Lichenologist **33**(5): 419–441 (2001)
doi:10.1006/lich.2001.0337, available online at http://www.idealibrary.com on IDEAL®

Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time

C. M. van HERK

Abstract: The lichen composition on wayside *Quercus robur* in the Netherlands was related to bark properties (pH, EC, NH_4^+, SO_4^{2-}, NO_3^-) and levels of air pollution (SO_2 and NH_3). The pH of the bark and the susceptibility to toxic substances appear to be the two major primary factors affecting epiphytic lichen composition. These factors have independent effects on the lichen composition. Most of the so-called nitrophytic species appear to have a low sensitivity to toxic effects of SO_2; their only requirement being a high bark pH. An increased bark pH appears to be the primary cause of the enormous increase in nitrophytic species and the disappearance of acidophytic species over the last decade in the Netherlands. Measurements of ambient NH_3 concentrations in air show that there is a nearly linear relationship between the NH_3 concentration and the abundance of nitrophytes on *Quercus*. The abundance of nitrophytes was not correlated with SO_2 concentrations. Most of the acidophytic species appear very sensitive to NH_3 since in areas with concentrations of 35 $\mu g\ m^{-3}$ or more, all acidophytic species have disappeared. Current methods using species diversity to estimate or monitor SO_2 air pollution need some modification, otherwise the air quality may be erroneously considered to be relatively good in areas with high NH_3 levels.

Introduction

During the last century numerous studies have successfully used epiphytic lichens to estimate sulphur dioxide (SO_2) air pollution levels. Several methods were introduced, such as de Sloover & Leblanc's (1968) Indices of Atmospheric Purity and Hawksworth & Rose's (1970) qualitative scale. In recent decades, however, with decreasing SO_2 concentrations and other factors apparently becoming more important, species diversity counts and distribution patterns no longer demonstrate a clear correlation with SO_2 (Seaward 1997).

The effect of SO_2 air pollution on epiphytic lichens is well-known and the number of published and unpublished studies on this topic is very large. During the second half of the last century, several sensitivity scales were introduced for a number of epiphytic lichen species (e.g. Barkman 1958; Hawksworth & Rose 1970; de Wit 1976). Hawksworth & Rose related their scale to absolute SO_2 concentrations, indicating that the toxic nature of SO_2 is probably the primary factor affecting lichen species rather than acidified bark. Several workers attempted to find a physiological basis for the drastic effects of SO_2 on lichens. The sensitivity of lichen species from acid bark, tested in the laboratory for their respiratory responses to sulphur dioxide, appeared to correlate well with the data on field sensitivity. For species from eutrophicated bark, however, the correlation appeared to be poor (Baddeley *et al.* 1972; Ferry & Coppins 1979).

Unlike the toxic effects of SO_2, acidity is a natural phenomenon. It proves to be highly

C. M. van Herk: Lichenologisch Onderzoekbureau Nederland, Goudvink 47, NL-3766 WK Soest, The Netherlands.

0024-2829/01/050419+23 $35.00/0

influential to species composition and is probably the most important factor determining the natural lichen floras of tree species (Bates & Brown 1981). Many studies have shown a switch of lichen species from trees with acid bark to trees with a normally neutral bark in the presence of increasing SO_2 pollution. Harmful effects of the acid nature of SO_2 resulting in a very acid bark with only a few lichen species have also been reported. A low bark pH, however, is not the principal reason for a poor lichen flora because in areas with low SO_2 air pollution levels, well developed lichen communities may occur at a pH of 4 or even lower (Gauslaa 1985). Damage by SO_2 to lichen species appears to be more severe at low pH values (Türk & Wirth 1975). In addition to this, many acidophytic species seem to be more susceptible to toxic effects of SO_2 than species preferring eutrophic conditions. Gilbert (1976) described an SO_2-polluted environment with *Fraxinus* trees having a very acid bark (pH 3) and only few lichens. In the surroundings of a limestone quarry the situation appeared to be quite different due to alkaline dust. Here a *Xanthorion* occurred with more than 20 species at a bark pH of more than 6. A species-poor situation apparently occurred only at high SO_2 levels in combination with low pH values.

More recently, ammonia (NH_3) air pollution caused by intensive cattle breeding has been recognized as an important factor affecting epiphytic lichen vegetation, first and principally in the Netherlands (van der Knaap 1984; de Bakker & van Dobben 1988; van Herk 1990), but to a lesser extent also in other European countries such as Denmark (Søchting 1991), Italy (Nimis *et al.* 1991), Spain (Berdowski & Aptroot 1991), Belgium (Hoffmann 1993), Great Britain (Benfield 1994; Wolseley & Pryor 1999), Switzerland (Ruoss 1999) and Germany (Zimmer 2000). The epiphytic lichens of large parts of the Netherlands have already been mapped to investigate the effects of NH_3 (van Herk 1999). Most authors observed a positive response of nitrophytic species to NH_3, usually combined with a negative response of acidophytic species.

Although a response of nitrophytic species to ammonia air pollution may be a relatively new and increasingly important phenomenon, the influence of nitrogen in general has been known for a long time and discussed by many authors. Barkman (1958) summarized the most important circumstances encouraging nitrophytic species: (1) bark wounds; (2) salt spray; (3) dust; and (4) dung from birds, cattle and dogs. Other agricultural practices that have an impact on lichens have been discussed by Brown (1992). According to Nienburg (1919) the ammonium (NH_4^+) content of sources encouraging nitrophytes is more important than the nitrate (NO_3^-) content. As a consequence, Räsänen (1927) suggested the adjective 'ammoniophytic' instead of 'nitrophytic'. Recent measurements of bark pH, however, suggest that the effect of airborne ammonia on nitrophytic lichens is probably caused by producing high pH values rather than an increased availability of ammonium on the bark (de Bakker & van Dobben 1988; van Herk 1990). This is supported by field observations since species that react to alkaline dust (Gilbert 1976) are largely the same as those now reacting positively to ammonia (van Herk 1999), i.e., mainly *Xanthorion* species.

Van Dobben & ter Braak (1999) calculated a sensitivity scale to NH_3, calibrated with abiotic data from the Dutch Air Quality Network (Asman & van Jaarsveld 1990*a*). On their scale, not only the usual *Xanthorion* species such as *Xanthoria parietina* and *Phaeophyscia orbicularis* are recognized as reacting to ammonia, but surprisingly the *Lecanorion carpineae* species of neutral to slightly basic bark such as *Lecanora carpinea*, *Lecidella elaeochroma* and *Cliostomum griffithii* yielded also very high NH_3 indicator values. These latter species are most often not considered as nitrophytic (see for example Wirth 1991). This seeming paradox can be explained by hypothesizing that in the Dutch situation nitrogen sources are not limiting growth because there is an excess available. In such an environment, acidity is probably the limiting factor for the growth of nitrophytic species and the difference between species needing nitrogen (*Xanthorion*) and species tolerating nitrogen (*Lecanorion carpineae*) may not be detectable. Therefore, the species reacting positively to ammonia on the scale of van Dobben & ter Braak are primarily basiphytic species.

The effect of nitrogen oxides (NO_x), mainly emitted by road traffic, on lichens remains poorly understood. In some recent studies an increase in nitrophytic species in urban areas has been observed, which was attributed to NO_x. Van Dobben & ter Braak (1999) went as far as to calculate a sensitivity scale for NO_x. However, attempts to find correlations between lichen distribution and NO_x concentrations may be confounded by strong positive correlations between SO_2 and NO_x concentrations. Accordingly, more fundamental work is probably necessary to decide whether NO_x encourages or is harmful to certain lichen species.

This paper is based on a statistical treatment of data on lichen community composition, chemical properties of bark, and SO_2 and NH_3 air pollution. Results of this work were first described in some internal Dutch reports (van Herk 1990, 1991). A more or less similar approach was chosen by van Dobben & Wamelink (1992) and Hoffmann (1993). In 1996 field measurements of NH_3 became feasible and were carried out at selected lichen monitoring sites (van Herk 1998). This paper integrates data on bark properties, measurements of ambient NH_3 concentrations in air and long-term monitoring of epiphytic lichens on wayside trees. It supplements an earlier paper in which a mapping programme on NH_3 effects was reported (van Herk 1999). The aims of this paper are (1) to discuss the effects of different air pollutants on lichen composition, (2) to find primary factors affecting the lichen community composition, (3) to evaluate bioindicators for NH_3 proposed earlier (NIW and AIW in van Herk 1999), and (4) to find an explanation for observed changes in lichen composition with time.

Multivariate analysis is used to gain an insight into the relative influence of several sources of air pollution and bark properties as possible intermediate factors. Evidence will be provided for the increasing importance of ammonia as an air pollutant.

Materials and Methods

Lichen recording

Each monitoring site consists of 10 wayside *Quercus robur* trees on which all lichen species were recorded. Lichens were identified to species level where possible using standard microscopic techniques and spot tests for their chemistry. Nomenclature follows Aptroot *et al.* (1999). The following abundance scale was used: (1) only one thallus present; (2) more thalli present on one tree; (3) present on 2–5 trees, less than 1dm^2 per tree; (4) present on 2–5 trees, more than 1dm^2 per tree; (5) present on 6–10 trees, less than 1dm^2 per tree; (6) present on 6–10 trees, more than 1dm^2 per tree. From 1989 onwards approximately 6000 sites were surveyed. The sites are widely distributed over areas with acid sandy soils (poor in nutrients and calcium) in the eastern parts of the Netherlands (Fig. 1A). Approximately 3500 of these sites continue to be involved in a monitoring programme with re-assessment at approximately 5 yearly intervals.

At each site abundance of nitrophytic species was taken as a measure for species reacting positively to NH_3 and abundance of acidophytic species was taken as a measure for species sensitive to NH_3. Both species groups were united into the integrated parameters, NIW (derived from Dutch 'Nitrofiele Indicatie Waarde') and AIW ('Acidofiele Indicatie Waarde') respectively, both calculated in accordance with van Herk (1999). NIW and AIW are defined as the mean number of nitrophytic and acidophytic species respectively, found on one tree. Species covering more than 1 dm^2 (class 4 and 6 on the abundance scale), however, were counted twice (i.e. 2·0 points when present on all trees). Thus, common species present on all trees add much more to NIW or AIW scores than species present in small numbers on one out of ten trees only (i.e. 0·1 point).

The following species were considered as nitrophytes and used to calculate the NIW: *Caloplaca citrina*, *C. holocarpa*, *Candelariella aurella*, *C. reflexa*, *C. vitellina*, *C. xanthostigma*, *Lecanora muralis*, *L. dispersa* s.lat. (incl. *L. hagenii*), *Phaeophyscia orbicularis*, *P. nigricans*, *Physcia adscendens*, *P. caesia*, *P. dubia*, *P. tenella*, *Rinodina gennarii*, *Xanthoria candelaria*, *X. calcicola*, *X. parietina* and *X. polycarpa*. The species used to calculate the AIW were *Cetraria chlorophylla*, *Chaenotheca ferruginea*, *Cladonia* spp. (all species taken together), *Evernia prunastri*, *Hypocenomyce scalaris*, *Hypogymnia physodes*, *H. tubulosa*, *Lecanora aitema*, *L. conizaeoides*, *L. pulicaris*, *Le-praria incana*, *Ochrolechia microstictoides*, *Parmelia saxatilis*, *Parmeliopsis ambigua*, *Placynthiella icmalea*, *Platismatia glauca*, *Pseudevernia furfuracea*, *Trapeliopsis flexuosa*, *T. granulosa* and *Usnea* spp. (all species taken together).

