provide a nitrophyte index (Nitrofiele Indicatie Waarde, NIW) and an acidophyte index (Acidofiele Indicatie Waarde, AIW). This allowed detection of shifts on naturally acid bark of oak (*Quercus robur*) from a high AIW (low NH_3 concentrations) to a high NIW (high NH_3 concentrations). Van Herk recognized that nitrophytes require a basic substratum as well as nitrogen. Because NH_3 is the only component of N and S pollutants that is a base, it may uniquely cause an increase in bark pH, which favours the nitrophyte species (van Herk 2001). Bark pH will also vary with tree species and in the Netherlands sampling was restricted to acid-barked species of *Quercus* (van Herk 1999). However, bark pH may also be influenced by calcareous dust around quarries (Gilbert 1976) and in dry areas of southern Europe (Loppi & Pirintsos 2000), as well as by geology, rainfall and age of the bark surface. In regions of high rainfall the bark is leached of basic ions so that in upland and western Atlantic oceanic regions, the bark tends to be more acid (Bates 1992).

The impacts of air pollution on lichen diversity have varied over a time scale of increasing or decreasing pollutant levels. The relationship established between lichens and SO_2 concentrations in the 1960s and 1970s has been shown not to apply in the last decade, so that despite continuing falling SO_2 levels the reappearance of lichen species does not follow an expected pattern (Hawksworth & McManus 1989; Rose & Hawksworth 1981; Davies *et al.* 2004). Gilbert (1992) attributed this to varying rates of recolonization by lichen species. The application of lichens as bioindicators of pollution impacts has focused on tree trunks (girth >1 m), but sampling of lichens on oak twigs in a range of environmental conditions on a nature reserve in west Wales (UK) showed a similar shift from acidophyte to nitrophyte lichen communities on the margins of agricultural land (Wolseley & Pryor 1999). This finding was tested on twigs and trunks of acid-barked tree species in the vicinity of known NH_3 emissions (Wolseley & James 2002*a*, *b*; Sutton *et al.* 2004; Wolseley *et al.* 2004). The results suggested that lichen communities of newly available bark surfaces such as twigs are sensitive indicators of the current air pollution climate, while those on trunks may contain species and communities that are relicts of previous conditions.

The objective of this study was to sample epiphytic lichen communities on trunks and twigs of *Quercus* species in sites in the vicinity of known atmospheric NH_3 concentrations in two different geographic regions of England, in order to test and evaluate the use of epiphytic indicator species defined as acidophytes and nitrophytes in continental Europe (van Herk 1999). Lichen species, diversity and cover are investigated for the first time in relation to bark pH of trunks and twigs and NH_3 concentration for the first time.

Methods

Study sites

Two study areas were selected in the vicinity of ammonia recording stations at Thetford, Norfolk

TABLE 1. *Lichen species identified as Nitrophytes and Acidophytes according to van Herk (1999, 2002). Species in bold were recorded in this study*

Nitrophytes (NIW)	Acidophytes (AIW)
Caloplaca citrina (Hoffm.) Th. Fr.	*Tuckermanopsis chlorophylla* (Willd.) Vain.
C. holocarpa (Hoffm.) A.E. Wade	*Chaenotheca ferruginea* (Turner ex Ach.) Mig.
Candelariella aurella (Hoffm.) Zahlbr.	***Cladonia* sp.**
***C. reflexa* (Nyl.) Lettau**	***Evernia prunastri* (L.) Ach.**
***C. vitellina* (Hoffm.) Müll. Arg.**	*Hypocenomyce scalaris* (Ach. ex Lilj.) M. Choisy
C. xanthostigma (Ach.) Lettau	***Hypogymnia physodes* (L.) Nyl.**
Lecanora muralis (Schreb.) Rabenh.	***H. tubulosa* (Schaer.) Hav.**
***L. dispersa* grp. (incl. *L. hagenii* auct.**	*Lecanora aitema* (Ach.) Hepp
***Phaeophyscia orbicularis* (Neck.) Moberg**	*L. conizaeoides* Nyl. ex Cromb.
P. nigricans (Flörke) Moberg	*L. pulicaris* (Pers.) Ach.
***Physcia adscendens* H. Olivier**	***Lepraria incana* (L.) Ach.**
P. caesia (Hoffm.) Fürnr.	*Ochrolechia microstictoides* Räsänen
P. dubia (Hoffm.) Lettau	***Parmelia saxatilis* (L.) Ach.**
***P. tenella* (Scop.) DC.**	*Parmeliopsis ambigua* (Wulfen) Nyl.
Rinodina gennarii Bagl.	***Placynthiella icmalea* (Ach.) Coppins & P. James**
***Xanthoria candelaria* (L.) Th. Fr.**	*Platismatia glauca* (L.) W.L. Culb. & C.F. Culb.
X. calcicola Oxner	*Pseudevernia furfuracea* (L.) Zopf
***X. parietina* (L.) Th. Fr.**	*Trapeliopsis flexuosa* (Fr.) Coppins & P. James
X. polycarpa **(Hoffm.) Th. Fr. ex Rieber**	*T. granulosa* (Hoffm.) Lumbsch
	***Usnea* sp.**

(0°49′E, 52°27′N) in a continental climate where annual rainfall is *c.* 500 mm, and at North Wyke, Devon (3°54′W, 50°27′N) with an oceanic climate where annual rainfall is *c.* 1000 mm. Aerial photographs (http:www.multimap.com) were used to identify suitable stations with sufficient exposed oak trees (*Q. robur* L. and *Q. petraea* L.) in and around sites where ammonia monitoring was already established.

