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Re-mapping epiphytic lichen biodiversity in The Netherlands: effects of decreasing SO_2 and increasing NH_3

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SUMMARY

In a follow-up of De Wit's (1976) inventory of epiphytic lichens in The Netherlands, *c.* 17% of the area covered in that study was re-inventoried, using a comparable method. A strong increase in species diversity became apparent, probably caused by a decrease in SO_2 concentration. In a comparison of various indicators for SO_2 concentration derived from the epiphytic vegetation (number of species per sample point and number of species per 5×5 km^2, or composite measures based on multivariate statistics), the number of species per 5×5 km^2 grid square had the strongest correlation with the measured SO_2 concentration. A second factor affecting epiphytic vegetation was the atmospheric NH_3 concentration. High concentration of this compound caused a dominance of nitrophytic species. The rate of change in the epiphyte vegetation between 1988 and 1989 was consistent with the rate of decrease of the SO_2 concentration during the preceding 5 years.

Key-words: air pollution, ammonia, biodiversity, epiphyte, lichen, recolonization.

INTRODUCTION

In The Netherlands, large-scale inventories of epiphytic lichens were undertaken in 1940–53 (Barkman 1958) and in 1970–73 (De Wit 1976). The conclusion of both studies, and particularly the latter, was that the epiphytic lichen biodiversity was low and declining, probably as a result of high SO_2 concentrations. After the 1970s a number of changes took place that raised the interest in an update of the older studies.

1. The SO_2 concentration had been steadily decreasing from *c.* 1970 onwards. As a result, some of the species that had become rare or extinct during the period of high SO_2 concentrations had returned (e.g. De Bakker 1986). Comparable observations were made in other countries, e.g. England (Hawksworth & McManus 1989) or France (Letrouit-Galinou *et al.* 1992).

2. Until *c.* 1980 the NH_3 concentration had increased. The strong increase of many nitrophytic species was ascribed to this rise in NH_3 (Van Dobben 1987; De Bakker 1989).

3. A country-wide, high-density air quality monitoring network was installed during the 1970s. Since then the concentrations of SO_2, NO_x and O_3 are known with a high resolution in both space and time (Anonymous 1978–90). These new data allowed quantification of the relationship between lichen biodiversity and actual measured pollutant concentrations. Former studies had to rely to a large extent upon relative measures, such as the distance to cities or industrial centres.

To study the changes in the epiphytic lichen diversity, and its relation with atmospheric quality in detail, a partial re-mapping was carried out in 1988 and 1989, on the basis of the sample points used in De Wit's (1976) study. The latter study was undertaken with a large number of amateur fieldworkers and comprised a huge number of sample points (*c.* 10 000). For the present study it was felt necessary to use a less time-consuming and more standardized method. As in De Wit's study the 5×5 km^2 grid square was used as a basic unit for the inventory, but a selection of the squares was used instead of a country-wide survey. The number of surveyed tree species was also limited, and a stricter standardization of the sample points was used. Moreover, the abundance of the species was estimated in a semi-quantitative scale instead of only presence or absence. The collected data were used to find answers to the following questions.

1. What is the quantitative relationship between the number of species per sample point and per grid square, and the air pollutant concentrations? A conclusion of De Wit's study was that the number of species per grid square was a good measure for atmospheric quality. However, an important reason for using this simple biodiversity measure was the unavailability of sophisticated methods for data processing at that time. Therefore, a re-evaluation of this method with present-day techniques was felt useful.

2. What is the relationship between the species composition of the epiphytic lichen vegetation and air pollutant concentrations? Individual species may react differently to various pollutants. An example is the increase of the nitrophytic species, ascribed to high NH_3 concentrations. We used multivariate techniques to make a general picture of the relationship between vegetation composition and pollutant concentrations. The response of the species to pollutants was compared with the simple and widely accepted ecological classification as nitrophytes, acidophytes or indifferent species.

3. What is the rate, direction and geographical variation of the change in epiphytic vegetation over a 1-year period? Rapid changes have been reported after 1980, mostly an increase in the frequency and abundance of nitrophytic species. If ammonia is the cause of this increase, the highest rate of change would be expected in areas with high concentrations of this pollutant.

We used a statistical approach to model both species numbers and the composition of the epiphytic vegetation (i.e. the abundances of the individual species) at the sample points as a function of measured air quality and a few other ecologically relevant variables, such as tree species and distance to the coast. The outcome of these models was used for a ranking of the environmental variables (air quality, and other) with respect to their importance in determining the epiphytic lichen biodiversity at a given site.

MATERIAL AND METHODS

A sample point consisted of a group of 10 trees of the same species (but fewer in places where not enough were available). More than one sample point could be present in a given locality if there were adjacent trees of different species. The abundance of all lichen species present on the trunks of these trees from the base up to a height of 2 m was estimated using a six-point scale (see Van Dobben 1993). Most species were identified in the field, but individuals that were not readily recognizable were sampled for later identification. The 5 × 5 km^2 squares of the local 'Amersfoort' grid were used as a basis for the sampling.

Samples were taken from the following tree species: *Quercus robur* L., *Populus* × *canadensis* Moench, *Salix alba* L., *Ulmus* × *hollandica* Miller, *Fraxinus excelsior* L., and *Tilia* spp. We attempted to sample about five different tree species in each grid square, but this number could vary according to local circumstances. The sampled tree species were regionally different according to their ecological behaviour and the prevailing abiotic conditions. In order to minimize the influence of environmental variables other than air quality, the sample points were selected to comply with certain standardization criteria. All sample points were free-standing trees, most of them wayside trees. Slanting trees and very thick or very slender trees were avoided, and so were strongly eutrophicated trees in farmyards, etc.

Eight regions that were uniformly spread over the country were selected for re-mapping. The sample points were preferably those used by De Wit (1976), but others were chosen in places where they could not be traced or did not comply with the uniformity criteria. Most of these points were sampled in both 1988 and 1989. The sampled grid squares are shown in Fig. 1, together with the names of the regions used to refer to them. A total of 266 squares containing 1216 points was sampled in 1989, yielding a total of 104 species (in 1988: 266 squares, 1199 points, 98 species). The mean number of trees per sample point was 7·3, the total number of sampled trees *c.* 8500. For the detection of the changes between 1988 and 1989 only the 1164 points were used that were sampled in both years. In this comparison six species that were probably overlooked or misidentified in 1988 were excluded, leaving a total of 98 species.