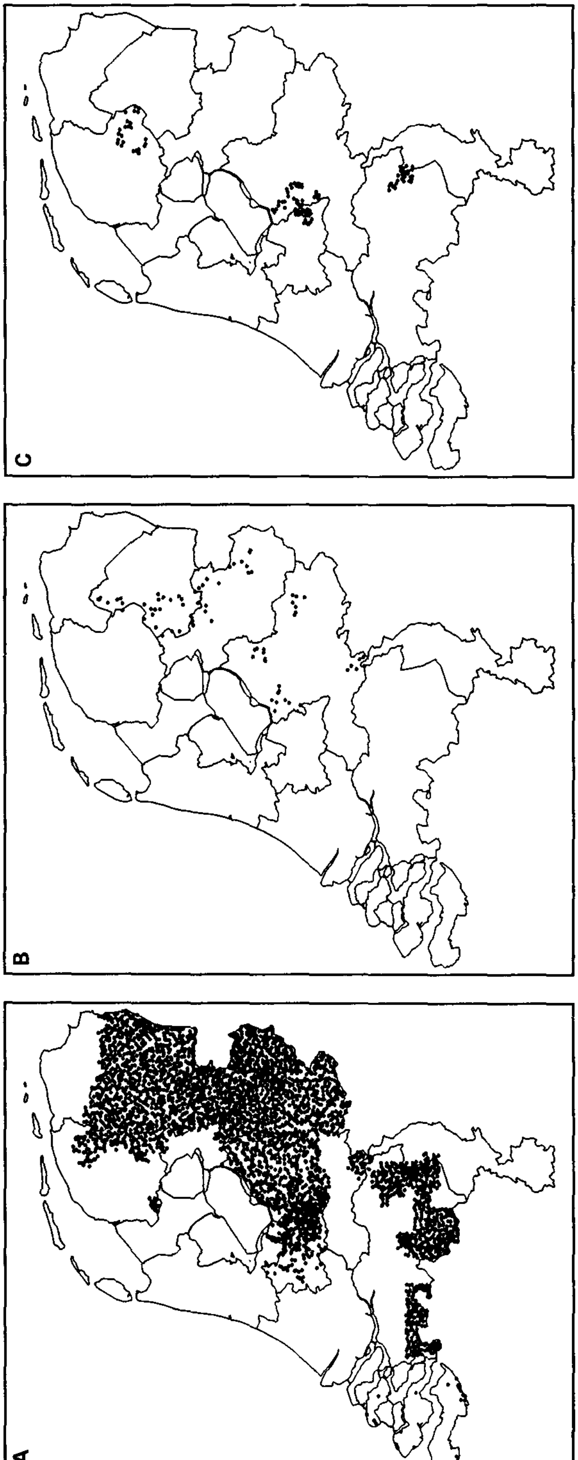


FIG. 1. A, lichen survey sites with *Quercus robur* as phorophyte in the Netherlands; B, sites where bark properties were also determined; C, sites where NH_3 concentrations in air were also determined.

Bark samples

Bark samples were collected from 76 lichen monitoring sites (Fig. 1B) in October 1989 from a wide range of habitats: from areas with mainly arable land (5), from moderately (31) to very intensively (21) used agricultural areas, villages (6), towns (6) and forests (7). The number of sites per habitat reflect its relative abundance. Only standard *Quercus robur* wayside plots were sampled and, wherever possible, only trees from exposed and well-illuminated sites. Within these restrictions, the sampled sites were selected at random in the area mapped for lichens in 1989 (van Herk 1990).

Bark slices *c.* 3 mm thick were taken from the southwest side of the trunk of all 10 trees at a height of 1·5 m (*c.* 20 g bark in total per site). Sampling was undertaken under dry, uniform weather conditions over a period of four days and each sample was stored in a separate plastic bag. In the laboratory the samples were dried at 20°C for two weeks, then ground with knife and grinder to <3 mm and 5·00 g of each suspended in 50 ml distilled water. The suspensions were shaken for one hour, left to stand for one day, then shaken again for one hour and centrifuged. The supernatant was analysed for electrical conductivity (EC) and pH using appropriate digital measuring instruments with platinum and glass electrodes, respectively. Soluble NH_4^+, SO_4^{2-} and NO_3^- were measured spectrophotometrically; NH_4^+ colorimetrically using Nessler's reagent, SO_4^{2-} turbidimetrically using Sulfaver—4 reagent and NO_3^- colorimetrically using Nitraver—4 reagent (only from 36 sites), all according to the Hach Kit Users Manual.

Air pollution

Measurement of the NH_3 concentration in air was performed *in situ* at 104 lichen monitoring sites (Fig. 1C) and carried out by the Dutch Institute for Technical Research, TNO (Duyzer *et al.* 1997). The records were taken during one year (February 1996 to January 1997) using passive samplers (diffusion tubes) specially developed by GRADKO Co. Inc. and described by Duyzer *et al.* (1998). These tubes were suspended monthly from the trunks of the monitored trees. Monthly measured values were used to calculate the mean annual NH_3 concentration in air for the whole period. This method measures the NH_3 concentration only; the NH_4^+ concentration is not recorded. The selection of the sampling sites and areas (Brabant, Gelderse Vallei and Friesland) was mainly determined by the Dutch government, practically all sites being standard wayside plots in agricultural areas.

Estimates of NH_3 and NH_4^+ concentrations (μg m^{-3}, 1988) were produced by the Dutch Air Quality Network and utilising a 5 × 5 km^2 grid census of cattle density and the atmospheric transport model TREND (Asman & van Jaarsveld 1990*a*). Ammonia concentrations range from 3 to 17 μg m^{-3} and ammonium concentrations from 4 to 7 μg m^{-3}. Most of the ammonium is actually deposited as ammonium sulphate [$(NH_4)_2SO_4$], which largely originates from an atmospheric chemical reaction between NH_3 and SO_4^{2-} (Asman & van Jaarsveld 1990*b*).

Data on SO_2 air pollution were also obtained from the Dutch Air Quality Network (Anonymous 1989–1993). SO_2 concentrations in air (μg m^{-3}, 1988) and deposition values (mol ha^{-1}, 1992) were provided per grid square of 5 × 5 km^2 for the whole of the Netherlands, using hourly measured concentrations at SO_2 monitoring stations. Concentrations range from *c.* 8 μg m^{-3} in clean areas to *c.* 12·5 μg m^{-3} in relatively polluted sites. Deposition data range from 420 to 690 mol/ha.

Girth of the sampled trees was recorded. Exposures to wind, rain and sunlight were estimated at the sites where ammonia measurements were taken using a six point scale (van Herk 1999).

Data processing

All species and abiotic data from the 76 sites with bark samples were entered into a database, and subjected to the ordination technique Canonical Correspondence Analysis (CCA) (Jongman *et al.* 1995). This was carried out with CANOCO, version 3·12 (ter Braak 1987, 1991). Tree girth was treated as a co-variable in order to exclude variation in lichen composition which has no relation to air pollution. Biplots are given with species scores and arrows of abiotic variables. Also biplots are given with sample scores and abundance values for selected species. Multiple regression analysis was performed to separate the effects of several air pollution-related factors.

The species and abiotic data from the 104 sites with NH_3 air concentration measurements were also entered into a database. Again regression analysis was carried out to separate the effects of ammonia, sulphur dioxide, tree girth and the degree of exposure.

Species data from *c.* 3500 monitoring sites were used to calculate changes in occurrence between 1989 and 1999 for three selected species, viz. *Phaeophyscia orbicularis*, *Hypogymnia physodes* and *Ramalina farinacea* and three parameters, viz. the Acidofiele Indicatie Waarde (AIW), the Nitrofiele Indicatie Waarde (NIW) and the number of lichen species per site.

Results

Bark properties and species data

All data on bark properties at the 76 investigated sites are shown in Table 1. The pH of the bark appears to be low in forests (3·65–4·40) and towns (3·80–4·95), and unusually high in some sites in intensively used agricultural areas (5·40–6·40). The lowest pH (3·65) was found in the only site with some old woodland species (Norgerholt with *Enterographa crassa*, *Lecanactis abietina*, *Thelotrema lepadinum*, Table 2). Also other

TABLE 1. *Bark properties of 76 selected sites with* Quercus robur *in the Netherlands*

Habitat type	Grid ref*	Locality	pH	EC ($\mu S\ cm^{-1}$)	NH_4^+ ($mg\ g^{-1}$)	SO_4^{2-} ($mg\ g^{-1}$)	NO_3^- ($mg\ g^{-1}$)
Predominantly arable land							
	1231	Veenhuizen	4·60	233	0·74	0·100	0·88
	1232	Zuidvelde	4·85	331	0·89	0·075	1·06
	1711	Smilde	4·90	257	0·95	0·075	0·88
	1721	Geeuwenbrug	4·60	216	0·75	0·050	0·88
	1732	Spier	4·60	209	0·76	0·075	0·97
	Average		**4·71**	**249**	**0·82**	**0·075**	**0·93**
Moderately used agricultural area							
	0752	Roderwolde	5·25	303	0·99	0·100	1·06
	1212	Foxwolde	5·15	370	0·95	0·100	0·79
	1212	Peize	4·95	220	0·79	0·075	0·88
	1221	Roderesch	5·15	193	0·81	0·025	0·79
	1636	Eesveen	5·05	244	0·77	0·050	0·88
	1646	Havelterberg	5·10	216	0·66	0·075	0·88
	1722	Ter Horst	5·50	264	0·80	0·050	0·97
	1732	Lheebroek	5·20	182	0·70	0·100	1·06
	1742	Pesse	5·10	311	1·01	0·050	1·14
	1752	Hoogeveen I	5·05	185	0·72	0·025	—
	2117	Halfweg	5·50	204	0·76	0·075	—
	2118	De Wijk	5·40	234	0·76	0·100	—
	2127	Staphorst	5·65	202	0·90	0·100	—
	2138	Balkbrug	5·50	302	0·90	0·050	—
	2147	Ruitenveen	5·45	320	1·11	0·025	—
	2223	Braamberg	5·40	282	0·87	0·050	—
	2231	Ommerschans	5·30	332	0·82	0·075	—
	2743	Epe	4·50	275	0·69	0·025	—
	2744	Boshoek	5·10	210	0·69	0·100	—
	2744	Wijnbergen	5·50	232	0·95	0·125	—
	2753	Vaassen II	5·00	282	0·70	0·050	1·14
	2754	Vaassen III	5·25	358	0·86	0·025	1·06
	2754	Vaassen IV	5·50	384	1·02	0·075	1·06
	2826	Tubbergen	5·20	287	1·08	0·025	—
	2837	Het Stift	5·30	230	0·66	0·100	—
	3215	Nijkerk	5·00	429	1·11	0·100	—
	3338	Warken	5·25	319	0·95	0·100	—
	3348	Vorden	5·10	334	0·90	0·100	—
	3441	Mossel	5·45	228	0·81	0·075	—
	4613	Groesbeek	4·95	306	1·01	0·100	0·88
	4623	Bredeweg	5·75	193	0·99	0·050	—
	Average		**5·24**	**272**	**0·86**	**0·068**	**0·97**
Intensively used agricultural area							
	1231	Nieuw Weper	6·30	245	1·34	0·150	1·14
	1618	Wateren	5·95	290	0·93	0·100	1·06
	1656	Nijeveen	5·70	417	0·90	0·100	0·70
	1721	Leggeloo	5·50	251	0·90	0·100	1·23
	2221	Nolde	5·70	240	0·83	0·075	—
	2234	Lutten	5·50	485	1·30	0·025	—
	2234	Hardenberg I	5·75	284	0·96	0·050	—
	2234	Hardenberg II	5·70	435	1·18	0·025	—
	2254	Bergentheim	5·80	293	0·82	0·025	—