Ammonia exposure estimates

At North Wyke, NH_3 concentrations have been measured on a monthly basis since 1996 at one site upwind of an experimental livestock farm of the Institute of Grassland and Environmental Research, as part of the UK National Ammonia Monitoring Network, using the DELTA active sampling denuder system (Sutton *et al.* 2001*b*). In order to estimate NH_3 concentrations at different locations around the farm, the SCAIL model (Simple Calculation of Ammonia Impact Limits) (Theobald & Sutton 2002) was applied. SCAIL calculates NH_3 concentrations with distance using input data of the NH_3 source emission rates, distance from the sources, wind speed and wind direction probabilities.

At Thetford NH_3 concentrations were available directly from monthly measurements at 25 sites as part of the GANE LANAS project for a period of 9 months (Theobald *et al.* 2004). The measurements were made with ALPHA high-sensitivity passive samplers (Tang *et al.* 2001), the main objective being to investigate the local variability of NH_3 concentrations and test a local scale atmospheric dispersion model (LADD, Local Atmospheric Dispersion and Deposition) (Dragosits *et al.* 2002). Of the sites, 11 had oak trees in the vicinity permitting assessment of lichen species occurrence. The main pollutant sources were intensive poultry rearing sheds. To deal with the potential lack of comparability of using measured NH_3 concentrations at Thetford and modelled NH_3 concentrations at North Wyke, it was considered of interest to apply the SCAIL model to the Thetford area. This was not feasible given the complex multi field and farm sources contributing to NH_3 in Thetford. However, the NH_3 concentrations at Thetford have been modelled in detail using LADD (Theobald *et al.* 2004), which was also applied to North Wyke allowing comparison with the SCAIL estimates.

Lichen recording

At each sampling station, lichens were recorded on up to five exposed *Quercus* tree trunks up to 2 m high using the van Herk 6 point cover and abundance scale: 1: only one lichen thallus present, 2: more thalli on one tree, 3: present on up to 50% of trees, $<10\ dm^2$ per tree, 4: present on up to 50% of trees, $>10\ dm^2$ per tree, 5: present on more than 50% of trees, $<10\ dm^2$ per tree, 6: present on more than 50% of trees, $>10\ dm^2$ per tree.

Selected species scored as nitrophytes or acidophytes (Table 1) were used in calculating the NIW and AIW indices for each station (frequencies of species in abundance classes 4 and 6 are multiplied by 2, and the index is the average of the total per tree for each site). The girth of each tree was recorded. The twig survey was carried out on exposed and accessible twigs of trees in

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the same sampling station. All lichens were recorded on five exposed twigs up to 15 years old (using girdle scars as indicators of annual increments) or *c.* 2 cm thick, and given a DAFOR (Dominant=5, Abundant=4, Frequent=3, Occasional=2, Rare=1) scale for each twig investigated. Total values for each twig are averaged for each site. Lichen names follow the checklist of lichens of Great Britain and Ireland (Coppins 2002)

Bark pH

Samples of tree bark with a flat surface and of twigs *c.* 5–7 mm diameter were collected and dried at air temperature and stored in paper bags. The pH was recorded in the laboratory using a flat-head electrode and a method adapted from Kermit & Gauslaa (2001) for assessing pH of twigs. Twigs 5–7 mm thick were cut into 6 cm lengths, the cut ends sealed with paraffin wax and placed in a tube with 6 ml of 25 mM KCl. Samples were shaken well and incubated for 1 hour at room temperature in test tubes and shaken at regular intervals. After one hour portions of KCl were extracted and pH measured using a flat-head electrode. Samples were measured after 2 hours to ensure that equilibrium values were obtained. For trunk bark pH the surface was wetted with 25 mM KCl for 5–10 minutes and the pH of the supernatant liquid measured with a flat-head electrode (Farmer *et al.* 1990). At least 3 samples were taken from each tree.

Results

The sites

Sampling stations at North Wyke (Fig. 1) cover an area approximately 1·5 × 1 km where the farm unit housing winter stock and slurry tank was identified as the point source. At Thetford the sampling area is approximately 9 × 7 km where sources of NH_3 from intensive poultry rearing units were spread over a wider area. Lichen data were recorded at 7 stations at North Wyke (Table 2) and 11 stations at Thetford (Table 3).

Estimated ammonia concentrations

The measured NH_3 concentrations at 11 locations at Thetford (Table 3) and one location in North Wyke have been made using the methods of the National Ammonia Monitoring Network (Sutton *et al.* 2001*a*) so that the mean concentrations at each site are estimated to be accurate to better than ±5%. By contrast, much higher uncertainty is associated with the SCAIL modelled estimates for the other locations at North Wyke (Table 2). The comparison of the SCAIL estimates with those derived from the LADD model for North Wyke provides some estimate of uncertainty. This provided the relationship for estimated ammonia concentrations of: LADD=1·11 × SCAIL, where the intercept was not significantly different from zero, with r^2=0·62. The mean concentration of the SCAIL estimates was 1·65 µg m^{-3} and that from LADD was 1·78 µg m^{-3} (i.e. on average 7% difference) with maximum differences at individual sites of −32% to +46%.

The comparison of the LADD model with the measured NH_3 concentrations at the 11 sites at Thetford provided a relationship of LADD=0·91 × Measured, with the intercept not significantly different from zero; r^2=0·94. The mean concentration of the measured values was 3·12 µg m^{-3} and that from LADD was 2·73 µg m^{-3} (i.e on average 13% difference), with maximum differences at individual sites of −39% to +12%.