Air pollution data were obtained from the Dutch Air Quality Monitoring Network. SO_2 and NO_2 were estimated as means of hourly measured concentrations (SO_2 April–September 1988, NO_2 June 1988–May 1989) at monitoring stations (Anonymous 1989, 1990), followed by interpolation (Van Egmond *et al.* 1978) of the concentrations at the sample points. Mean NH_3 concentrations were estimated on a 5 × 5 km^2 grid basis using the 1988 emission data and the atmospheric transport and deposition model TREND (Asman & Van Jaarsveld 1990). Figure 2 gives the spatial patterns of SO_2, NO_2 and NH_3. Other environmental variables used in the statistical analyses were tree circumference, distance to the coast (=nearest salt water basin), number of trees per sample point, dummy variables for the six tree species, and a dummy variable to indicate pollard trees. All explanatory variables were approximately normally distributed except tree circumference which was logarithmized. The other variables were analysed untransformed.

The effect of the environmental variables on species numbers was analysed by multiple linear regression, using the program GENSTAT5 version 2.2 (Payne *et al.* 1987). The effects of the environmental variables on the abundance of all species simultaneously (further referred to as 'composition of the vegetation') were analysed by redundancy analysis (RDA) and a permutation significance test, using the program CANOCO version 3.10 (Ter Braak 1988). Technical details of RDA and permutation, and its application to the present data, are given by Van Dobben (1993). The results of the RDA analyses are given in the form of biplots; for their interpretation, see Jongman *et al.* (1987) or Ter Braak (1994). The significance of the effects of SO_2 and NH_3 on the

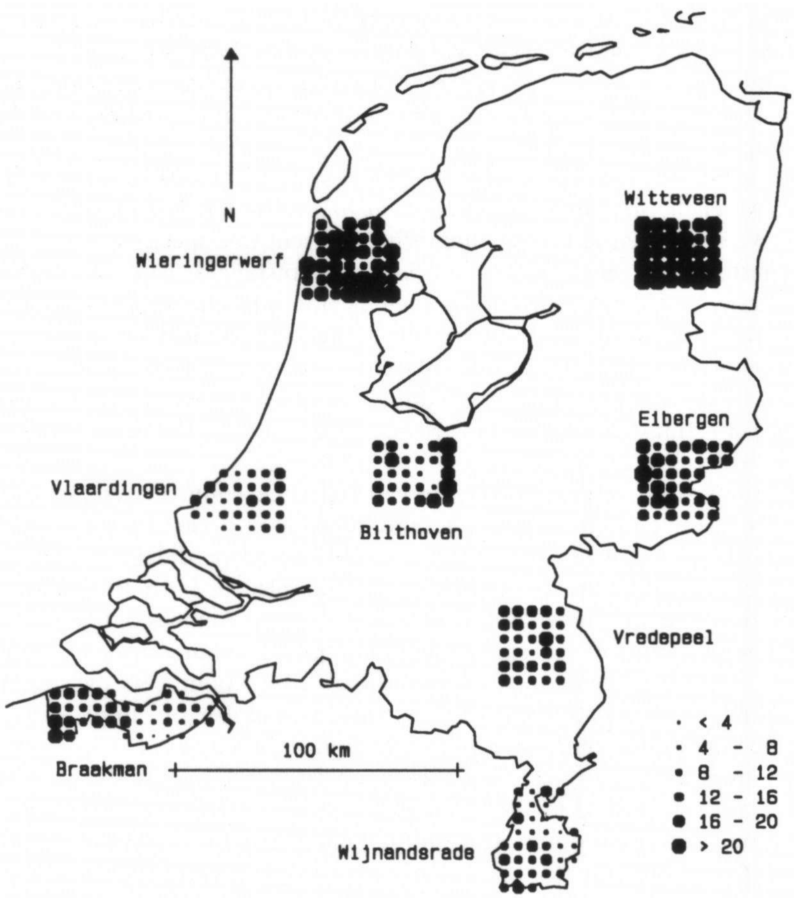

Fig. 1. Location of the investigated grid squares and names used to refer to the regions. The size of each dot corresponds to the mean number of species per sample in a square.

abundance of the species is presented in the form of a *t*-value biplot; for details on this type of plot, see Ter Braak & Looman (1994).

To analyse the change in vegetation composition and to obtain some indication of its possible cause, the samples from 1988 were projected into the RDA ordination diagram of the samples from 1989. For the construction of this diagram the environmental variables that change over time (atmospheric quality and tree circumference) were the explanatory variables, using the values from 1989. The variables that were constant over time (distance to the coast, tree species, and number of trees) were used as covariables. The sample scores were then determined for both years using the species scores determined for the 1989 data (i.e. the samples from 1988 were made 'passive'; see Ter Braak 1988).

The response of the species to the environmental variables was compared with the classification in ecological groups according to pH preference, viz. nitrophytic (preference for bark pH *c.* 5–7), acidophytic (preference for bark pH <4), or indifferent/undetermined. The classification used in this paper is similar to the one used by Van Dobben (1993), which was based on data in Wirth (1980) and Brand *et al.* (1988).

