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Effects of NO_x and NH_3 on lichen communities and urban ecosystems

A Pilot Study

A report produced by Imperial College
& The Natural History Museum, as partners in the

A.P.R.I.L.

Network for the Department for Environment, Food and Rural Affairs

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A.P.R.I.L.
AIR POLLUTION RESEARCH IN LONDON
A research network supported by EPSRC, DEFRA & EA

APRIL is a multidisciplinary research network that aims to deliver research projects identified by academics, local and national government and other stakeholders working in urban environments. Through regular seminars, workshops and meetings a large research programme has arisen covering the following major topics: Modelling, Measurements, Meteorology, Natural Environment, Health, Planning, Economic & Social Issues, Transport and Indoor Air Quality. A key element of the research programme is collaboration between many different disciplines and organisations in London and other parts of the UK. By combining elements of each specialist group's research programme two large consortium proposals have arisen and several more are in preparation. The first, Meteorology and Air Pollution in London's Environment (MAPLE, measures meteorological and pollution parameters of the air mass coming into London, in and above the city itself and the air mass leaving the city. It involved climatic and pollutant measurements at three sites, Cliffe on the southeastern perimeter of the city, Regents Park in the centre of London and Silwood Park to the West. Campaigns to assess pollution at ground level and in the vertical profile are proposed to provide a greater understanding of the sources of particulates and oxides and nitrogen and their chemistry and dispersion over a large city to specifically inform decisions on the most appropriate abatement strategies to meet London's air quality objectives and generally to improve scientific understanding of the urban environment. Funding is required.

The second consortium proposal, Dispersion of Air Pollution & Penetration into the Local Environment (DAPPLE), funded by EPSRC, investigates the finer scale pollution problems at street corners and junctions where the highest concentrations from traffic emissions arise and where human exposure is potentially greatest. This study is located in the Marylebone Road, Westminster and combines expertise from five universities. It will provide much needed measurements and lead to improvements in the modelling tools used to assess the impact of pollutants on health, including the accidental or terrorist releases of toxic or flammable gases, spatial design and the location of buildings. Other funded projects are studying the impact of pollution on plants and birds in the city and a major health study is currently in preparation.

APRIL host seminars and conferences, specialist workshops and meetings, and members participate at a national and international level at air quality events. The Network, established in 1999, is led by a steering committee chaired by Professor Helen ApSimon of Imperial College, London, supported by: Professor Mike Batty, UCL, Professor Bernard Fisher (Environment Agency), Professor Frank Kelly (King's College), Professor Alan Robins (Surrey), Professor Lord Julian Hunt (UCL), Dr. Roy Colvile (Imperial), Dr. Steve Smith (King's), Dr. Claire Burton (EPSRC), Dr. Janet Dixon (DEFRA), Jim Storey (Environment Agency (EA), David Hutchinson (GLA), Chris Lee (ALG), Steve Hedley (King's College), Linda Davies (Network Co-ordinator, Imperial College).
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Background and Executive Summary

The increase of oxidised nitrogen in urban areas and reduced nitrogen in rural areas has been widely recognised in Europe. This pilot study investigates the impact of reduced and oxidised nitrogen on sensitive vegetation at selected sites in England. The Review and Assessment of Air Quality (DETR, 2000) resulted in Air Quality Management Areas (DEFRA, 2000) being declared by twenty-nine of the thirty-three local authorities in London making it the largest urban area in the UK in breach of the Health Objectives for nitrogen dioxide of 21 ppb (annual mean). Objectives for total oxides of nitrogen (16 ppb) and sulphur dioxide (8 ppb) have been determined to protect sensitive vegetation and ecosystems from harm and although not applicable in urban environments, nevertheless are relevant. Both sites of conservation interest (e.g. SSSIs, cSACs and NNRs) and priority Biodiversity Action Plan (BAP) species require special protection from nutrient (especially NH_3) nitrogen deposition and it is therefore essential to develop sensitive biomonitoring methods appropriate for such habitats and species groups.

Data collected by Clapp (Clapp & Jenkin 2001[*]) of measurements of total NOx at the roadside in the centre of Westminster averaged 210 ppb NOx, falling to 73 and 23 ppb respectively at the outer London background sites of Hillingdon and Teddington. There are huge diurnal and seasonal variations in values. The modelling of nitrogen dioxide concentrations by the Greater London Authority (GLA, 2001) reproduced in Section 1, Figure 1.1 clearly demonstrates the gradient in nitrogen dioxide concentrations across London and was used to select three inner London sites and three outer London sites where the diversity, frequency and vitality of lichens and bryophytes were recorded on a single phorophyte, ash (*Fraxinus excelsior)*. Lichen diversity on oak (*Quercus* spp*)* at one central location was also recorded. Background concentrations of nitrogen dioxide decline along a transect from central London through the suburbs and into Surrey where only three local authorities needed to declare Air Quality Management Areas. Heathland communities are particularly sensitive to nitrogen input and formed the basis of a second study looking at nitrogen accumulation in *Calluna vulgaris* and the use of isotopic signatures as an index of traffic-derived pollution in this species.

The primary emission sources of nitrogen in London are transport and heating, contrasting sharply with rural areas where reduced nitrogen from farming activities is frequently the most common form of atmospheric pollution. Intensive agricultural activity in the form of a poultry unit in Norfolk and a dairy farm in Devon provide the third study area where the influence of ammonia on bark pH and lichen communities as determined by van Herk (1999, 2001) was assessed.