Continued

TABLE 1. *Continued*

Habitat type	Grid ref*	Locality	pH	EC ($\mu S\ cm^{-1}$)	NH_4^+ ($mg\ g^{-1}$)	SO_4^{2-} ($mg\ g^{-1}$)	NO_3^- ($mg\ g^{-1}$)
Intensively used agricultural area							
	2816	Langeveen	5·70	279	0·95	0·025	—
	2816	Veldhoek	5·80	304	1·00	0·075	—
	2836	Mariaparochie	5·40	270	1·01	0·125	—
	2838	Weerselo	5·85	254	1·13	0·150	—
	3216	Putten	5·80	248	1·29	0·150	—
	3216	Gerven	6·00	203	0·99	0·100	—
	3236	Barneveld	6·20	297	1·02	0·075	1·14
	3246	Renswoude	6·40	256	1·23	0·050	1·23
	3431	Lochem	5·55	347	0·80	0·100	—
	3432	Zwiep	5·80	215	0·73	0·025	—
	3442	Boschheurne	5·50	326	0·80	0·075	—
	3452	Ruurlo	5·60	195	0·61	0·025	—
	Average		**5·79**	**292**	**0·99**	**0·074**	**1·08**
Forest							
	1232	Norgerholt	3·65	310	0·53	0·100	0·70
	1628	Diever I	4·05	238	0·55	0·075	0·62
	1628	Diever II	3·80	162	0·36	0·100	0·35
	2753	Vaassen I	3·80	215	0·48	0·050	0·70
	3217	Koudhoorn	4·35	102	0·34	0·100	—
	3227	Boeschoten	4·40	140	0·41	0·050	—
	3228	Stroe	4·30	225	0·52	0·100	—
	Average		**4·05**	**199**	**0·46**	**0·082**	**0·59**
Village							
	1627	Vledder	4·80	315	0·89	0·100	1·14
	1731	Dwingeloo	5·10	370	1·10	0·050	1·14
	1751	Echten	5·30	240	0·79	0·100	1·06
	2223	Slagharen	5·35	284	0·76	0·050	—
	3226	Voorthuizen	5·50	532	1·25	0·900	1·14
	4622	Molenhoek	4·90	426	1·03	0·025	0·97
	Average		**5·16**	**361**	**0·97**	**0·200**	**1·09**
Town							
	1752	Hoogeveen II	4·95	172	0·63	0·050	—
	2835	Almelo I	4·15	900	1·52	2·500	—
	2835	Almelo II	5·15	175	1·09	0·100	—
	4052	Nijmegen I	4·45	480	1·16	0·850	0·88
	4052	Nijmegen II	3·80	933	1·51	6·500	1·06
	4612	Nijmegen III	3·90	573	1·52	2·250	0·79
	Average		**4·40**	**539**	**1·24**	**2·041**	**0·91**

*Dutch Topographic System.

forest sites (Diever II, Vaassen I) with a low pH (3·80) have a well-developed and lush lichen flora with *Platismatia glauca*, *Pseudevernia furfuracea*, *Ochrolechia microstictoides* and even *Leproloma membranaceum*. Urban sites with a similar pH (3·80–3·90) such as Nijmegen II and III, however, have a very impoverished flora with only three species each, *Lecanora conizaeoides* being the most abundant. An important difference in bark properties between these rich forest sites and poor urban sites was found to be the sulphate (SO_4^{2-}) content, which is extremely high in some urban areas (2·25–6·50 mg

TABLE 2. *Species composition at 76 selected sites with* Quercus robur *in the Netherlands where bark samples were taken (Table 1). The sites are arranged according to 6 different habitat types*

	Habitat type*						
	ara	**mod**	**int**	**for**	**vil**	**twn**	**tot**
Number of sites	5	31	21	7	6	6	76
Species							
Anaptychia ciliaris	0	0	0	0	1	0	1
Arthonia radiata	0	0	2	0	0	0	2
Buellia griseovirens	5	27	12	0	4	1	49
B. punctata	5	30	21	0	6	4	66
Candelaria concolor	0	2	4	0	0	0	6
Candelariella reflexa	0	2	12	0	3	0	17
C. vitellina	0	11	15	0	3	1	30
C. xanthostigma	0	8	7	0	1	0	16
Cetraria chlorophylla	4	0	0	0	0	0	4
Chaenotheca chrysocephala	0	0	0	1	0	0	1
C. ferruginea	0	0	0	5	0	1	6
Chrysothrix candelaris	0	0	0	1	0	0	1
Cladonia sp.	2	9	1	7	3	0	22
Dimerella pineti	0	1	0	3	0	0	4
Diploicia canescens	0	0	2	0	1	0	3
Enterographa crassa	0	0	0	1	0	0	1
Evernia prunastri	5	29	12	5	5	3	59
Haematomma ochroleucum	1	9	5	1	3	0	19
Hypogymnia physodes	4	19	5	6	4	3	41
H. tubulosa	1	4	0	1	2	0	8
Hypocenomyce scalaris	5	7	1	6	1	1	21
Lecanactis abietina	0	0	0	1	0	0	1
Lecanora aitema	3	3	0	0	0	0	6
L. argentata	0	1	0	0	0	0	1
L. carpinea	0	3	4	0	0	0	7
L. chlarotera	2	21	19	0	5	1	48
L. conizaeoides	5	15	0	6	2	5	33
L. dispersa s. lat.	0	10	8	0	0	5	23
L. expallens	5	31	21	7	6	2	72
L. muralis	0	1	0	0	0	1	2
L. pulicaris	5	13	2	2	1	1	24
L. symmicta	1	4	6	0	0	1	12
Lecidella elaeochroma	0	9	15	0	1	0	25
Lepraria incana	5	27	16	7	6	3	64
Leproloma membranaceum	0	0	0	1	0	0	1
Ochrolechia androgyna	1	11	1	1	3	1	18
O. microstictoides	2	0	0	1	0	0	3
Parmelia acetabulum	5	16	9	0	4	1	35
P. caperata	1	7	1	0	0	1	10
P. elegantula	2	1	0	0	1	0	4
P. exasperatula	0	3	7	0	1	0	11
P. glabratula	2	3	0	0	0	0	5
P. laciniatula	3	3	0	0	3	1	10
P. revoluta	2	14	0	2	0	1	19
P. saxatilis	3	4	2	4	0	0	13
P. subaurifera	5	26	16	0	4	2	53
P. subrudecta s. lat.	3	23	11	1	4	1	32
P. sulcata	5	30	21	5	5	3	69
P. tiliacea	0	0	0	0	1	0	1

Continued

TABLE 2. *Continued*

	Habitat type*						
	ara	**mod**	**int**	**for**	**vil**	**twn**	**tot**
Number of sites	5	31	21	7	6	6	76
Species							
Parmeliopsis ambigua	0	0	0	3	0	0	3
Pertusaria albescens	0	2	0	0	0	0	2
P. amara	3	11	2	2	4	1	23
P. coccodes	2	9	1	0	4	0	16
P. pertusa	2	6	0	1	3	0	12
Phaeophyscia orbicularis	0	6	11	0	1	2	20
Phlyctis argena	3	16	4	0	3	1	27
Physcia adscendens	1	10	16	0	2	2	31
P. caesia	1	8	11	0	4	3	27
P. dubia	0	8	11	0	1	0	20
P. tenella	4	30	21	0	5	4	64
Physconia grisea	0	1	4	0	1	0	6
Placynthiella icmalea	0	2	1	0	0	0	3
Platismatia glauca	0	0	0	3	0	0	3
Protoparmelia hypotremella	0	2	0	0	0	1	3
Pseudevernia furfuracea	4	6	0	3	1	1	15
Pyrrhospora quernea	2	8	3	0	4	1	18
Ramalina farinacea	5	20	12	0	4	1	42
R. fastigiata	3	14	13	0	5	1	36
R. fraxinea	0	3	1	0	0	0	4
Thelotrema lepadinum	0	0	0	1	0	0	1
Trapeliopsis granulosa	0	1	0	1	0	0	2
Usnea sp.	1	3	0	0	0	2	6
Xanthoria calcicola	0	0	1	0	0	0	1
X. candelaria	1	26	19	0	5	1	52
X. parietina	1	18	20	0	4	2	45
X. polycarpa	5	30	21	0	5	3	64

***ara**=predominantly arable land, **mod**=moderately used agricultural areas, **int**=intensively used agricultural areas, **for**=forest, **vil**=village, **twn**=town, **tot**=total). The figures given represent the number of sites where a species is present.

g^{-1}) and scarcely detectable in many other places (0·05–0·10 mg g^{-1}). Apparently a low pH alone does not provide an explanation for a very impoverished lichen flora, a combination with other factors is decisive.

Sites with a very high pH in intensively used agricultural areas (e.g. 6·20–6·40 in Nieuw Weper, Barneveld and Renswoude) invariably have lichen communities dominated by nitrophytic species such as *Candelariella reflexa*, *Lecanora dispersa*, *Physcia caesia*, *P. dubia*, *Phaeophyscia orbicularis* and *Xanthoria parietina*. The ammonium (NH_4^+) content of such sites is not markedly high (*c.* 1·13 mg g^{-1}), being only slightly higher than average values for arable land (0·82 mg g^{-1}), moderately used agricultural areas (0·86 mg g^{-1}) or intensively used agricultural areas (0·99 mg g^{-1}). The highest values for NH_4^+, however, were found in urban sites where at the same time high SO_4^{2-} values were found, like Nijmegen II and III and Almelo I. These figures suggest a high ammonium sulphate [$(NH_4)_2SO_4$] deposition in urban areas. The lowest bark NH_4^+ contents were found in forests.

Nitrate (NO_3^-) behaves more or less like NH_4^+ in rural areas, being lowest in forests

(0·59 mg g^{-1}), average in areas of arable land (0·93 mg g^{-1}) and moderately used agricultural areas (0·97 mg g^{-1}) and relatively high in intensively used agricultural areas (1·08 mg g^{-1}). In built-up areas NO_3^- appears especially high in villages (1·09 mg g^{-1}), rather than in towns (0·91 mg g^{-1}). The electrical conductivity (EC) is very high at some urban sites (500–900 μS cm^{-1}) where a very high SO_4^{2-} and NH_4^+ content was also found. Very low EC values (100–160 μS cm^{-1}) were found in some forests sites.