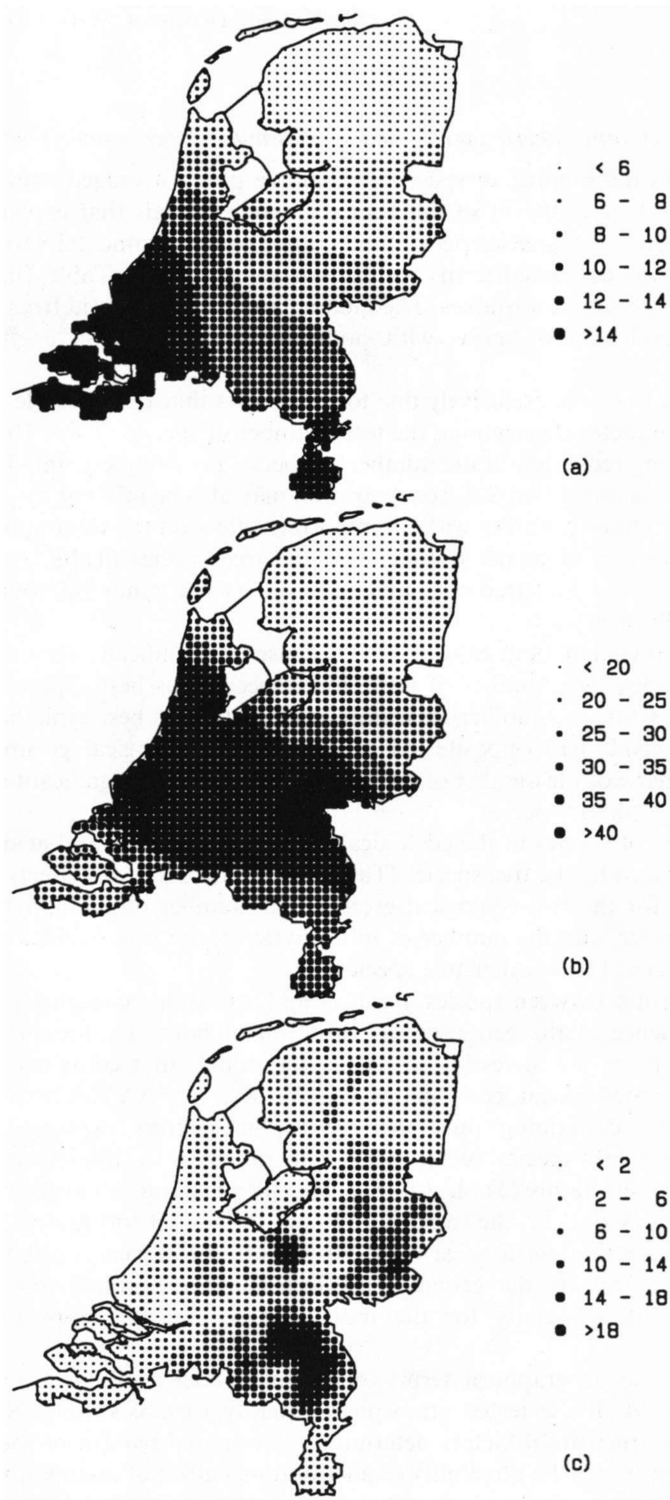

Fig. 2. Spatial pattern of pollutant concentrations: (a) SO_2, mean over April–September 1988; (b) NO_2, mean over June 1988–May 1989; (c) NH_3 (1988). (a) and (b), calculated after Anonymous (1989 and 1990); (c) after Asman & Van Jaarsveld (1990). Units=$\mu g\ m^{-3}$.

RESULTS

Relation between atmospheric quality and species number per sample point

Figure 1 shows the number of species per sample point, averaged over the $5 \times 5\ km^2$ squares. Table 1 gives the fit of different regression models that explain the relation between the number of species per sample point and environmental variables. The full model (Table 1a) contained terms for the variables listed in Table 1b, plus squared terms for all quantitative variables. The minimal model was derived from the full model by stepwise exclusion of terms with non-significant regression coefficients (*t*-test, $P \leqslant 0{\cdot}001$).

The large fit that was exclusively due to SO_2 shows that this variable was by far the most important factor determining the total number of species (Table 1b). The effect of SO_2 was a strong reduction in the number of species per sample point. The correlation between species number and SO_2 concentration may also be inferred by comparing Fig. 1 with Fig. 2a. Other variables with an important effect on the total number of species were the number of trees per sample, and the tree species (Table 1b). NO_2, NH_3, distance to the coast and tree circumference had only a minor (although statistically significant) influence.

Table 1b shows that both SO_2 and NO_2 caused a significant decrease in the total number of species. The number of nitrophytic species was best explained by the SO_2 concentration, while the number of acidophytic species was best explained by the NO_2 concentration. NH_3 had opposite effects on the two ecological groups: it caused a significant increase of the number of nitrophytic species, and a significant decrease of the number of acidophytic species.

The numbers of species in the ecological groups (nitrophytes and acidophytes) were strongly influenced by the tree species (Table 1b). As for NH_3, the effects of tree species were opposite for the two ecological groups. The number of acidophytic species was significantly larger, and the number of nitrophytic species was significantly smaller on *Quercus* compared to the other tree species.

The relationship between species numbers and atmospheric quality parameters is a direct consequence of the geographical variation of both. The importance of (bio)-geographical effects was investigated by testing models that contained the first- and second-order geographical coordinates (X, Y, X^2, Y^2, XY) as explanatory variables. A model containing only the geographical terms explained 30% of the variance in the 'all species' case, nearly as much as a model containing only atmospheric quality terms (Table 1a). Statistically speaking, atmospheric quality can therefore be replaced in the models by any other set of factors that have a geographical variation on a scale of *c.* 50–200 km. However, replacing the atmospheric quality terms by the geographical terms in the minimal model resulted in a decrease in fit, especially for the individual ecological groups ('geo+trees' in Table 1a).

Addition of the geographical terms to the full model resulted in a slight (*c.* 2%) increase of the fit. If the tested atmospheric quality variables (SO_2, NO_2, NH_3) are accepted as the true causal factors determining the spatial pattern of species diversity, there is apparently still the possibility of an additional effect of an unknown factor with a comparable type of geographical variation. Such a factor might be (i) a biogeographical effect, or (ii) any other unknown factor affecting species diversity, including unknown pollutants.

Table 1. Multiple regression of number of species per sample point on explanatory variables. Regression models were tested for three selections of species: all species=total number of species, nitrophytes=number of nitrophytic species, acidophytes=number of acidophytic species. Number of observations=1208. (a) Fit ($R^2_{adj} \times 100$) of different models derived for the three species groups. The full model contained the terms listed in Table 1b, and quadratic terms for all quantitative variables. The 'full model+geo' contained the terms of the full model plus the first- and second-order geographical terms (coordinates: X, Y, X^2, Y^2, XY). The minimal model was derived from the full model by stepwise exclusion of non-significant (P>0·001) terms. The terms of the minimal model were the terms with a significant effect in the corresponding column in Table 1b. The atmospheric quality model contained only terms for SO_2, NO_2 and NH_3. The geography model contained only the geographical terms. The 'geo+trees' model contained the geographical terms and terms for tree species, circumference and number of trees. (b) Extra fit of explanatory variables in the minimal model. The absolute value of each column entry is the decrease in fit of the minimal model when omitting the term(s) for a single variable; the sign is the sign of the regression coefficient of a linear term for that variable. NS=variable that does not belong to the minimal model for a species group because it has no significant contribution (P>0·001). !=variable with non-linear effect; the sign indicates whether the effect is monotonously decreasing (−) or increasing. !! or ?!!?=variable with unimodal effect, reaching a maximum (!!) or a minimum (?!!?) within its range in our data; the sign indicates whether the modelled effect at the highest value in our data is lower (−) or higher than the modelled effect at the lowest value in our data. (c) Extra fit of interaction terms. The absolute value of each column entry is the increase in fit when adding a single term to the minimal model; the sign is the sign of the regression coefficient of that term. NS=term with no significant effect (P>0·001)