Lichen species diversity and differences between study areas

The North Wyke and Thetford sites both support open-grown mature oaks in a range of stations, allowing sampling to be conducted at a range of stations across the site. However, where sampling is restricted to isolated trees it is often not possible to find 10 trees in one station, and in practice to allow differentiation of the data across a limited transect up to 5 trees were sampled at each station. The age range of exposed trees selected was often considerable, especially at Thetford where exposed trees were frequently <150 cm girth (Fig. 2). However there was no correlation between age (girth) and lichen diversity in either site, younger trees <2 m girth often having rather high diversity. Although the range of tree girths was similar in both sites the diversity of lichens on trunks was very different; at North Wyke lichen diversity was high on trunks with a mean of 18·2 species, and at Thetford a mean of 7·8 species (Table 4). The diversity of the twig flora at both sites

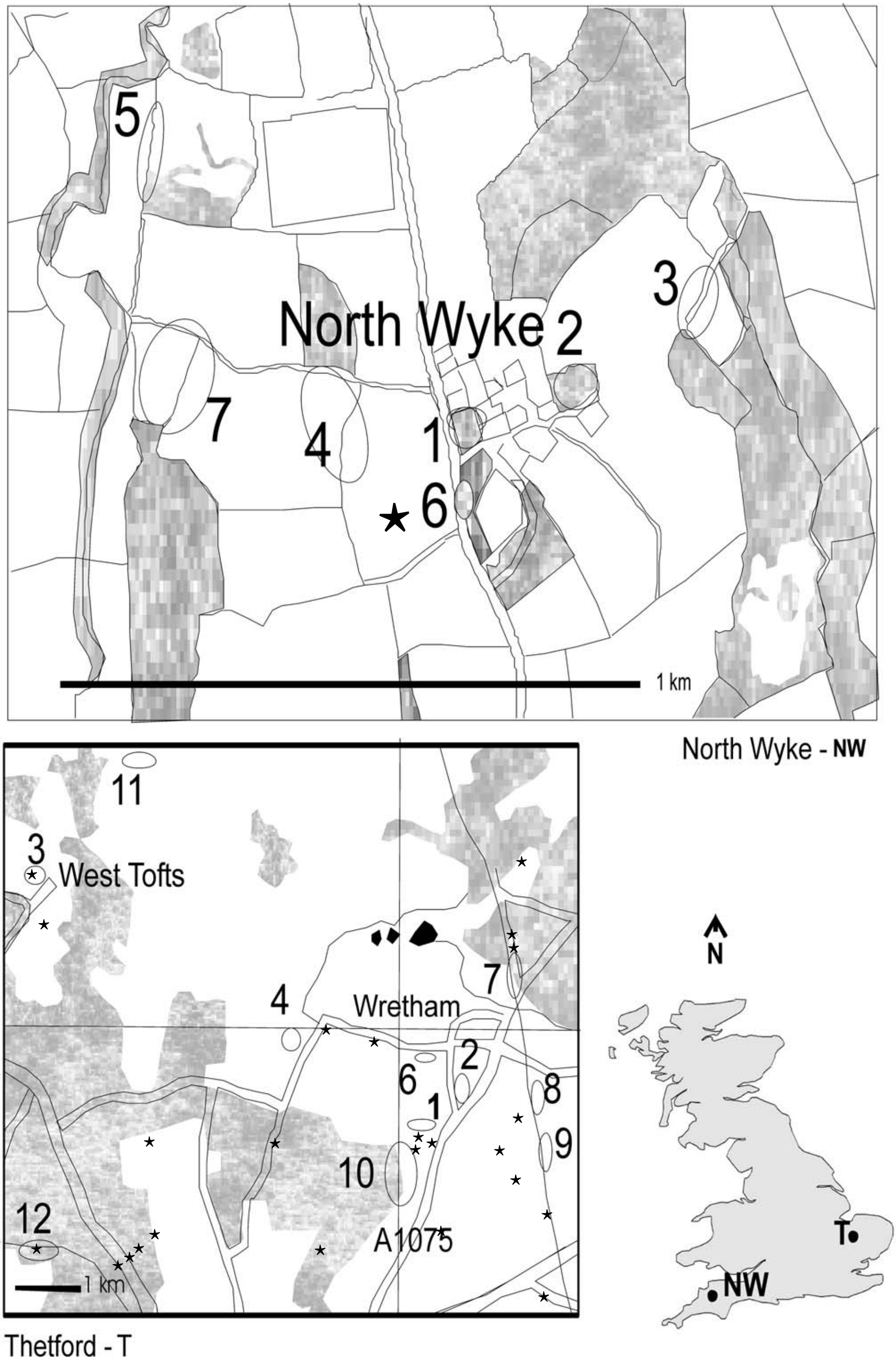

FIG. 1. Distribution of sampling sites (dotted areas) and ammonia sampling stations (★) at North Wyke and Thetford. Wooded areas shaded.