Ordination analysis

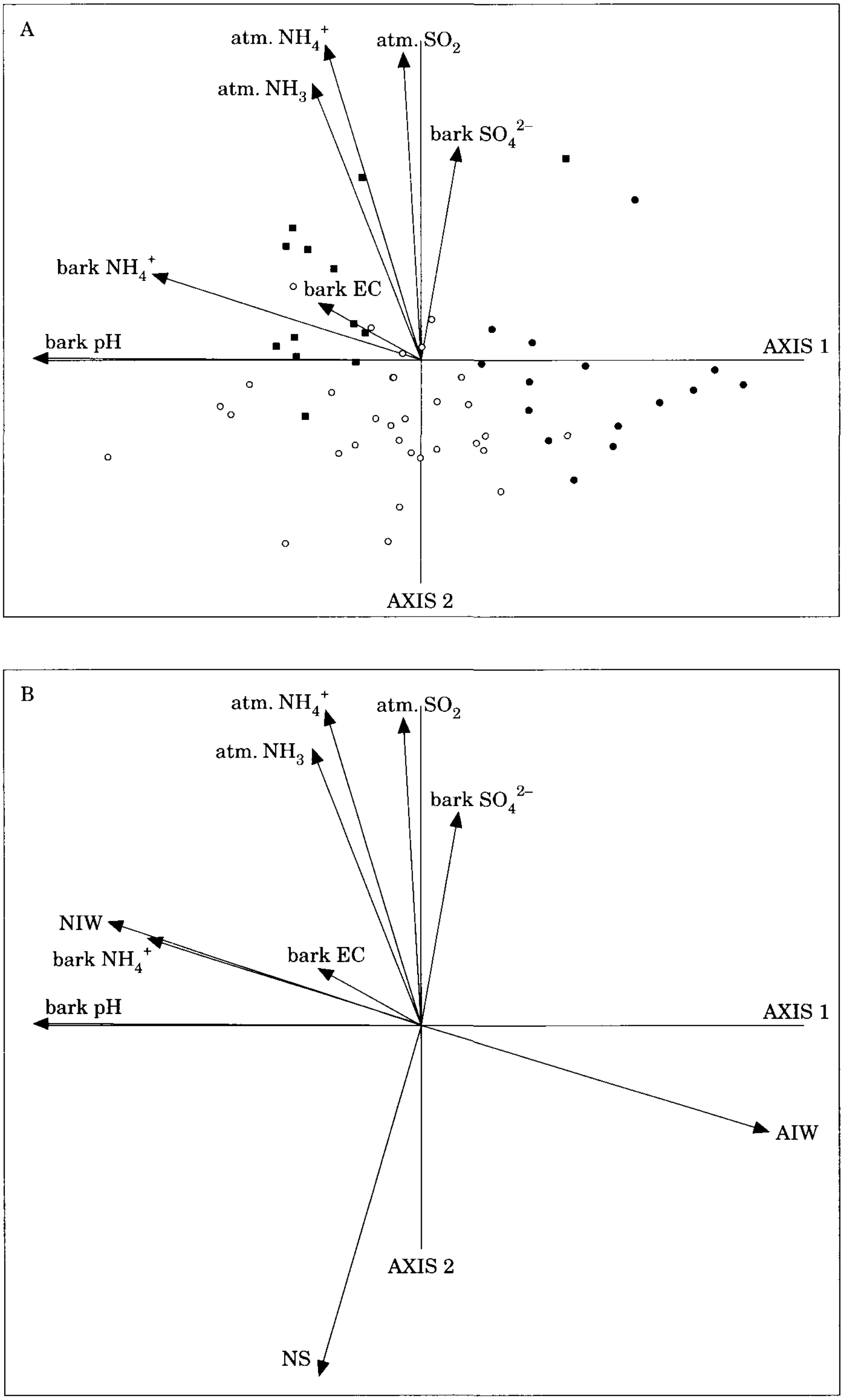
Biplots with results of the CCA ordination are given in Fig. 2 (centroïds of species) and Fig. 3 (sample scores). The length of each arrow shows to what extent an abiotic variable is recognizable in the two main components (axis 1 and axis 2) of the species composition. The first axis appears to be dominated by the bark pH ($r = -0{\cdot}89$, $P < 0{\cdot}0001$), the arrow coincides nearly entirely with axis 1 (Fig. 2). Bark NH_4^+ has also a high biplot score on axis 1 ($r = -0{\cdot}61$, $P < 00001$). Axis 2 has high biplot scores for several variables: atmospheric SO_2 ($r = +0{\cdot}63$, $P < 0{\cdot}0001$), atmospheric NH_4^+ ($r = +0{\cdot}63$, $P < 0{\cdot}0001$), atmospheric NH_3 ($r = +0{\cdot}55$, $P < 0{\cdot}0001$) and bark SO_4^{2-} ($r = +0{\cdot}41$, $P < 0{\cdot}0001$). The EC seems to be of minor importance for species reacting to axis 1 and 2. Bark NO_3^- is not represented in Fig. 2 due to missing values (Table 1).

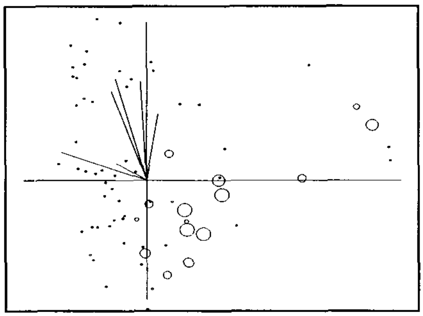
Regression analysis shows that bark pH is probably the primary factor affecting the species along axis 1 [76·7% variance (r^2)]. Bark NH_4^+ is able to add a small, but significant contribution, with both factors accounting for 81·4% of the variance. Axis 2 appears to be determined by atmospheric SO_2, atmospheric NH_4^+ and bark SO_4^{2-}, all three together explaining 70·4% of the variance. It is difficult to estimate the relative importance of SO_2, NH_4^+ and SO_4^{2-}. All three add a substantial part to the variance, but bark SO_4^{2-} is probably only important in some extremely polluted urban sites (Table 1). Atmospheric NH_3, although correlating with axis 2, adds no extra fit to this model. Thus, axis 1 is clearly identifiable as a response to acidity, while axis 2 can be identified as a response to pollution-stress. Apparently acidity and pollution-stress (susceptibility to toxic substances) behave as independent factors.

The positions of the centroïds of the species in Fig. 2A show that most of the acidophytic species are found in the right hand 'acid' part of the diagram, opposite the pH arrow. Nitrophytic species are found mainly in the left hand part above axis 1. This shows not only their preference for a high pH, but also suggests a toxitolerant behaviour, at least when ammonium is present. Most of the remaining species, found in the bottom part of the diagram, can be regarded as susceptible to toxic substances.

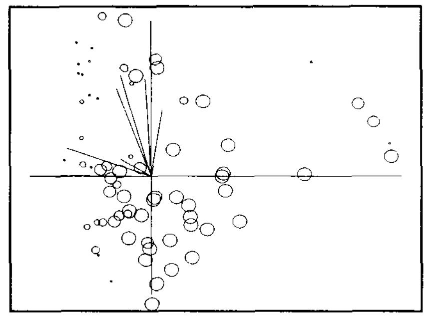
Figure 2B is similar to Fig. 2A, but instead of the centroïds of species, arrows of the number of lichen species per site (NS), the abundance of acidophytes (AIW) and the abundance of nitrophytes (NIW) are shown. All three were added as 'passive' variables. The number of species per site points to low scores on axis 2, demonstrating the susceptibility to toxic substances of many species. In addition to this, there is in general a slight increase in species diversity with higher pH values. As was to be

FIG. 2. Biplots of the Canonical Correspondence Analysis (CCA), using the 76 *Quercus* sites where bark samples were taken. Tree girth was entered as a co-variable. Eigenvalues: 0·323 (axis 1), 0·096 (axis 2), species–environment correlations: 0·929 (axis 1), 0·797 (axis 2). A, biplot with arrows for abiotic variables (atm.= air concentration, bark= bark content) and centroïds of lichen species (■=nitrophytic species, ●=acidophytic species, ○=remaining species), a few species with an extreme position are not shown; B, same as A but instead of lichen centroïds, now with the arrows for the abundance of nitrophytes (Nitrofiele Indicatie Waarde, NIW), the abundance of acidophytes (Acidofiele Indicatie Waarde, AIW) and the number of lichen species per site (NS), all three entered afterwards as passive variables.

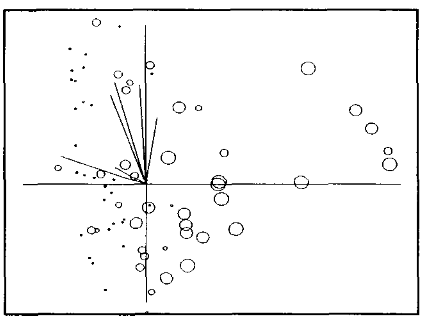

A
atm. NH4+
atm. SO2
atm. NH3
bark SO42−
bark NH4+
bark EC
bark pH
AXIS 1
AXIS 2
B
atm. NH4+
atm. SO2
atm. NH3
bark SO42−
NIW
bark NH4+
bark EC
bark pH
AXIS 1
AIW
AXIS 2
NS



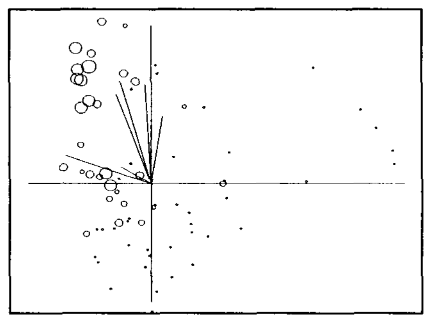
A. *Pseudevernia furfuracea*



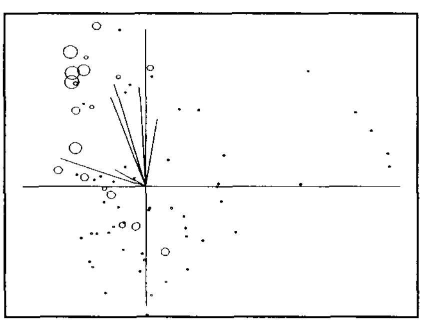
B. *Evernia prunastri*



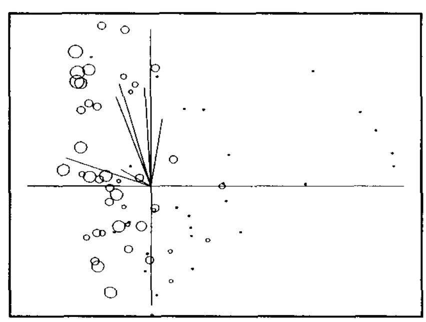
C. *Hypogymnia physodes*



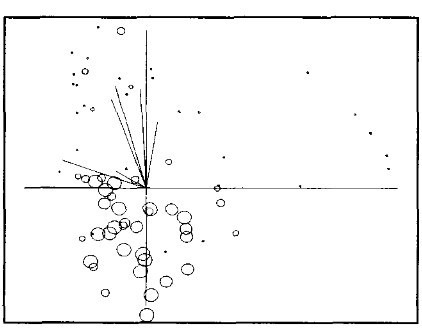
D. *Physcia adscendens*



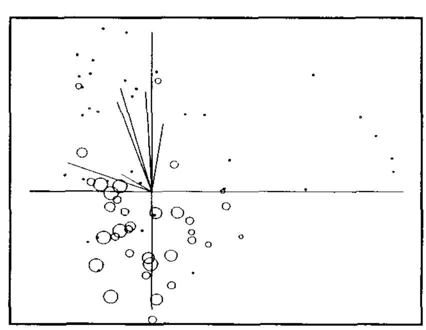
E. *Phaeophyscia orbicularis*



F. *Xanthoria parietina*



G. *Ramalina farinacea*



H. *Parmelia acetabulum*

expected, multiple regression shows significant effects of atmospheric SO_2 (negative) and bark pH (positive) on the species diversity, together accounting for 49·4% of the variance.