	All species	Nitrophytes	Acidophytes
(a) Model			
Full model	45	36	42
Full model+geo	48	38	44
Minimal model	44	35	41
Atmospheric quality	31	19	23
Geography	30	14	19
Geo+trees	40	21	29
(b) Variable			
SO_2	−15·1	−14·0!	NS
NO_2	−1·7	NS	−10·3?!!?
NH_3	0·5	5·5	−5·2
Populus	NS	3·4	−4·1
Salix	NS	1·7	−1·5
Ulmus	3·1	8·9	−3·6
Fraxinus	0·9	1·5	−0·6
Tilia	NS	1·1	−2·0
Pollard	NS	NS	−1·0
Distance coast	−1·4	−1·3?!!?	0·7
Tree circumference	0·4	NS	NS
Number of trees per sample	7·9!!	4·6!!	6·0
(c) Term			
Quercus. NH_3	NS	NS	NS
Quercus. SO_2	−0·7	NS	−3·1
Distance coast. SO_2	NS	NS	NS
$SO_2.NH_3$	NS	NS	NS

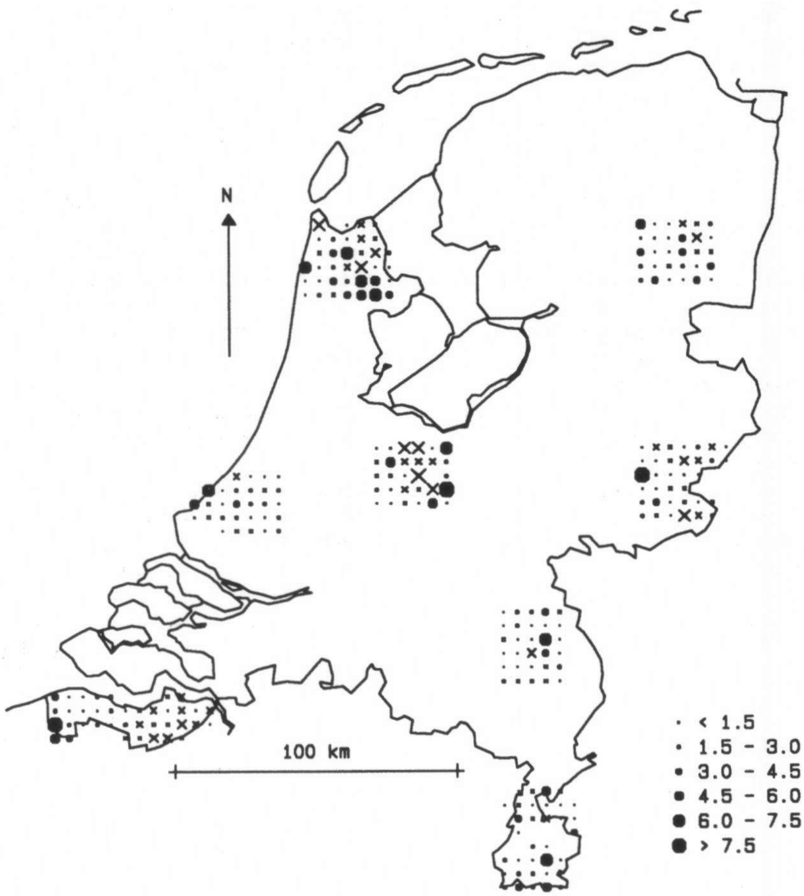

Fig. 3. Residual (observed – fitted) number of species per sample point, averaged per grid square. The fitted values were determined with the minimal model (see Table 1b). The size of each symbol refers to the magnitude of the residual; dot=positive, X=negative residual.

Biogeographical effect. In our models (including the minimal model) a term for distance to the coast has been used to account for a biogeographical effect. Many epiphytic lichens have a strongly coastal ('atlantic') distribution (see, e.g. distribution maps in Barkman 1958 or Seaward & Hitch 1982), and the general species diversity may therefore decrease on moving inland. Indeed, the minimal model showed a small but significant negative effect of distance to the coast on the total number of species (Table 1b).

Unknown factors. The effect of unknown factors was further investigated by mapping the 'residual' (observed minus fitted) number of species after fitting the minimal model. The residuals did not show a clear spatial pattern (Fig. 3), although there were clusters of squares with a positive (dots) or a negative mean residual (X). Although such clusters might be due to local pollution sources, differences in land use seem a more probable explanation. In a number of cases the boundaries between clusters with a positive and a negative residual coincide with geographical boundaries. For example, this is the case in the area Bilthoven, where the central part is mostly afforested (negative residual),

Table 2. Multiple regression of number of species per grid square on explanatory variables, for the three species groups as in Table 1. Number of observations=264. (a) Fit ($R^2_{adj} \times 100$) of full and minimal model. The full model contained the terms listed in Table 1b, and quadratic terms for all variables. The minimal model was derived from the full model by stepwise exclusion of non-significant ($P>0{\cdot}001$) terms. The terms of the minimal model were the terms with a significant effect in the corresponding column in Table 2b. The atmospheric quality model contained only terms for SO_2, NO_2 and NH_3. (b) Extra fit of explanatory variables in the minimal model. The absolute value of each column entry is the decrease in fit of the minimal model when omitting the term(s) for a single variable; the sign is the sign of the regression coefficient of a linear term for that variable. NS=variable that does not belong to the minimal model for a species group because it has no significant contribution ($P>0{\cdot}001$). !! or ?!!?=variable with unimodal effect, reaching a maximum (!!) or a minimum (?!!?) within its range in our data; the sign indicates whether the modelled effect at the highest value in our data is lower (−) or higher than the modelled effect at the lowest value in our data

	All species	Nitrophytes	Acidophytes
(a) Model			
Full model	64	50	62
Minimal model	62	48	60
Atmospheric quality	53	37	58
(b) Variable			
SO_2	−27·5	−27·8!!	NS
NO_2	−1·3	NS	−30·1?!!?
NH_3	NS	5·6	−1·7
Distance coast	3·1	−7·8?!!?	11·5!!
Number of samples	8·0!!	5·4	NS

while the eastern part is mainly in agricultural use (positive residual). Another example is the area Wieringerwerf, where a cluster of squares with a negative residual coincides with the Wieringermeer polder, where recolonization started after an inundation in 1945.