TABLE 2. *Modelled ammonia concentrations around a livestock farm at North Wyke, together with mean lichen diversity scores and mean bark pH at stations in the North Wyke study area*

Site no.	Site location	Modelled NH_3 conc. (μg m^{-3})	Distance from source (m)	No. of trees sampled	Diversity*		NIW†		AIW†		pH‡	
					Trunk	Twig	Trunk	Twig	Trunk	Twig	Trunk	Twig
1	Standard trees between farm buildings	2·4	30 W	5	18	4·4	0·4	3·5	1·4	0·2	5	5·3
2	Standard trees adjacent to slurry tank	2·64	20 E	5	18·2	5	0·8	7·1	1	0	4·7	5·9
3	Hedgerow trees	1·32	300 E	4	15·7	9·3	0	3·5	0	0·2	4·9	5·5
4	Isolated trees in pasture	1·47	300 W	4	20·7	11·5	1·25	5	3	0	4·8	5·4
5	Trees on edge of *Molinia* wetland reserve	0·89	750 NW	3	18·6	12·3	0	3	5	6	4·25	5·1
6	Boundary oaks on road	1·9	150 SW	2	16·5		0		1			
7	Trees in pasture	0·95	700 W	4	18	11·2	0	3	3·5	0	4·4	5·9

*Mean lichen diversity on sampled trunks and twigs at each station; †NIW and AIW refer to the scores for nitrophyte and acidophyte lichen species, respectively, according to the method of van Herk (1999) for trunks and as modified here for twigs; ‡bark pH of trunks and twigs measured in KCl.

was more similar, North Wyke having a mean of 8·3 species and Thetford having a mean of 4·65 species. Acidophyte and nitrophyte species selected by van Herk (Table 1) formed the basis of AIW and NIW indices (van Herk 1999) for both sites (Tables 2 & 3). At North Wyke the AIW was consistently higher than the NIW on trunks but this situation was reversed on twigs except in station 5 in the wetland reserve area.

The high number of acidophyte species on trunks at North Wyke suggests that this site is less affected by nitrogen compounds than Thetford (Table 4). However, very few of the indicator species identified by van Herk (1999, 2002) were present at North Wyke where only two species were present on twigs and four on trunks. At Thetford four indicator species were present on twigs and eight on trunks. The mean bark pH of oak trunks was the same in both sites although the range of bark pH was greater at Thetford. Bark pH of twigs was consistently higher in both sites so that the mean is similar in both sites but higher than that of trunks in both sites (Tables 2 & 3). As oak bark tends to increase in basic ions with age (Bates 1992; Farmer *et al.* 1991), both sites represent an abnormal condition.

Relationships between local ammonia, bark pH and lichen species diversity

Bark pH and ammonia

Levels of ammonia recorded at North Wyke were low throughout the year, the highest being *c.* 3·5 μg m^{-3} in August and September each year. The modelling of ammonia concentrations at each station was based on stocking levels, slurry storage and deposition in the farm units (Table 2). However, in agricultural areas there may be considerable variation in NH_3 emissions over the year as illustrated by the maximum values recorded at Thetford (Table 3) which may affect lichen communities. At Thetford ammonia records were available for all stations where lichens were sampled and showed considerable variation in recorded concentrations (Table 3). At both sites bark pH of twigs was higher than that of trunks. At North Wyke bark pH of twigs was correlated with modelled ammonia concentration whereas trunk pH showed little variation in pH with increasing ammonia concentration (Fig. 3A). At Thetford bark pH of trunks and twigs was correlated with ammonia concentrations but at levels above 4·5 μg m^{-3} no further increase in bark pH was recorded (Fig. 3B).

Lichen diversity and ammonia

At North Wyke lichen diversity on trunks was not related to modelled ammonia concentration while lichen diversity on twigs was significantly negatively related to modelled ammonia concentration (r^2=0·89) (Fig. 3C). At Thetford lichen diversity on trunks and twigs was lowest at stations with the highest recorded ammonia concentration, but relatively high diversity was recorded on trunks at levels up to 4·5 μg m^{-3} (Fig. 3D). Diversity on twigs was negatively related to ammonia concentration, and at the highest concentrations there were no species present on the twigs.

Nitrophyte and Acidophyte Lichen Indices and ammonia

Using nitrophyte and acidophyte species defined by van Herk (Table 1) NIW and AIW indices were calculated for trunks and twigs of the sampled oaks at each station at both sites (Tables 2 & 3) and values plotted against ammonia concentrations. At North Wyke the AIW on trunks decreases with increasing ammonia concentration (r^2=0·40) whereas the absence or low frequency of nitrophytes on the trunk gives zero or low NIW values (Fig. 4A). In contrast, the absence or low frequency of acidophytes on twigs provides zero or low AIW values while the NIW values on twigs are related to ammonia concentration (r^2=0·49) (Fig. 4C). At Thetford rising NIW values for both trunks and twigs are clearly related to NH_3 concentrations except at the highest levels where there is a drop in NIW values (Figs 4B & D) indicating that high levels of ammonia might be toxic to both nitrophytes and acidophytes.

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TABLE 3. *Ammonia concentrations, mean lichen diversity scores and mean bark pH at stations in the Thetford study area, except station 5 where no trees were found*