Acidophytic species not only show a preference for an acid environment (accounting for 44·7% of the variance), but a multiple regression with AIW as dependent variable shows that these lichens are also susceptible to atmospheric NH_4^+ and bark NH_4^+ (67·2% of variance for all three). Evidence to suggest that these species are susceptible to SO_2 or bark SO_4^{2-} was not found, perhaps because *Lecanora conizaeoides* was included in the AIW. Multiple regression with the NIW values showed a significant contribution only of bark pH (accounting for 53·6% of variance). None of the other variables improved the fit. If the insignificant positive effect of bark NH_4^+ ($P=0{\cdot}0967$, partial correlation) is incorporated into the model, the explained variance increased to 55·3%.

In Figure 3 biplots are shown from which the response of separate species to pH (axis 1) and toxic substances (axis 2) may be derived. Within the main ecological groups (acidophytic, nitrophytic and remaining species, mainly 'neutrophytic' species) several specific response patterns can be distinguished. For instance, *Pseudevernia furfuracea* (Fig. 3A) is strictly acidophytic and toxiphobous, while *Evernia prunastri* (Fig. 3B) has a wide pH range in less polluted sites but appears acidophytic under pollution-stress. *Hypogymnia physodes* (Fig. 3C) is typical of all acid habitats, irrespective of pollution-stress. All common nitrophytic species appear to be toxitolerant providing the pH is high. For example *Physcia adscendens* (Fig. 3D), *Phaeophyscia orbicularis* (Fig. 3E) and *Xanthoria parietina* (Fig. 3F) all range with high abundance values into sites with pollution-stress. *Ramalina farinacea* (Fig. 3G) has a wide pH range and clearly avoids pollution-stress. It appears to be slightly more toxitolerant at high pH values, although only with small occurrences. *Parmelia acetabulum* (Fig. 3H) behaves very much like *R. farinacea.*

Ammonia measurements

Annual mean NH_3 concentrations, measured at 104 sites (Fig. 1C), ranged between 3 and 47 µg m^{-3}. Values of 20 µg m^{-3} or more are very high by international standards and probably occur in only a few western European countries. High NH_3 concentrations were found in areas with large bioindustries involving livestock rearing.

The responses of the lichens to the measured NH_3 values are illustrated by two nitrophytic and two acidophytic species (Fig. 4). *Phaeophyscia orbicularis* showed a clear positive response to NH_3. Within the concentration range, the average abundance increased from 0·4 (nearly absent) to 6·0 (i.e. in large quantities on most of the trees). *Xanthoria parietina* also showed a positive but less pronounced response. By contrast *Hypogymnia physodes* appears to be very sensitive to NH_3 being absent at mean annual concentrations >13 µg m^{-3}. *Evernia prunastri* is less sensitive; it gradually decreased from 5 µg m^{-3} to be absent at levels >29 µg m^{-3}.

Figure 5 shows that there is a negative response to NH_3 when all acidophytic species (AIW) are taken together. At low NH_3 concentrations a slight increase in NH_3 leads to a more pronounced decrease in the AIW than at high NH_3 levels. At approximately 35 µg m^{-3} all acidophytic species had disappeared; even the relatively insensitive species *Lepraria incana* did not occur at this level. A linear regression of AIW on NH_3 accounts for 42·1% of the variance, but such a model is certainly an underestimation

FIG. 3. Biplots of the Canonical Correspondence Analysis (CCA), using the 76 *Quercus* sites where bark samples were taken (axis 1 vs. 2 as in Fig. 2). In all biplots the sample scores of the sites are plotted, showing the abundance values of eight selected species. Small dot=species is absent at this site, large circle=species is present in a large quantity. A few sites with an extreme position are not shown. A–C, acidophytic species; D–F, nitrophytic species; G–H, remaining species.

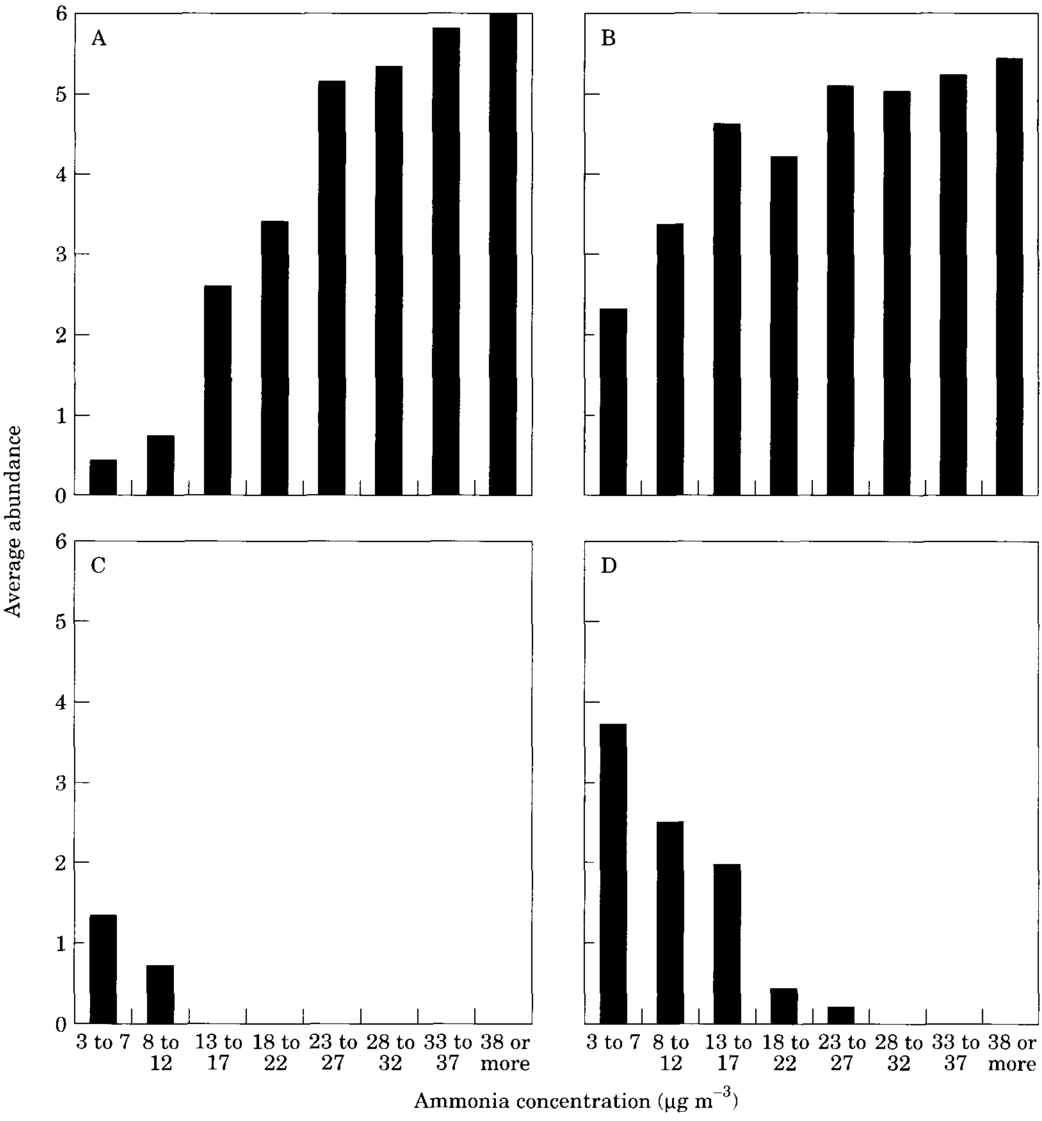


FIG. 4. Abundances of four lichen species on *Quercus robur* at different levels of NH_3 pollution. For each class of annual mean NH_3 concentration (μg m^{-3}), the average abundance value for that species is given. A, *Phaeophyscia orbicularis*; B, *Xanthoria parietina*; C, *Hypogymnia physodes*; D, *Evernia prunastri*.

of the relationship. For example, a third order polynomial regression curve accounts for 57·6% of the variance. Figure 6 shows the abundance of nitrophytic species (NIW) as a function of NH_3 concentration. Unlike Fig. 5 concerning the acidophytes, the relationship between NH_3 and NIW is nearly linear. At sites with high ammonia levels nitrophytes are dominant. A linear regression of the NIW on NH_3 accounted for 59·3% of the variance. Other significant effects (Table 3) can be attributed to the girth of the trees (NIW higher on slender trees) and to the exposure of the trees (NIW higher on exposed trees). The joint variance accounted for by these three factors is

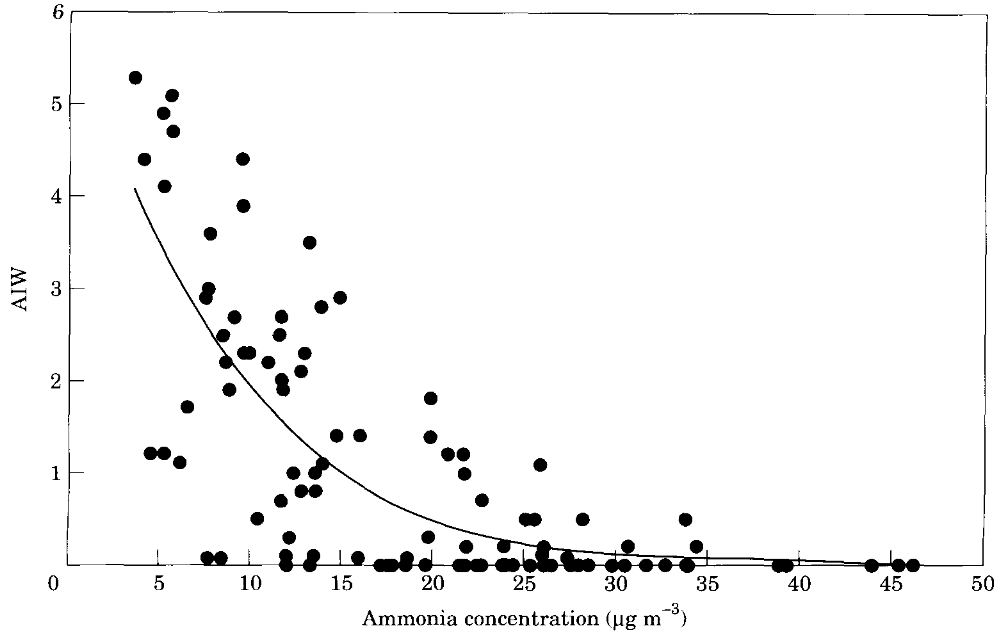


FIG. 5. The abundance of acidophytes (AIW) as a function of annual mean NH_3 concentration (μg m^{-3}). The line is a 3rd order polynomial regression curve (r^2=0·58, n=104). At approximately 35 μg m^{-3} all acidophytic species have disappeared.