The minimal model explaining the total number of species did not contain squared terms except for the number of trees per sample. Apparently, the effect of all other tested variables was close to linearity. The minimal model for the numbers of species in the ecological groups (nitrophytes and acidophytes) contained some more non-linear terms (Table 1b). The effect of some of the interaction terms was tested by adding single terms to the minimal model (Table 1c). The only important interaction effect was that of SO_2 on the number of acidophytes occurring on oak (decrease, 3% extra fit). The effects of the other tested interaction terms were not significant.

Relation between atmospheric quality and species number per grid square

The same statistical procedures were used as for the number of species per sample point, and the results (Table 2) were largely comparable (decreasing number of species at higher SO_2 or NO_2 concentration or at greater distance to the coast). However, the percentage explained variance was appreciably higher in this case (62% instead of 44%), and so was the fit that was exclusively due to SO_2 (*c.* 27% in the 'all species' case). Both in the 'all species' case and for the nitrophytes the fit of SO_2 far exceeded the fit of all other variables, but for the acidophytes NO_2 was the most important explanatory

Table 3. Forward selection of environmental variables in FDA. Extra fit=increase in fit (percentage explained variance) when adding a term to a model containing the terms above this term; cumulative fit=fit of a model containing this term and all terms above. The full model contained all terms listed in Table 1b+c, except the number of trees per sample point which was used as a covariable. Selection was stopped when the increase in fit on including a term was less than 1·0%. The significance of the effects was tested by means of permutation, and exceeded P=0·01 for all terms after 99 permutations. Differences in the cumulative fit and the sum of the extra fit and the cumulative fit in the preceding row are due to rounding errors

Term	Extra fit	Cumulative fit
SO_2	7·9	7·9
NH_3	2·8	10·7
Quercus	3·1	13·9
Tilia	2·0	15·8
Circumference	2·0	17·8
NO_2	1·3	19·1
Distance coast	1·1	20·2
Pollard	1·1	21·3
Quercus. SO_2	1·1	22·3
Full model		25·2

variable (Table 2b). The effect of NH_3 on the total number of species per grid square was not significant. Some non-linear effects were found (Table 2b).

Relation between atmospheric quality and vegetation composition

Table 3 gives the result of a forward selection of the environmental variables according to the importance of their effect, using RDA and permutation testing. All terms listed in Table 1b, and also the interactions term of oak and SO_2 had a significant (P<0·01) effect. Figure 4 shows the species scores for a selection of common species, together with the canonical (regression) coefficients of the environmental variables. The significance of the effects of SO_2 and NH_3 is presented in the form of a t-value biplot (Fig. 5).

Lecanora conizaeoides and *L. muralis* were the only species whose abundances were significantly positively correlated with the SO_2 concentration (Figs 4 and 5). The abundances of nearly all other species were negatively correlated with both SO_2 and NO_2 (Fig. 4) and for all species (except *Parmelia caperata* and *P. saxatilis*) the correlation with SO_2 was significant at P<0·05 (Fig. 5). The negative correlation of the abundance of most species with SO_2 and NO_2 causes a decrease of the total number of species at high concentrations of these compounds, which is consistent with the findings presented above. Similarly, the negative correlation of the abundance of most species with distance to the coast (Fig. 4) is consistent with the decrease in species number at greater distance to the coast (Table 1b).

The effect of NH_3 was radically different from the effects of SO_2 and NO_2. The numbers of species that were positively or negatively related with this compound were approximately equal (Fig. 4). All nitrophytic species were positively correlated with