Site no.	Site location	NH_3 μg m^{-3}*			No. of trees sampled	Diversity†		NIW‡		AIW‡		pH§	
		max.	mean	min.		Trunk	Twig	Trunk	Twig	Trunk	Twig	Trunk	Twig
1	Norfolk Wildlife reserve 1	2·9	2·64	0·8	5	7·4	5	3·4	7	1·8	0	4·5	5·2
2	Abrey field	3·13	2·9	1·86	3	10	2·7	5·3	5	0	0	6·3	5·7
3	West Tofts	2·7	1·9	0·99	4	8·5	6·6	1·75	6·1	2	0·3	4	5·3
4	Thorpe Great heath	2·95	2·2	1·32	3	7·7	7·7	2·3	7	4	3	3·5	5·4
6	Military camp	3·91	2·8	1·44	3	7	3	2	9·7	1·3	0	5·2	6·4
7	Roadside trees	124·7	43	27·27	3	8	4	4·2	8·5	0·25	0	6	6·3
8	Peddars way adj. poultry	16·46	9·2	5·63	3	5·3	0·5	1·7	1·7	0·7	0	5·7	6·3
9	Peddars way adj. pigs	9·81	4·54	1·4	3	10·3	2	6	2·2	0·3	0	6	6·3
10	Norfolk Wildlife reserve	4·45	1·92	1·6	4	7·2	3·5	1·2	5·5	2	0	4·4	5·5
11	Stanford area	2·03	1·4	0·86	3	10	6·1	0·7	2	0	0	3·9	5·5
12	Santon Downham FC	3·32	2·05	1·2	3	7·3	8	0·3	1·8	1·7	5·7	3·9	5·1

*Monthly values from 9 months of monitoring; †mean lichen diversity on sampled trunks and twigs at each station; ‡NIW and AIW refer to the scores for nitrophyte and acidophyte lichen species, respectively, according to the method of van Herk (1999) for trunks and as modified here for twigs; §bark pH of trunks and twigs was measured in KCl.

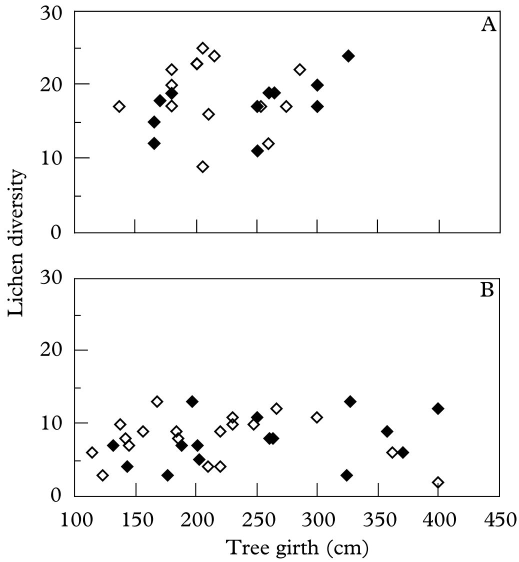

FIG. 2. The relationship between lichen diversity on trunks and tree girth distinguished by mean NH_3 concentrations of <3·5 (◆) and >3·5 (◇). A, North Wyke; B, Thetford.

Nitrophytes, acidophytes and bark pH

Loss of acidophytes on the trunks at North Wyke occurred between pH 4–5 but twigs with higher bark pH supported no acidophytes in most stations (Fig. 5A). Both trunks and twigs at Thetford showed a loss of AIW species corresponding to a change in bark pH and very low or zero AIW above pH 5.5 (Fig. 5B). At North Wyke NIW values were zero or very low on trunks where pH was between 4 and 5, but on twigs increased rapidly between pH 5 and 6 (Fig. 5C). At Thetford NIW values occurred on trunks with bark pH as low as pH 3·5 and increased with increasing bark pH. In contrast, NIW values for twigs were variable between pH 5–6·5, suggesting that bark pH was not the only factor affecting NIW values. Indeed, when bark pH is plotted against NH_3 concentration (Fig. 3B) at Thetford only a weak relationship is apparent for both twigs or trunks. However AIW and NIW values at North Wyke show that bark pH is correlated with loss of acidophytes on the trunk and increasing nitrophytes on the twigs (Fig. 5A). At North Wyke no acidophytes were present on trunks or twigs above pH 5·3 and no nitrophytes present below pH 5 whereas at Thetford nitrophytes were present even at pH 3·5 and acidophytes were present at pH 5·7. In contrast, acidophytes on twigs at Thetford were present only in stations receiving <2·5 μg m^{-3} NH_3 (Table 3). These results suggest that the new substratum is more rapidly affected by atmospheric ammonia than the old, and that bark pH can provide only an approximate indication of the nitrogen status.

Discussion

Differences between study areas

Differences in climate and pollution histories as well as methodological differences in the ammonia estimates (Fournier *et al.* 2002) may affect the results. The comparison of the SCAIL model (North Wyke) and measured NH_3 concentrations (Thetford) with the common LADD model approach provided encouraging results. Although, individual site differences were up to ~40% on average the differences were ~10%, demonstrating that the uncertainties in the SCAIL modelled estimates would not cause a major bias in the results. In addition, it should be noted that bark pH was measured for both study areas, which provides a common data set to link the interpretation of results.

Both sites offered a similar age range of trees, including veteran trees, in sites with very different climatic conditions and pollution histories. It is apparent that the high rainfall and low levels of SO_2 pollution at North Wyke have allowed many sensitive species to survive on trunks of veteran trees in close proximity to intensive stock-keeping and slurry storage. This farm has been intensively managed for several decades, the only low-level management being around station 5, a *Molinia* moor and former Site of Special Scientific Importance (SSSI). At Thetford intensive poultry-rearing sheds are adjacent to a large area of land on the edge of Thetford Forest, a large area of wood

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TABLE 4. *Comparison of minimum, maximum and mean lichen species diversity and standard deviation (SD) of the sample, total nitrophyte (NIW) and acidophyte (AIW) species and bark pH at Thetford and North Wyke*

		Species diversity				NIW	AIW	Bark pH		
	Sites	Min.	Max.	Mean	SD	spp/20	spp/20	Min.	Max.	Mean
Oak trunks	North Wyke	9	25	18·2	4·25	6	12	4·25	5·5	4·7
	Thetford	2	13	7·8	3·2	9	4	3·5	6·3	4·8
Oak twigs	North Wyke	2	20	8·3	4·1	4	2	5·1	5·9	5·5
	Thetford	1	12	4·65	2·8	8	4	5·1	6·4	5·7

Lichen species diversity was assessed from each tree list; NIW and AIW refer to the scores out of 20 for nitrophyte and acidophyte lichen species, respectively, according to van Herk (see Table 1).