68·1%, while no effect could be attributed to SO_2. Thus, in areas with high SO_2 levels nitrophytes are as common as in areas with low SO_2 levels (Table 3).

Separate regression lines were calculated for three areas where NH_3 measurements were taken (Brabant, Gelderse Vallei and Friesland). There is a close co-incidence between these three regression lines (Fig. 6), suggesting that the relationship between NH_3 and NIW is probably comparable in the three areas. When NH_3 and NIW are aggregated to an average per 5 × 5 km square, the NIW appears to be a very good predictor of ammonia air pollution with *c.* 90% explained variance (Fig. 7).

Changes with time

Some results of the monitoring over the period 1989–1999 are shown in Figs 8 and 9. A considerable decrease in *Hypogymnia physodes* (Fig. 8) occurred, while *Phaeophyscia orbicularis* increased very rapidly over the same period. *Ramalina farinacea* has also increased. More or less similar results were found when all acidophytic (AIW) and nitrophytic (NIW) species were taken together. A strong decrease in AIW (Fig. 9A) as well as a strong increase in NIW (Fig. 9B) are both apparent. The total number of lichen species per site increased dramatically over the period 1989–1999 from 14·3 to 21·4 (Fig. 9C).

Discussion

This paper records striking changes in the epiphytic lichen composition on *Quercus* in the Netherlands as a result of severe NH_3 pollution: nitrophytes such as *Phaeophyscia orbicularis* have become dominant and acidophytes such as *Hypogymnia physodes* have largely disappeared. The changes in these species over the period 1989–1999 strongly suggest that a marked de-acidification of the *Quercus* bark has occurred (cf. with Fig. 3). An increase in neutrophytic species like *Ramalina farinacea*, however, is probably

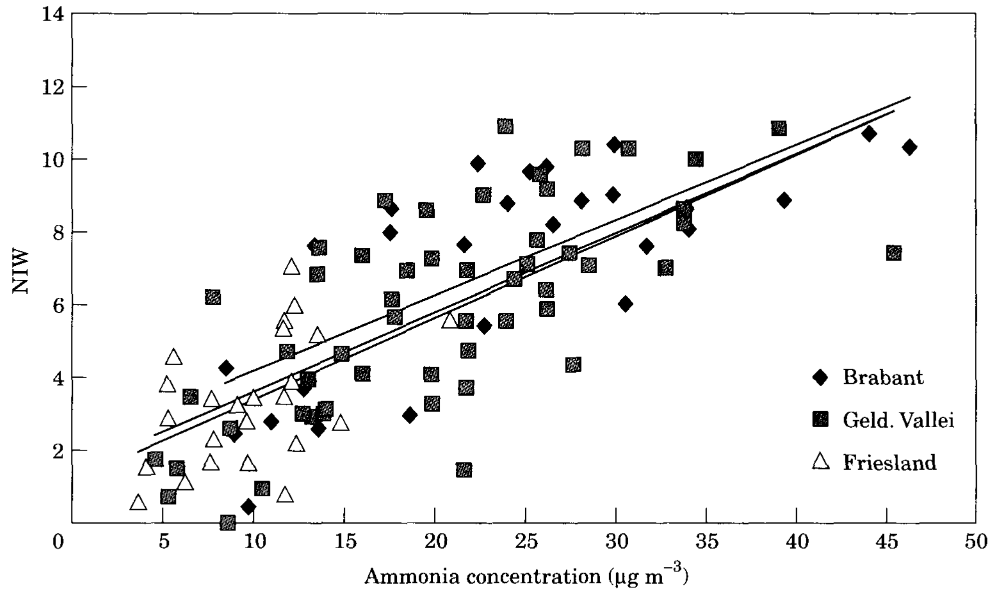


FIG. 6. The abundance of nitrophytes (NIW) as a function of annual mean NH_3 concentration (µg m^{-3}). Linear regression analysis is performed for all sites together (r^2=0·59, n=104, line not shown), and for three separate areas in the Netherlands: Brabant (Fig. 1C, in the south), Gelderse Vallei (in the centre) and Friesland (in the north).

TABLE 3. *Multiple regression using Nitrofiele Indicatie Waarde (NIW) as dependent variable and four environmental factors (NH_3, SO_2, tree girth and exposure) as independent variables. This regression is carried out to investigate which factors have a direct effect on NIW*

In model:‡	Regression coefficient (r^2)	F	t	Significance P
Constant	1·29		1·1	0·2792 n.s.
NH_3 concentration	0·187	80·60	9·0	<0·0001**
Tree girth	− 0·146	8·93	− 3·0	0·0035*
Exposure	0·621	14·70	3·8	0·0002**
Not in model:	Correlation coefficient (r)	F		Significance P
SO_2 deposition	0·103	1·05		n.s.

‡r^2=0·68; degrees of freedom=100.
*=P<0·005; **=P<0·0005; n.s.=not significant.

related primarily to a decreasing effect of toxic substances (Fig. 3), although such species may show a slight positive effect of the de-acidification of the bark as well.

Strong correlations have been demonstrated between the spatial patterns of lichen composition and NH_3 concentrations (Figs 5–7). It was initially hypothesised that SO_2

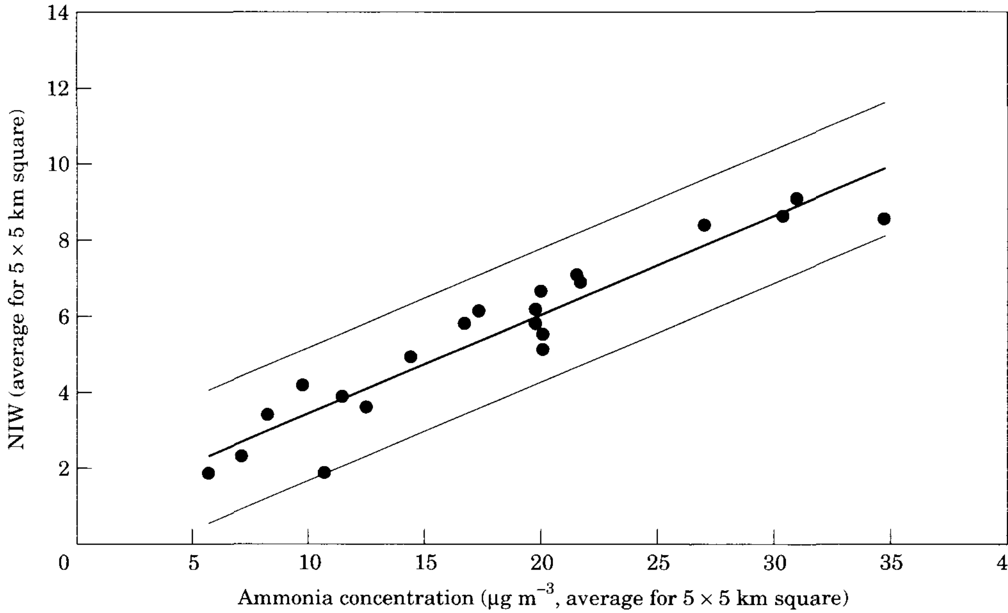


FIG. 7. The abundance of nitrophytes (NIW) as a function of annual mean NH_3 concentration (µg m^{-3}), both aggregated to an average per square of 5 × 5 km. Linear regression analysis is performed, the upper and lower lines show the 95% confidence limits (r^2=0·90, n=21).

concentration would account for at least some spatial differences in the occurrence of nitrophytes, whereas in fact it has not proved possible to attribute any effects to SO_2 (Table 3, see also van Herk 1999). An explanation may be that SO_2 concentration, which is now much lower than in former times, no longer causes acidification of *Quercus* bark (see also discussion below). Neither are direct toxic effects of SO_2 on nitrophytes apparent at the current levels (Fig. 3).

Although an increased bark pH is very likely to be the primary cause of the shift in species composition from acidophytic to nitrophytic (Fig. 2) during the last decade, it is difficult to address this temporal change unequivocally to an increase in NH_3, or to changes in other factors as well. Nitrophytes have undoubtedly increased very rapidly (Figs 8 & 9B) and according to the regression analysis (Table 3, Fig. 7), the increase in the NIW between 1989 and 1999 (being 1·3 points in Fig. 9B) coincides with an increase of the NH_3 concentration of 5–7 µg m^{-3}, assuming that the effects of Δ SO_2 (not significant), Δ tree girth (significant, but only a slight increase) and Δ exposure (remained the same) are negligible (Table 3). Changes in AIW indicate a similar increase in atmospheric NH_3. However, the Dutch Air Quality Network data (Anonymous 2000; van Jaarsveld *et al.* 2000) suggest that NH_3 emissions and NH_x (=NH_3+NH_4^+) deposition levels (all derived from computer models) remained about the same, or slightly decreased, over the period of 1987–1997.

The way in which a high pH is caused by atmospheric NH_x pollution may provide more insight into this apparent discrepancy. Recent data on the NH_3/NH_4^+ deposition ratio, provided by the Dutch Air Quality Network (van Jaarsveld *et al.* 2000) show that during the last decade, due to the reduction of SO_2, the atmospheric NH_4^+ concentration decreased by about 2–3 µg m^{-3} (1987–1997) in favour of the NH_3 concentration. Such a shift may partly explain the increase in nitrophytic lichens (NIW) because atmospheric NH_3 causes

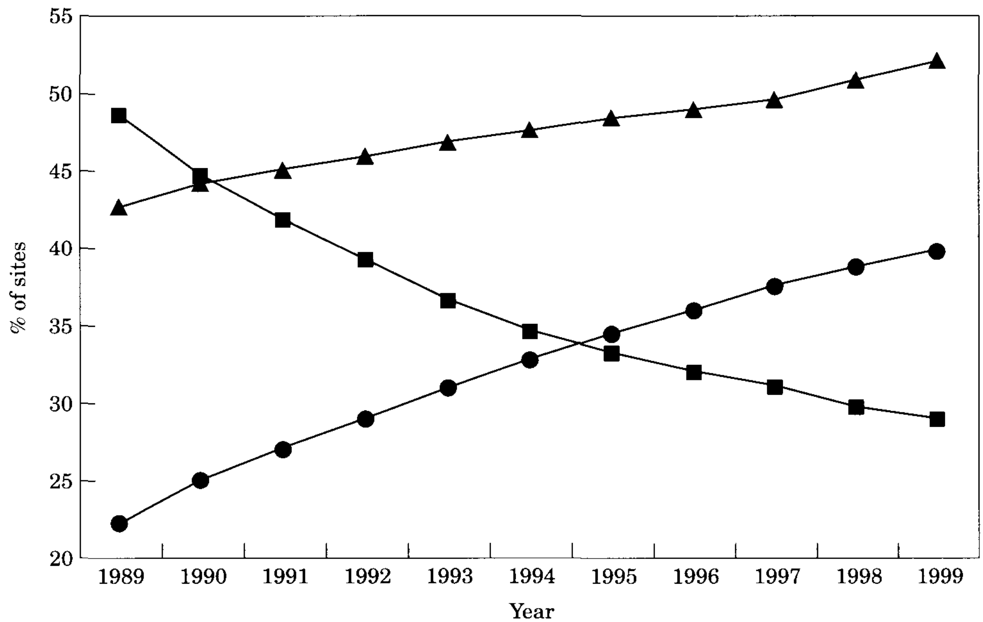


FIG. 8. Changes in the occurrence of three lichen species on *Quercus robur* over the period of 1989–1999. For each species the average percentage of the lichen monitoring sites at which it is present is given. ■ = *Hypogymnia physodes*, ● = *Phaeophyscia orbicularis*, ▲ = *Ramalina farinacea*.