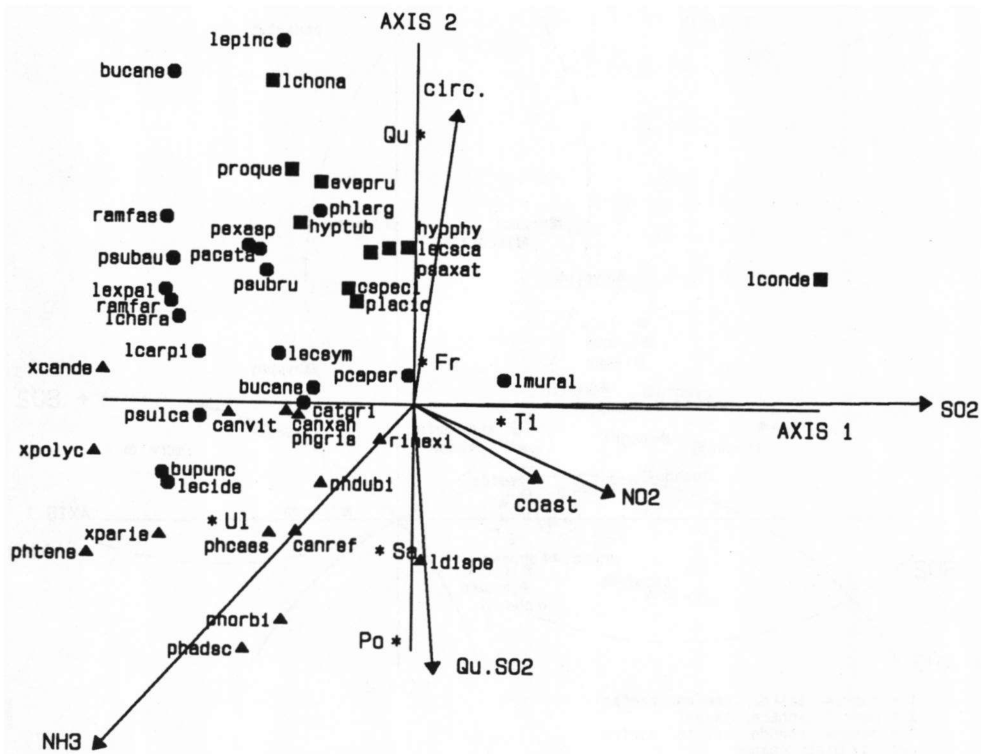

Fig. 4. Regression biplot of species scores and environmental variables, based on redundancy analysis with the plotted variables as explanatory variables and number of trees per sample as covariable. The tree species are given as the centroids of the sample scores per tree species (asterisks, species names abbreviated to the first two characters), plotted on a scale that is 0·5 times the scale of the species scores. Species scores are plotted for species with more than 30 occurrences: triangle=nitrophyte; square=acidophyte; circle=indifferent or undetermined. The biplot explains 72% of the variance in the fitted abundance values (eigenvalues of the first three axes are 0·11, 0·06 and 0·03, respectively). Species: bucane, *Diploicia canescens*; bugris, *Buellia griseovirens*; bupunc, *Buellia punctata*; canref, *Candelariella reflexa*; canvit, *Candelariella vitellina*; canxan, *Candelariella xanthostigma*; catgri, *Cliostomum griffithii*; cspeci, *Cladonia* spec.; evepru, *Evernia prunastri*; hypphy, *Hypogymnia physodes*; hyptub, *Hypogymnia tubulosa*; lcarpi, *Lecanora carpinea*; lchera, *Lecanora chlarotera*; lchona, *Lecanora pulicaris*; lconde, *Lecanora conizaeoides*; ldispe, *Lecanora dispersa*; lecide, *Lecidella elaeochroma*; lecsca, *Hypocenomyce scalaris*; lecsym, *Lecanora symmicta*; lepinc, *Lepraria incana*; lexpal, *Lecanora expallens*; lmural, *Lecanora muralis*; paceta, *Parmelia acetabulum*; pcaper, *Parmelia caperata*; pexasp, *Parmelia exasperatula*; phadsc, *Physcia adscendens*; phcaes, *Physcia caesia*; phdubi, *Physcia dubia*; phgris, *Physconia grisea*; phlarg, *Phlyctis argena*; phorbi, *Phaeophyscia orbicularis*; phtene, *Physcia tenella*; placic, *Placynthiella icmalea*; proque, *Pyrrhospora quernea*; psaxat, *Parmelia saxatilis*; psubau, *Parmelia subaurifera*; psubru, *Parmelia subrudecta*; psulca, *Parmelia sulcata*; ramfar, *Ramalina farinacea*; ramfas, *Ramalina fastigiata*; rinexi, *Rinodina exigua*; xcande, *Xanthoria candelaria*; xparie, *Xanthoria parietina*; xpolyc, *Xanthoria polycarpa*.

NH_3, while all acidophytic species were negatively correlated. Most of these correlations were significant at $P<0{\cdot}05$ (Fig. 5).

Figures 4 and 5 indicate that the variation in the epiphytic vegetation entails (i) variation in species diversity (the species-poor sites being dominated by *Lecanora conizaeoides*), and (ii) dominance by either nitrophytic or acidophytic species. Variation (i) is reflected by the first (horizontal) RDA axis in Figs 4 and 5, and is primarily determined by SO_2 and NO_2 concentration. Variation (ii) is reflected by the second (vertical) axis, and is primarily determined by NH_3 concentration and tree species. For

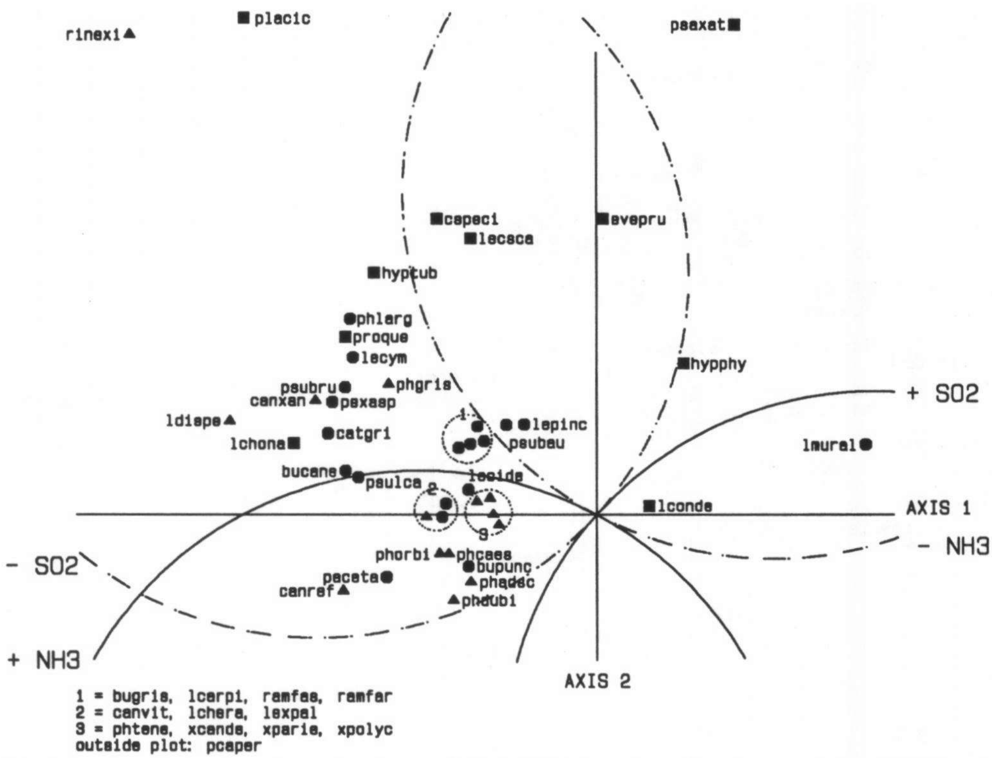

Fig. 5. T-value biplot of species and environmental variables, based on redundancy analysis with SO_2 and NH_3 concentration as explanatory variables and the other variables used in Fig. 4 (except the interaction term) as covariables. Species are indicated as in Fig. 4. The significance of the effects of SO_2 and NH_3 in the model of Fig. 4 can be read from this figure for each species with the aid of the plotted 'Van Dobben circles' (Ter Braak & Looman 1994). Species located inside the circle segment marked '$+SO_2$' are significantly ($P \leqslant 0.05$) positively correlated with SO_2; those located inside the circle segment marked '$-SO_2$' are significantly negatively correlated with SO_2. Similarly, the circle segments marked '$+NH_3$' and '$-NH_3$' indicate the significance of the effects of NH_3. Eigenvalues are 0·07 and 0·01 for the first and second axis, respectively.

the third and higher axes no meaningful ecological interpretations could be found. The low eigenvalue of these axes (0·03 for the third and 0·02 for the fourth axis) shows that these axes do not reflect important sources of variation.