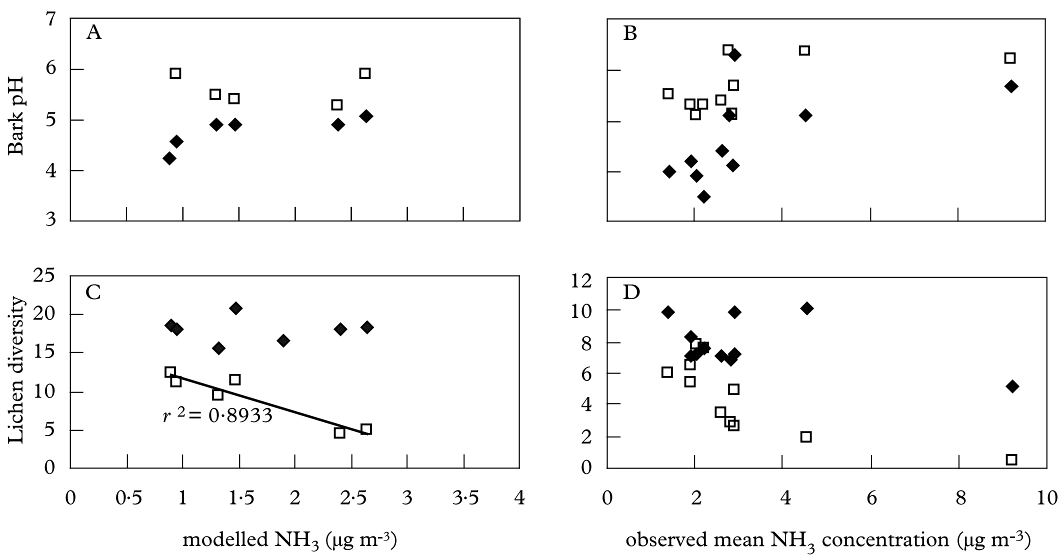

FIG. 3. The relationship of mean bark pH and ammonia concentrations (A & B) and mean lichen diversity and ammonia concentrations (C & D) for trunks (◆) and twigs (□). A & C, modelled ammonia concentrations at North Wyke (linear regression values given where relevant); B & D, measured ammonia concentrations at Thetford (site 7 omitted).

pasture owned by the Ministry of Defence and used for training purposes. This extensive area of parkland and woodpasture is managed with low intensity sheep grazing. Despite the presence of large ancient veteran trees the diversity in this site was rather low, and included species characteristic of former acidification (Wolseley & James 2002*b*).

Lichen diversity and ammonia

Lichen diversity was widely used when mapping zones of pollution due to high levels of SO_2 deposition (Hawksworth & Rose 1970). The present survey has shown that lichen diversity on tree trunks may remain the same or even increase at NH_3 concentrations between 2–3 µg m^{-3} with a loss of diversity at concentrations above 3–4 µg m^{-3}. While species diversity might increase or remain the same at lower levels there is a conspicuous shift in the lichen community to include more nitrophytic species. On twigs at both sites there was a good relationship between increasing ammonia levels and loss of diversity which

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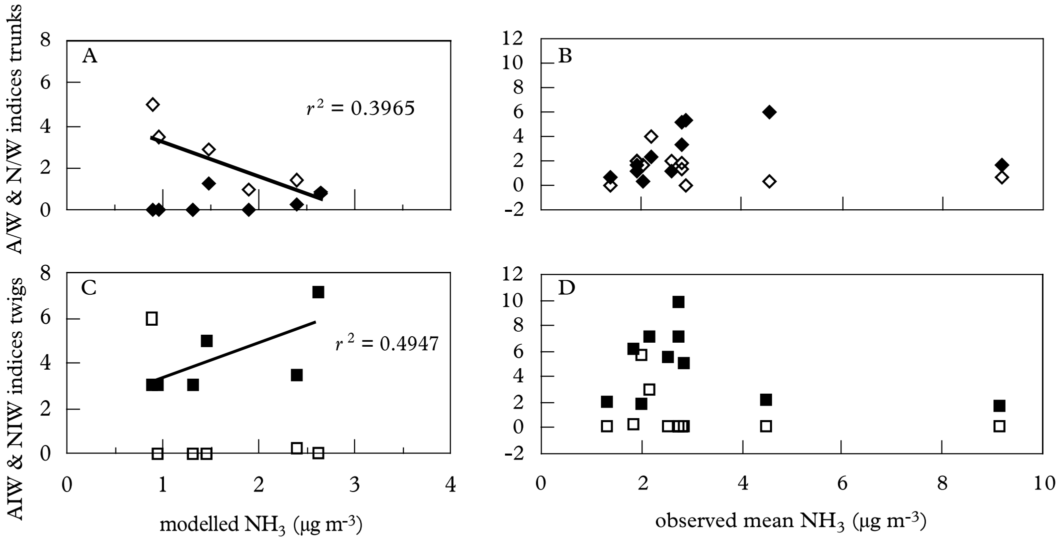

FIG. 4. Relationships between mean nitrophyte (NIW solid symbols) and acidophyte (AIW open symbols) indices on trunks (◆, ◇) and twigs (■, □) and ammonia concentrations (coefficient of determination given where relevant). A & C, modelled ammonia concentrations at North Wyke; B & D, observed concentrations at Thetford.

suggests that conditions for colonization might be more similar on the new bark substratum of twigs. At North Wyke the greatest loss in diversity on twigs occurred between 0–300 metres from the point source, after which there was no further loss. This corresponds well with estimates of the range of ammonia deposition from a point source by Fowler *et al.* (1998).