a considerable rise in bark pH whereas atmospheric NH_4^+, deposited mainly as $(NH_4)_2SO_4$, affects bark pH only slightly. It has already been shown that nitrophytic lichens appear to be encouraged by atmospheric NH_3 only, whereas atmospheric NH_4^+ shows no significant effect (van Herk 1999). Another part of the increase in NIW may be explained by the relatively high deposition rate of NH_3 compared to NH_4^+, resulting in a shorter atmospheric transport distance (Asman & van Jaarsveld 1990*b*). Thus, the reduction of SO_2 probably caused a concomitant increase in the NH_3/NH_4^+ ratio and a greater proportion of the emitted NH_3 to remain within the Netherlands, both of which may have aided the increase in nitrophytes.

There is currently a considerable debate about the reliability of the NH_x data provided by the Dutch Air Quality Network. Measured NH_3 concentrations (Duyzer *et al.* 1998) were *c.* 35% higher than the calculated values based on livestock numbers (van Jaarsveld *et al.* 2000) giving rise to the so-called 'ammonia hole' (Anonymous 2000). Due to this uncertainty it is not possible to test satisfactorily the presumed cause of changes in nitrophytes.

The Dutch SO_2 emissions decreased between 1987 and 1997 from 250 million kg to 115 million kg (Anonymous 2000). Over the same period, the mean SO_2 concentration decreased from 17 to 5 $\mu g\ m^{-3}$. The decreasing SO_2 levels, therefore, are well correlated with the increase in nitrophytes. This led van Dobben (1993) and van Dobben & ter Braak (1998) to ascribe the increase in nitrophytes in the Netherlands between 1977 and 1990 to the decrease in SO_2. The spatial distribution of nitrophytes, however, does not show a correlation with the spatial distribution of SO_2: at least during the last decade no spatial SO_2 pattern is apparent from the distribution of nitrophytes (Table 3; van Herk 1999: table 2). It may be concluded that the data on SO_2 air pollution and the occurrence of nitrophytes do not match when comparing their temporal changes to their spatial patterns. Thus, the

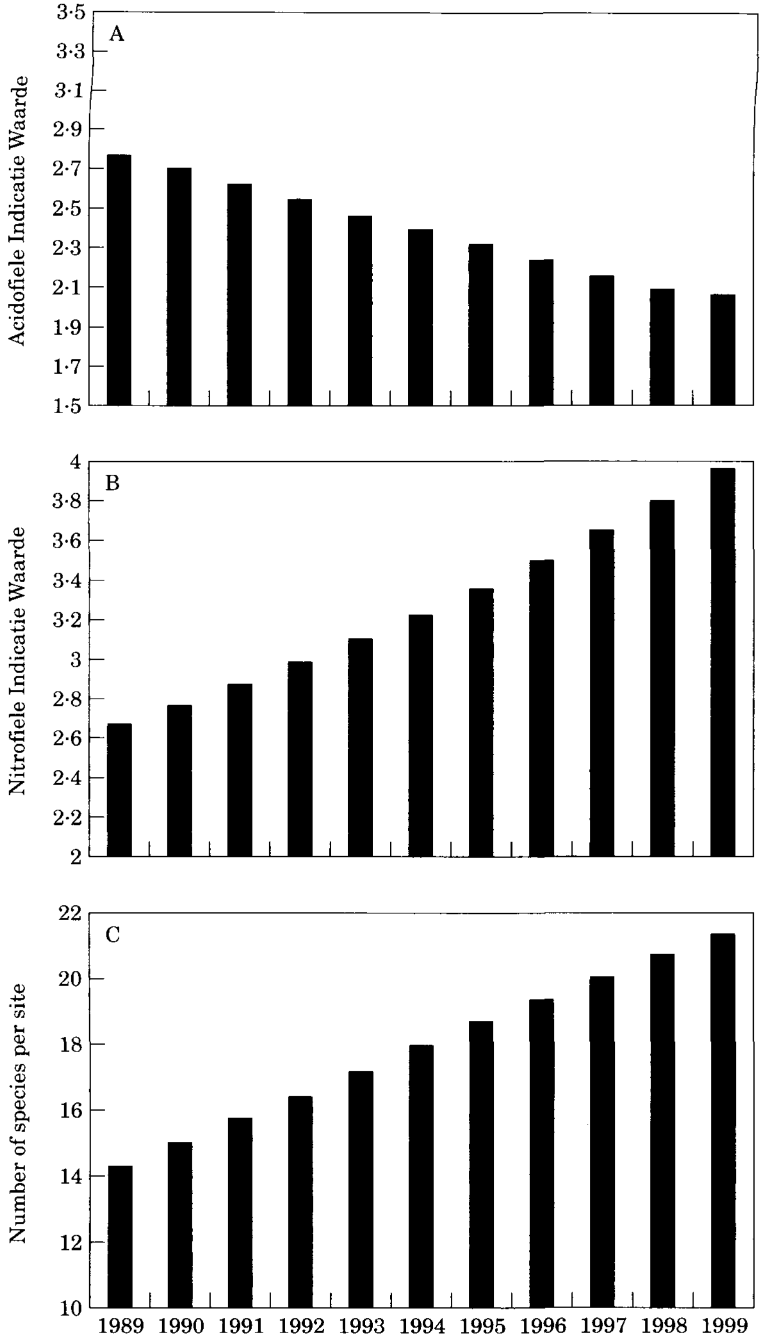


FIG. 9. Changes in lichen communities on *Quercus robur* during the period of 1989–1999. A, abundance of acidophytes (Acidofiele Indicatie Waarde, AIW); B, abundance of nitrophytes (Nitrofiele Indicatie Waarde, NIW); C, the number of lichen species present per site.

SO_2 concentration itself, which decreased from 17 to 5 μg m^{-3} between 1987 and 1997, does not provide an acceptable explanation for the increase of nitrophytes. It remains, however, unclear whether the increase in nitrophytes (Fig. 9B) may be attributed entirely to an increase in NH_3, due to a decrease in SO_2. When considering also the decrease in acidophytes (Fig. 9A) the same may be concluded. It appears very unlikely that a decrease in SO_2 caused such a strong negative effect (if any) on, for example, *Hypogymnia physodes* (Fig. 8).

In this study no effect of SO_2 on bark pH became apparent since no correlation was found between bark pH and atmospheric SO_2 concentration ($r = -0{\cdot}03$, $P = 0{\cdot}7846$), and the ordination (Fig. 2) showed the arrows of bark pH and SO_2 to be pointing in different directions. Acidification may now play an insignificant role because SO_2 concentrations are much lower than in former times. These results are, nevertheless, probably highly correlated in *Quercus robur*, because it has an acid bark by nature. At one site supporting some old woodland lichens, a pH as low as 3·65 was found (Table 1, Norgerholt). Such low pH values may be partly due to the poor nature of the acid sandy soils in the Netherlands. Studies in Norway (Gauslaa 1985) and Scotland (Bates 1992) showed that the pH of *Quercus* bark was also dependent on the calcium content of bark and soil.

When surveying tree species with less acid bark, such as *Populus* × *canadensis*, some interference with acidification due to SO_2 may obscure the effects of NH_3, as can be seen from results of van Dobben & Wamelink (1992) and Hoffmann (1993). In bioindication studies, it may therefore be necessary to compensate for the effects of SO_2 when tree species with a less acid bark are investigated. In areas with a warm and dry climate such as the Mediterranean, the effect of dust may dominate. A proper registration of the effects of NH_3 may appear difficult or impossible in these countries (Loppi & De Dominicis 1996). However, obvious effects of NH_3 were found by Berdowski & Aptroot (1991) when surveying *Pinus nigra*, a tree with very acid bark, in a warm and dry area of Spain.

Most of the species defined as nitrophytic appear to be toxitolerant, the only factor with which they are universally associated is a high pH. Within high pH conditions they are ubiquitous (Figs 2 & 3), but this is probably only true because the Dutch environment supplies abundant ammonium: at high pH values nitrogen is usually not a limiting factor for the occurrence of nitrophytes. In more natural situations with high pH values (*c.* 6·0 in some *Lobarion* communities, see e.g. Gauslaa 1985), nitrophytic species are usually completely absent. Apparently a high pH alone does not provide a suitable explanation for the occurrence of nitrophytes. Therefore the term 'nitrophytic' must be perpetuated and alternatives like 'basiphytic' rejected.

The relatively low sensitivity of nitrophytes to toxic effects of SO_2 also follows from the observations made by Gilbert (1976) on calcareous dust-impregnated trees. The absence of nitrophytes on non-impregnated trees was not due to toxic SO_2 levels (*c.* 65 μg m^{-3} at that time), but was simply because the bark pH was too low. During periods with severe urban SO_2 pollution nitrophytes were able to establish and grow on calcareous stone, some species even flourished at sites infested by pigeons. Therefore the SO_2 sensitivity attributed to nitrophytes in some classic scales (Barkman 1958; de Wit 1976) is probably partly due to a sensitivity to acidification of the substratum rather than a direct sensitivity to pollutants. For many other species, especially neutrophytes, the opposite holds. Most species in this group are good indicators of toxic effects of SO_2 (Figs 2 & 3), and pH preference seems to be of minor importance. Apparent interaction between pH and SO_2 sensitivity, as described by Türk & Wirth (1975), was not found for most of the species in the present survey. Rather few species seem to be slightly more toxitolerant at high pH values (*Ramalina farinacea*, Fig. 3G), although, it is clear that more species are found under a combination of high SO_2 levels with high pH than under a combination of high SO_2 levels with low pH. The increased number of species per site (from 14·3 to 21·4, Fig. 9C) may therefore not only be attributable to a decrease in SO_2 levels, but also to higher bark pH values due to an increase in NH_3 concentrations.

A very high bark sulphate content appeared to correlate with inhibited lichen growth at urban sites. Sulphate was probably accumulated in former periods with high SO_2 concentrations. Thus it is probable that the present lichen composition does not reflect the current air quality, but merely that of former times. In the literature there is no evidence to suggest that sulphate is damaging to lichens. Perhaps SO_4^{2-} is an indication of other aspects of bark chemistry.