Changes between 1988 and 1989

The country-wide mean number of species per sample point significantly ($P<0.05$, paired t-test) increased from 11·4 in 1988 to 12·6 in 1989. The mean number of species per grid square also significantly ($P<0.001$) increased from 21·7 to 23·6. The regional increase in species number is given in Table 4. The increase in species number was smallest in the south (regions Wijnandsrade and Braakman) and largest in the centre of the country (regions Vlaardingen and Bilthoven).

Figure 6 is a PCA ordination diagram based on the species–environment relationship determined for 1989, with the sample scores as means for the eight areas in 1988 (passive) and 1989 (active). The sample scores for the 2 years were significantly different on the first axis (paired t-test, $P<0.001$), but not on the second axis. The shift on the first axis was also significantly different between the areas (ANOVA, $P \simeq 0.01$). The significance

Table 4. Regional increase in species numbers between 1988 and 1989. Mean incr. = mean absolute increase in number of species per sample point and per grid square, respectively. Different letters indicate significant differences between regions (ANOVA, $P \leqslant 0.05$). See Fig. 1 for the location of the regions

Region	Species per sample		Species per square	
	Samples (n)	Mean incr.	Squares (n)	Mean incr.
Wijnandsrade	141	0·9 ab	38	2·1 ab
Vredepeel	148	1·3 bc	30	2·1 ab
Braakman	142	0·8 a	38	1·3 a
Vlaardingen	143	2·0 d	30	2·8 b
Wieringerwerf	146	1·1 ab	36	1·4 ab
Bilthoven	149	1·6 c	29	2·4 b
Eibergen	149	1·1 ab	35	1·4 ab
Witteveen	146	1·0 ab	30	1·9 ab

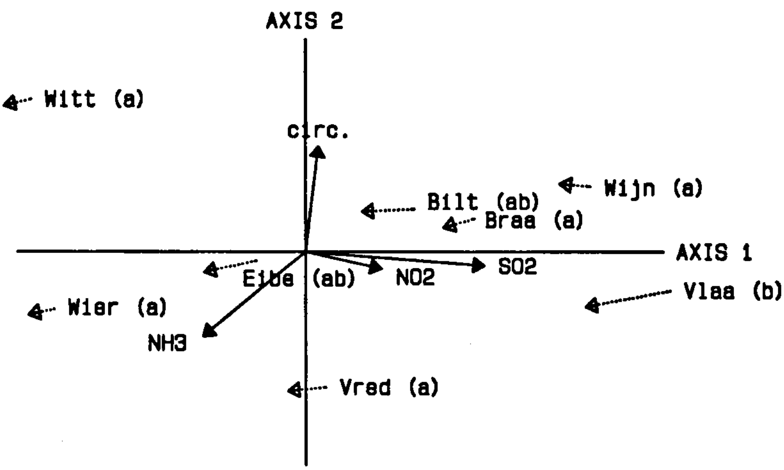

Fig. 6. Distance biplot of mean sample scores per region in 1989 (head of dotted arrows, active samples) and in 1988 (base of dotted arrows, passive samples). Regions: Wijn = Wijnandsrade, Vred = Vredepeel, Braa = Braakman, Vlaa = Vlaardingen, Wier = Wieringerwerf, Bilt = Bilthoven, Eibe = Eibergen, Witt = Witteveen; see Fig. 1 for the location of the regions. Letters indicate the significance of the differences in (1988–89) shift along the first axis (ANOVA, $P \leqslant 0.05$). The shift along the second axis was not significantly different from zero. Solid arrows: regression (canonical) coefficients of environmental variables. Tree species, number of trees and distance to the coast were used as covariables. Eigenvalues were 0·10 and 0·03 for the first and second axis, respectively.

of the area-to-area comparisons is indicated in Fig. 6. The first axis was primarily determined by SO_2 and NO_2, but also by NH_3 (see Fig. 6). Of these compounds NO_2 and NH_3 were virtually constant over time, but SO_2 decreased at a rate of *c.* 1·2 μg m^{-3} year^{-1} over the period 1979–89 (Anonymous 1978–90; Van Dobben 1993). If SO_2 concentration is assumed to be the only variable with a temporal change, its rate of change can be inferred by multiplying the shift in sample score on the first axis with the canonical (regression) coefficient of SO_2 on this axis, yielding a value of -0.76 μg m^{-3} year^{-1}.

Epiphytes are often assumed to react to changes in atmospheric quality over a period of *c.* 2–5 years (Henderson-Sellers & Seaward 1979; Showman 1981). The mean summer SO_2 concentration at the sample points decreased from $14{\cdot}4 \pm 3{\cdot}9\ \mu g\ m^{-3}$ to $10{\cdot}5 \pm 3{\cdot}9\ \mu g\ m^{-3}$ between 1983 and 1988, yielding an average change of $-0{\cdot}78\ \mu g\ m^{-3}\ year^{-1}$. Apparently the assumption of SO_2 as the main cause for the change in the epiphytic vegetation yields an estimate for the decrease in SO_2 concentration that is approximately correct.

The second axis was primarily determined by NH_3 and tree circumference (Fig. 6). Since the difference in sample score on this axis was not significant the vegetation changes were probably not caused by succession (correlated with or caused by the increase in tree circumference) or by NH_3. The shift in the composition of the vegetation in the eight regions (reflected by the shift in sample scores in Fig. 6) runs more or less parallel to the increase in species number per region (Table 4). Both were largest in the region Vlaardingen, which also had the highest SO_2 concentration (Fig. 2a). The regions with the highest NH_3 concentration (Vredepeel and Eibergen) did not have a strongly deviating increase in species number or change in vegetation composition compared to the other regions (Fig. 6, Table 4). This indicates that the changes in the epiphytic vegetation were caused by a decrease in SO_2 concentration rather than by high NH_3 concentrations.

DISCUSSION

A comparison of Fig. 1 and De Wit's (1976) Appendix 12 shows that in the period between De Wit's study and the present one the spatial pattern of epiphyte biodiversity has not dramatically changed. However, the absolute figures did change. Methodological differences prevent a direct comparison of De Wit's and our results, but a global comparison is possible. De Wit's 'classes of epiphyte richness' were computed per grid square for each tree species on the basis of the total number of epiphyte species occurring on that tree species in that square. There were six classes of epiphyte richness, and the class boundaries were different for the various tree species (dependent upon the country-wide mean number of epiphyte species on each tree species). The final class assigned to a square was the highest of the class numbers derived for each of the tree species in that square.