Lichen indicators

At North Wyke the presence of species such as *Lobaria pulmonaria* (L.) Hoffm., *Teloschistes flavicans* (Sw.) Norman, *Punctelia reddenda* (Stirt.) Krog and five species of *Usnea* on trunks of veteran trees suggests a long history of ecological continuity, whereas at Thetford the absence of indicators of ecological continuity on trunks of veteran trees suggests past environmental alteration (Rose 1992; Wolseley & James 2002*b*). Acidophyte species such as *Evernia prunastri* (L.) Ach. and species of *Hypogymnia*, and *Usnea* were frequent on trunks at North Wyke but nitrophyte species of *Xanthoria* and *Physcia* were frequent on the twigs. At Thetford the low lichen diversity and presence of acidophyte species of *Lepraria* combined with low mean bark pH of 4·7 suggest that this area has suffered from former acidification. In contrast 8 NIW species were present on twigs at Thetford compared with 4 at North Wyke.

Nitrophyte and acidophyte lichen indices and ammonia

Whereas SO_2 is highly toxic, nitrogen is a nutrient and only becomes toxic in excess to those species that are sensitive. Where nitrogen is available in areas of former lichen deserts a wide diversity of epiphytic species has begun to colonize, as on the branches at Thetford or in urban areas (Davies *et al.* 2004). In areas not formerly affected by SO_2, and where lichen communities are already well established on ancient trees, it seems that many 'sensitive' lichen species are able to tolerate additional nitrogen. At North Wyke lichen diversity and AIW values on trunks remained high even in sites immediately adjacent to the stockyard and slurry tank (Fig. 3A). The higher bark pH of the twigs discouraged colonization by acidophytes and encouraged colonization by

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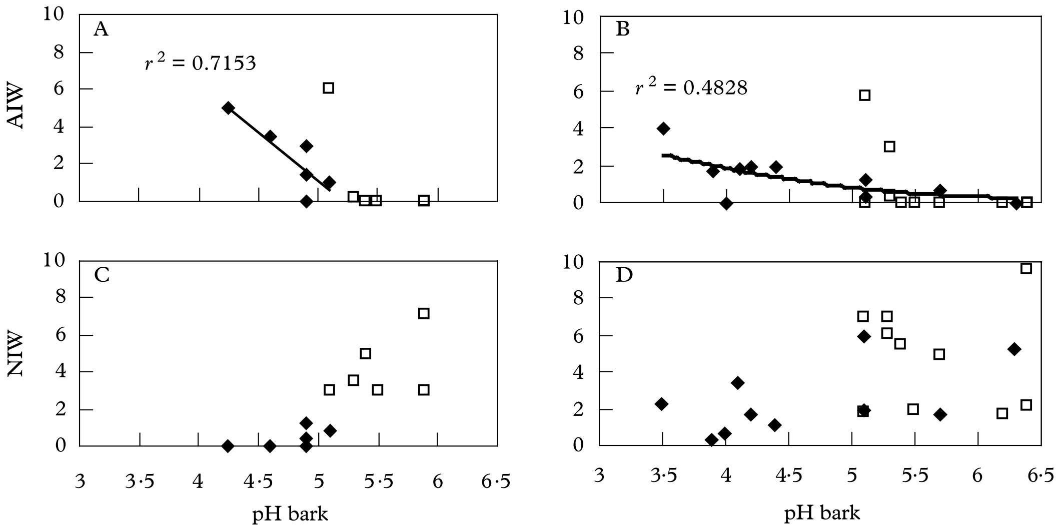

FIG. 5. Relationship between mean AIW and NIW indices and mean bark pH on trunks (◆) and twigs (□), coefficient of determination given for trunk stations. A & C, North Wyke; B & D, Thetford.

nitrophytes (Fig. 5A). On veteran trees the twig substratum provides information on the present conditions for lichen colonization while the trunk may continue to support communities that were established a hundred years or more ago!

The use of weighted cover values of indicator species provides a quantitative basis for comparing acidophyte and nitrophyte indices with ammonia concentrations. The relationship between ammonia concentrations and NIW values was good on trunks at Thetford but at concentrations higher than 4.5 μg m^{-3} there was a rapid drop in NIW suggesting that at higher concentrations ammonia is toxic to most species. In contrast, at North Wyke, where there were few nitrophyte species present on the trunk, and there was no relationship between NIW and modelled ammonia concentrations whereas the negative relationship between AIW and increasing NH_3 concentration was conspicuous. At Thetford AIW values were low throughout, even at low bark pH (Fig. 5B). The NIW values on twigs show a similar effect, increasing with increasing ammonia concentrations and decreasing rapidly above 4 μg m^{-3} immediately adjacent to poultry sheds. The results suggest that the effects of ammonia on lichens of twigs and trunks is similar; at low levels to increase growth of nitrophytes and at high levels to inhibit growth. It also suggests that the ammonia strongly affects colonizers of a new substratum. Further work in Scotland on lichens on twigs adjacent to a poultry unit where high levels of ammonia were recorded produced similar results (Sutton *et al.* 2004).