Recent changes in lichen composition observed in monitoring studies require careful interpretation. Some authors, such as Kirschbaum & Hanewald (1998), consider the decrease in acidophytes to be caused by an improvement in air quality while in fact an increase in NH_3 pollution may be the principal cause. Even the rapid decrease of *Lecanora conizaeoides* in several western European countries (e.g. Wirth 1993) is probably only partly caused by decreasing SO_2 concentrations because this species appears to be very sensitive to NH_3. Similarly, an increase in nitrophytes should not be interpreted as a result of an improvement of air quality without considering tree species and effects of eutrophication.

The inclusion of nitrophytic lichens in IAP-bioindication studies is not advisable, especially in urban areas where such methods are widely applied (e.g. Kirschbaum & Hanewald 1998). Relatively high IAP values may nowadays be found only due to eutrophication whereas the IAP is supposed to register toxic effects on lichens (e.g. Anonymous 1995).

Although practically all sites in this work are situated along roads, no effects on lichens caused by road traffic (i.e. NO_x), were apparent (van Herk 1991). The sites were divided into five categories according to the type of road concerned (i.e. ranging from cul-de-sacs to major highways). A multiple regression was carried out to consider the effects of SO_2, NH_3, NH_4^+ and tree girth. Only small differences were found with trees along busy roads supporting slightly more lichens (van Herk 1991). This is probably due to the manner in which the trees along busy roads are managed: for safety all low branches (<6 m) are usually cut away regularly. As a consequence the lichens receive more light, which favours most species.

I thank André Aptroot, Barbara Benfield, Piet Bremer and Leo Spier for their useful comments and the corrections of the English text. The provincial administrations of Groningen, Friesland, Drenthe, Overijssel, Gelderland, Utrecht, Zeeland and Noord-Brabant are thanked for making this study possible and providing the opportunity to publish this paper.

References

Anonymous (1989–1993) *Jaaroverzicht Luchtkwaliteit 1988–1992*. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieuhygiëne.

Anonymous (1995) *Ermittlung und Beurteilung phytotoxischer Wirkungen von Immissionen mit Flechten: Flechtenkartierung zur Ermittlung des Luftgütewertes.* VDI-Richtlinie 3799, Blatt 1. Berlin: Beuth-Verlag.

Anonymous (2000) *Milieubalans 2000, het Nederlandse milieu verklaard.* Alphen aan den Rijn: Samson bv.

Aptroot, A., van Herk, C. M., Sparrius, L. B. & van den Boom, P. P. G. (1999) Checklist van de Nederlandse lichenen en lichenicole fungi. *Buxbaumiella* **50:** 4–64.

Asman, W. A. H. & van Jaarsveld, J. A. (1990*a*) *A variable-resolution statistical transport model applied for ammonia and ammonium.* RIVM report no. 228471007. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieuhygiëne.

Asman, W. A. H. & van Jaarsveld, J. A. (1990*b*) *Gedrag van atmosferisch ammoniak. Proceedings Symposium Dierlijke Mest: Problemen en oplossingen.* Den Haag: KNCV.

Baddeley, M. S., Ferry, B.W. & Finegan, E.J. (1972) The effects of sulphur dioxide on lichen respiration. *Lichenologist* **5:** 283–291.

Barkman, J. J. (1958) *Phytosociology and Ecology of Cryptogamic Epiphytes.* Assen: Van Gorcum.

Bates, J. W. (1992) Influence of chemical and physical factors on *Quercus* and *Fraxinus* epiphytes at Loch Sunart, western Scotland: a multivariate analysis. *Journal of Ecology* **80:** 163–179.

Bates, J. W. & Brown, D. H. (1981) Epiphyte differentiation between *Quercus petraea* and *Fraxinus excelsior* trees in a maritime area of southwest England, UK. *Vegetatio* **48:** 61–70.

Benfield, B. (1994) Impact of agriculture on epiphytic lichens at Plymtree, East Devon. *Lichenologist* **26:** 91–96.

Berdowski, J. J. M. & Aptroot, A. (1991) Field verification study in the Maestrazgo area. *TNO report* **R91/046:** 1–44. Delft: TNO.

Brown, D. H. (1992) Impact of agriculture on bryophytes and lichens. In *Bryophytes and Lichens in a Changing Environment* (J. W. Bates & A. M. Farmer, eds): 259–283. Oxford: Clarendon Press.

de Bakker, A. J. & van Dobben, H. F. (1988) Effecten van ammoniakemissie op epifytische korstmossen, een correlatief onderzoek in de Peel. *RIN-rapport* **88/35:** 1–48. Leersum: Rijksinstituut voor Natuurbeheer.

de Sloover, J. & LeBlanc, F. (1968) Mapping of atmospheric pollution on the basis of lichen sensitivity. In *Proceedings of the symposium on recent advances in tropical ecology* (R. Misra & B. Gopal, eds): 42–56. Varanasi: The International Society for Tropical Ecology.

de Wit, A. (1976) Epiphytic lichens and air pollution in the Netherlands. *Bibliotheca Lichenologica* **5:** 1–115.

Duyzer, J., Weststrate, H. & Wyers, P. (1997) Karakterisering van regionale concentratievelden van ammoniak, 2^e fase: resultaten van concentratiemetingen. *TNO-rapport* **97/475:** 1–53. Apeldoorn: TNO.

Duyzer, J., Weststrate, H., Erisman, J. W., Bleeker, A. & van Jaarsveld, H. (1998) Karakterisering van regionale concentratievelden van ammoniak, 2^e fase: overzicht. *TNO-rapport* **98/004:** 1–53. Apeldoorn: TNO.

Ferry, B. W. & Coppins, B. J. (1979) Lichen transplant experiments and air pollution studies. *Lichenologist* **11:** 63–73.

Gauslaa, Y. (1985) The ecology of *Lobarion pulmonariae* and *Parmelion caperatae* in *Quercus* dominated forests in south-west Norway. *Lichenologist* **17:** 117–140.

Gilbert, O. L. (1976) An alkaline dust effect on epiphytic lichens. *Lichenologist* **8:** 173–178.

Hawksworth, D. L. & Rose, F. (1970) Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. *Nature* **227:** 145–148.

Hoffmann, M. (1993) *Verspreiding, fytosociologie en ecologie van epifyten en epifytengemeenschappen in Oost- en West-Vlaanderen.* Ph.D Thesis, University of Gent.

Jongman, R. H. G., ter Braak, C. J. F. & van Tongeren, O. F. R. (1995) *Data analysis in community and landscape ecology.* Cambridge: Cambridge University Press.

Kirschbaum, U. & Hanewald, K. (1998) Immissionsbezogene Flechtenkartierungen in hessischen Dauerbeobachtungsflächen. *Journal of Applied Botany* **72:** 212–227.

Loppi, S. & De Dominicis, V. (1996) Effects of agriculture on epiphytic lichen vegetation in central Italy. *Israel Journal of Plant Sciences* **44:** 297–307.

Nienburg, W. (1919) Studien zur Biologie der Flechten I. Nitrophile Rindenflechten. *Zeitschrift für Botanie* **11:** 1–38.

Nimis, P. L., Lazzarin, A., Lazzarin, G. & Gasparo, D. (1991) Lichens as bioindicators of air pollution by SO_2 in the Veneto region (NE Italy). *Studia Geobotanica* **11:** 3–76.

Räsänen, V. (1927) Über Flechtenstandorte und Flechtenvegetation im westlichen Nordfinnland. *Annales Societatis Zoologicae-Botanicae Fennicae Vanamo* **7:** 1–202.

Ruoss, E. (1999) How agriculture affects lichen vegetation in Central Switzerland. *Lichenologist* **31:** 63–73.

Seaward, M. R. D. (1997) Urban deserts bloom: a lichen renaissance. *Bibliotheca Lichenologica* **67:** 297–309.

Søchting, U. (1991) *Laver som kvælstofmonitorer i danske skove.* Kopenhagen: Institut for Sporeplanter.

ter Braak, C. J. F. (1987) *CANOCO, a Fortran Program for Canonical Community ordination by (Partial) (Detrended) (Canonical) Correspondence Analysis and Redundancy Analysis (Version 2.1).* Wageningen: TNO.

ter Braak, C. J. F. (1991) *Program CANOCO Version 3.12.* Wageningen: Agricultural Mathematics Group DLO.

Türk, R. & Wirth, V. (1975) The pH dependence of SO_2 damage to lichens. *Oecologia* **19:** 285–291.

van Dobben, H. F. & Wamelink, W. (1992) Effects of atmospheric chemistry and bark chemistry on epiphytic lichen vegetation in The Netherlands. *RIN-rapport* **92/23:** 1–34. Wageningen: Instituut voor Bos- en Natuuronderzoek.

van Dobben, H. F. (1993) *Vegetation as a monitor for deposition of nitrogen and acidity.* Ph.D Thesis, University of Utrecht.

van Dobben, H. F. & ter Braak, C. J. F. (1998) Effects of atmospheric NH_3 on epiphytic lichens in the Netherlands: the pitfalls of biological monitoring. *Atmospheric Environment* **32:** 551–557.

van Dobben, H. F. & ter Braak, C. J. F. (1999) Ranking of epiphytic lichen sensitivity to air pollution using survey data: a comparison of indicator scales. *Lichenologist* **31:** 27–39.

van der Knaap, W. O. (1984) Epifyten in de provincie Utrecht 1979–1984. *Buxbaumiella* **16:** 15–17.

van Herk, C. M. (1990) *Epifytische korstmossen in de provincies Drenthe, Overijssel en Gelderland.* Zwolle: Provincie Overijssel.

van Herk, C. M. (1991) *Korstmossen als indicator voor zure depositie.* Arnhem: Provincie Gelderland.

van Herk, C. M. (1998) *Onderzoek naar de relatie tussen de ammoniakconcentratie en epifytische korstmossen.* Soest: Lichenologisch Onderzoekbureau Nederland.

van Herk, C. M. (1999) Mapping of ammonia pollution with epiphytic lichens in the Netherlands. *Lichenologist* **31:** 9–20.

van Jaarsveld, J. A., Bleeker, A. & Hoogervorst, N. J. P. (2000) *Evaluatie ammoniak emissiereducties met behulp van metingen en modelberekeningen.* RIVM report no. 722108025. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieuhygiëne.

Wirth, V. (1991) Zeigerwerte von Flechten. *Scripta Geobotanica* **18:** 215–237.

Wirth, V. (1993) Trendwende bei der Ausbreitung der anthropogen geförderten Flechte *Lecanora conizaeoides*? *Phytocoenologia* **23:** 625–636.

Wolseley, P. A. & Pryor, K. V. (1999) The potential of epiphytic twig communities on *Quercus petraea* in a Welsh woodland site (Tycanol) for evaluating environmental changes. *Lichenologist* **31:** 41–61.

Zimmer, D. (2000) Dauerbeobachtung der epiphytischen Flechtenvegetation unter immisionsökologischen Aspekten an Boden-Dauerbeobachtungsflächen in Schleswig-Holstein. *Bibliotheca Lichenologica* **75:** 231–251.

Accepted for publication 26 *April* 2001