Table 5 shows for each region our mean numbers of species per sample point and per grid square, together with the approximate number of species per grid square on the 'richest' tree species in 1972, inferred from data given by De Wit (1976). The latter number was estimated from the class numbers for the squares in each region, the epiphyte-richest tree species in that region, and the numbers of epiphyte species for the classes on that tree species (Table 5). If there were no changes in epiphytic vegetation, De Wit's number of epiphyte species per 'richest' tree species per grid square is expected to be somewhat above our mean number of species per sample point (because De Wit's number represents a maximum and ours a mean value), but lower than our mean number of species per grid square (because some epiphyte species that do not occur on the richest tree species will still occur on other tree species). However, Table 5 shows that the number of species inferred from De Wit's classes is mostly in the same range as our mean number of species per sample point, and far lower than our number of species per grid square. The largest increase probably took place in the region Vlaardingen, where

Table 5. Comparison of De Wit's (1976) data and our data. Species per sample = number of species per sample point in our data (mean ± SD); species per square = number of species per grid square in our data (mean ± SD); richest tree sp. = tree species with the highest class of epiphyte richness (De Wit, Appendix 80–81); class = approximate 'epiphyte class' in De Wit's final map (Appendix 12); ep. spec. per tree sp. = approximate number of epiphyte species per grid square on the 'richest' tree species (De Wit, Appendix 6). See Fig. 1 for the location of the regions. Tree species: qu, *Quercus robur*; po, *Populus* × *canadensis*; sa, *Salix alba*; ul, *Ulmus* × *hollandica*, fr, *Fraxinus excelsior*; ti, *Tilia* spp.

	Our data		De Wit's data		
Region	Species per sample	Species per square	Richest tree sp.	Class	Ep. spec. per tree sp.
Wijnandsrade	9 ± 5	17 ± 6	fr, po	2–4	3–13
Vredepeel	12 ± 4	23 ± 4	po	3–4	7–13
Braakman	10 ± 5	18 ± 7	sa, po	1–4	4–13
Vlaardingen	9 ± 4	18 ± 4	ul, sa, ti	1–2	0–8
Wieringerwerf	18 ± 6	31 ± 7	ul, po	4–5	15–20
Bilthoven	12 ± 6	25 ± 8	qu, sa	2–4	4–15
Eibergen	14 ± 4	25 ± 5	qu	3–5	8–21
Witteveen	19 ± 6	37 ± 6	qu	4–6	13–23

in 1972/1973 only five species were present over large areas (De Wit 1976, Appendix 57), while presently the mean number of species per sample point in that region was *c.* 9 (Table 5).

The relatively strong increase of the nitrophytic species, which has become apparent from many other studies (e.g. De Bakker 1986, 1989; Van Dobben 1987) can also be inferred from a comparison of De Wit's and our data. In De Wit's data the nitrophytes *Physcia caesia*, *Ph. orbicularis* and *Ph. adscendens* occurred in *c.* 20–40% of the grid squares (De Wit 1976, Appendix 84), while in our data these species were present in *c.* 60–90% of the squares. The studies of Van Dobben (1987) and De Bakker (1989) ascribed this strong increase of nitrophytic species to high NH_3 concentrations. However, our study did not yield direct evidence to support this hypothesis. Other factors, such as differences in dispersal capacity, may also have caused differences between the species in their rate of increase. The study of such factors was outside our present scope.

Our regression models (Tables 1–3) support De Wit's (1976) conclusions that (i) the spatial pattern of epiphyte diversity is primarily determined by the SO_2 concentration, and (ii) the number of species per grid square is a good measure for the SO_2 concentration. Of all studied parameters the number of species per grid square had the strongest correlation with atmospheric quality (62% variance accounted for by the minimal model, and 53% by a model containing only atmospheric quality terms; Table 2). These percentages of explained variance were lower for the number of species per sample point (minimal model 44% and atmospheric quality model 31%; Table 1) and the composition of the vegetation (full model *c.* 25% and atmospheric quality model *c.* 12%, using RDA; Table 3). Both the number of species per sample point and the abundances of the individual species are apparently affected by local variation in ecological factors that is not accounted for by the measured variables. The number of species per grid square is the result of observations made at several sample points and is probably less affected by such local sources of variation.

The general decrease in number of species at greater distance from the coast is probably just a biogeographical effect. The 'coastal effect' reported by De Wit (1976) (a smaller effect of SO_2 in coastal regions) did not become apparent from our data as the effect of the interaction term of distance to the coast and SO_2 was not significant (Table 1c).

The presence of two distinct directions of variation in the epiphyte vegetation (species-rich vs. species-poor, and dominance of nitrophytes vs. dominance of acidophytes; Figs 4 and 5) suggests the presence of two 'master' factors affecting vegetation composition. These factors could be (i) the presence of toxic substances (SO_2, NO_2) determining species diversity, and (ii) bark pH (which in turn is dependent upon NH_3 concentration and tree species), determining the dominance of nitrophytes or acidophytes. It is now well established that the occurrence of 'nitrophytes' is not primarily determined by nitrogen but rather by bark pH (Wirth 1991).

Our results are very similar to the findings of Hawksworth & McManus (1989) in central London, and Letrouit-Galinou *et al.* (1992) in Paris. In both studies a rapid recolonization of epiphytic lichens during the 1980s was reported, although for a smaller number of species than in our study. This difference is probably due to the SO_2 concentration in London and Paris which is still high, albeit decreasing, compared to Dutch rural circumstances (in the order of 50 $\mu g\ m^{-3}$). The return of epiphytic lichens at decreasing SO_2 concentration may be a general phenomenon in north-western Europe.

Our data show that the general increase in species diversity that took place after 1973 still continued in 1989. The environmental circumstances for epiphytic lichens are apparently greatly ameliorated since the 1970s. The rate and direction of the changes in the epiphytic vegetation between 1988 and 1989 suggest the decrease in SO_2 concentration as the most probable cause for these changes. It is therefore tempting to ascribe the change over the complete period to the decrease in SO_2. However, if SO_2 is accepted as the main cause for the changes, there is still the apparent contradiction to be explained of a strong increase in nitrophytic species and no effect of an increase in NH_3 concentration. This problem is now subject to further research.

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