Bark pH

The relationship between bark pH and lichen communities has been described by Farmer *et al.* (1991), Bates (1992) and others who have demonstrated that increasing bark pH is associated with increasing base ions particularly calcium. In the Netherlands an increase in pH due to NH_3 is associated with an increase in nitrophytic communities of the *Xanthorion* (van Herk 1999, 2001). Conversely a low bark pH is associated with a rich *Usneion* and *Pseudevernion* in unpolluted areas of acidic soils and high rainfall, and in areas of former pollution due to SO_2 by species tolerant of industrial pollution (Bates 1992). Increasing bark pH is associated with loss of AIW species but the pH at which this change occurs varies with site and between trunks and twigs

Species selected as nitrophytes and acidophytes

Using NIW and AIW species defined by van Herk, a good relationship was demonstrated between NH_3 concentrations and bark pH at Thetford and at North Wyke. For nitrophytes this was due to the occurrence of widespread species such as: *Physcia adscendens* (Fr.) H. Olivier, *P. tenella* (Scop.) DC, *Xanthoria parietina* (L.) Th. Fr. and *X. polycarpa* (Hoffm.) Th. Fr. ex Rieber. However only nine NIW species were recorded at Thetford and only six at North Wyke. Several of the nitrophyte species identified in the Netherlands, such as *Candelariella aurella* (Hoffm.) Zahlbr., *Physcia caesia* (Hoffm.) Fürnr. and *P. dubia* (Hoffm.) Lettau and *Xanthoria calcicola* Oxner are rarely found on trees in the UK. In the UK these species are associated with calcareous rocks and man-made substrata. Their appearance on trees is associated with an increase in bark pH, which has been noted in the Netherlands (van Herk 2002). Although nitrophyte species may favour basic substrata there are several nitrophytes that grow well on acid substrata, for example *Xanthoria* spp. Recent work in London has demonstrated low pH values for oak trees despite the presence of nitrophyte species appearing in these areas. (R. S. Larsen *et al.* in press). Further work is necessary to understand the relationship of nitrophytes with pH.

Of the acidophytes only four AIW species were recorded at Thetford whereas 12 were recorded at North Wyke. Lichens in the acidophyte category include species that are rare on tree trunks in the UK (*Tuckermannopsis chlorophylla* (Willd.) Hale), those that occur on other substrata with a low pH such as lignin (*Trapeliopsis flexuosa* (Fr.) Coppins & P. James), earth or lignin (*Placynthiella icmalea* (Ach.) Coppins & P. James) and recently burnt heathland (*Trapeliopsis granulosa* (Hoffm.) Lumbsch). The list includes species of acid bark that are tolerant of SO_2 (*Lecanora conizaeoides* Nyl. ex Cromb.) and species that are highly sensitive such as *Usnea* spp., the latter being characteristic of acid bark in unpolluted areas, especially oceanic areas with a high rainfall. Other species that are candidates being designated for nitrophytes or acidophytes in Britain include species that are not included in the van Herk list such as *Diploicia canescens* (Dicks.) A. Massal., *Lecanora expallens* Ach. (nitrophytes), and *Dimerella pineti* (Ach.) Vězda and *Lecanora carpinea* (L.) Vain. (acidophytes). Further testing in Britain will determine appropriate indicator species.

Meanwhile this small data set has shown that the occurrence of widespread nitrophytes and acidophytes is related to increasing bark pH which appears to increase NIW and decreases AIW. Although the loss of acidophytes may be attributed to factors other than increasing ammonia (van Herk *et al.* 2002), the correlation between NIW, AIW and ammonia concentration at Thetford suggests that ammonia has a significant role to play in the shift from acidophyte to nitrophyte species.

Conclusions

The use of lichen indicators of acidophyte and nitrophyte epiphytic communities provides a useful tool for assessing changes associated with increased emissions of ammonia. The correlations obtained using indicators defined for the Netherlands depend on a few widespread species in both categories. Indicator species defined for continental conditions may be absent from acidophyte and nitrophyte associations in Britain and replaced by other species. Further analysis of more sites from a range of conditions is required in order to define appropriate indicators for Britain.

Lichens on twigs are a barometer of current atmospheric conditions whereas lichens on trunks may carry a relict flora reflecting previous conditions; in Thetford of SO_2 deposition and in North Wyke of ecological continuity. Invasive nitrophytes are associated with a higher bark pH and in both sites the pH recorded on twigs was consistently higher than that of trunks thus allowing rapid colonization by nitrophyte species. The presence of acidophytes on the trunks at North Wyke and their absence on twigs suggests that the lichens on twigs are responding more rapidly to current levels of ammonia. Where former SO_2 pollution created a lichen desert, invasion of younger substrata is occurring associated with an increase in bark pH. Although bark pH can be used as a guide to ongoing changes in atmospheric conditions in local areas other factors are also influencing the shift from acidophytes to nitrophytes. In high rainfall, oceanic areas of Britain where there is a source of ammonia sensitive acidophytes may persist on the trunk together with a low frequency of nitrophytes, while on twigs acidophytes are absent and nitrophytes frequent. In both sites the difference between the lichen floras of trunks and twigs allows an evaluation of the response of the lichen flora to changes in levels of ammonia concentration. This research has indicated that the loss of acidophytes and other sensitive species is occurring at lower ammonia concentrations than previously suggested, and that this could be applied in other sites with a rich lichen flora as a predictive tool in order to detect ongoing environmental changes associated with surrounding land management.

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