Protocols using lichens have been developed to monitor ammonia pollution in The Netherlands that have defined ‘nitrophyte’ and ‘acidophyte’ indicator species (van Herk) as well as an EU recording method to detect environmental changes. Part of this pilot study tests the application of these protocols in the UK in selected urban and rural sites. More detailed investigations at the microscale level were carried out using lichens transplanted from a rural site to the centre of London at the Natural History Museum wildlife garden (Cromwell Road) to assess eco-physiological changes in selected species. In addition, the impact of urban NOx emissions on higher plants was studied, along a transect of decreasing concentrations away from the city centre. The following methods were tested:-

- The EU Directional Quantitative Lichen Monitoring protocol was used to detect changes in epiphytic lichen diversity on trunks of ash trees between sites in inner and outer London.
- Twigs with a healthy lichen community were transplanted within a clean air site in Somerset and to the wildlife garden at the Natural History Museum adjacent to Cromwell Rd in the vicinity of NOx monitoring gauges. The health of individual thalli of selected species was assessed using chlorophyll fluorescence.
- A transect study was carried out during winter 2001/2, to assess changes in shoot chemistry of *Calluna* (heather) along a gradient of decreasing NO_x pollution from central London to rural Surrey. Shoots were analysed for concentrations of nitrogen, carbon, phosphorus, lead, zinc and stable isotopes of nitrogen.
- The van Herk method was tested on oak trees in rural localities in different climatic regions in SW (North Wyke, Devon) and E England (Thetford) in the vicinity of ammonia monitoring networks and Sites of Special Scientific Interest.

The pilot survey in London showed differences in lichen diversity on ash trees between inner and outer London sites correlating with concentrations of transport emissions. Foliar nitrogen concentrations in *Calluna* were positively related to NO_2 concentrations and also decreased significantly with increasing distance from central London. Carbon:nitrogen ratios showed the opposite relationship with both NO_2 and distance, with linear regressions again statistically significant. High phosphorus concentrations in central London were responsible for a significant increase in foliar N:P ratios with distance from the city centre and suggested that phosphorus availability would not limit plant response to nitrogen at any of the study sites. Unlike studies elsewhere using mosses, foliar lead and zinc concentrations do not appear to be a useful index of exposure to traffic-derived pollution in *Calluna.* This may be because the sites used in this study were typically located at distances of more than 150 m from major roads, or because higher plants are more effective at excluding heavy metals than mosses or lichens. Delta ^{15}N measurements in *Calluna* were positively related to NO_2 concentrations and declined significantly with increasing distance from central London and increasing distance from major roads. This may suggest a decreasing contribution of traffic-derived NO_x to the nitrogen nutrition of higher plants away from busy roads and in less urban areas, although nitrogen transformations within the soil (and plant) make it difficult to interpret this signal without further investigation.

The high diversity of lichens on oak trees in central London suggests that species recovery is not as limited by slow dispersal mechanisms as previously considered. Over a 3-month period transplanted material from Somerset to a roadside site across a NOx gradient in London showed great variation in persistence and vitality between species. The chlorophyll fluorescence data requires further testing and evaluation over a longer time period to develop its application as a biomarker of stress for NOx.

Foliar nitrogen concentrations in *Calluna* and nutrient ratios suggest that many sites in urban areas experience high deposition inputs of nitrogen and as such are at risk from changes in species interactions and ecosystem function. The widespread occurrence of 'nitrophyte lichens' (as defined by van Herk) in urban and rural sites investigated in the pilot project suggests that both oxidised and reduced nitrogen is readily assimilated by these lichens. Further work is required to establish the sensitivity of urban vegetation to realistic levels of both NO and NO_2, to determine the implications of

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[*] Daily daylight-averaged NOx, annual mean concentration using 1998-1999 data ppbv.

current and future emissions of traffic-derived pollution on urban ecology and to define indicators appropriate for London. It is to be expected that species new to Britain or science will be discovered as was found during surveys in the Netherlands.

Indices of nitrophyte and acidophyte species were correlated with ammonia levels (Thetford) and distance from source (North Wyke). However, many nitrophyte species defined in Holland were absent from the sites in the UK, suggesting that indicator species must be evaluated on a regional basis. The importance of both climate and pollution history in the UK was also highlighted by the survey. Highly pollution sensitive Lobarion species that are also indicators of ecological continuity were present at North Wyke whilst at Thetford acidophytes on ancient trunks in conservation sites testified to former acidification. In both sites younger trees and particularly twigs supported the majority of nitrophyte species.

NO_2 and NH_3 data from the nearest National Diffusion Tube / Ammonia Monitoring Network sites may not provide the most accurate representation of vegetation exposure to NO_x / NH_3 in urban green spaces and the wider countryside. This pilot study has highlighted the need for standardised biomonitoring recording techniques in combination with physico-chemical and modelling data. This is necessary to develop practical indicator scales appropriate to the UK that can be used to monitor the new pollution climate. The data from this pilot study offer an insight into temporal and spatial differences in selected sensitive species under changing environmental conditions. The project does not consider other primary or secondary pollutants from these sources, climate change or differences in management practice.

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This report has been compiled in three sections, corresponding to the separate projects funded under contract to DEFRA

Section 1. (two parts) Impacts of NO_x pollution on lichens

Part 1. Corticolous Lichens in London

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Section 3. Assessing the role of biological monitoring using lichens to map excessive ammonia (NH_3) deposition in the UK.

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SECTION 1

Impacts of NO_x Pollution on Lichens

PART 1: CORTICOLOUS LICHENS IN LONDON

Appendices

Appendix A

Appendix B

Appendix C

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1. INTRODUCTION

This pilot study investigates the diversity and distribution of corticolous lichens on a single phorophyte, ash (*Fraxinus excelsior*) at six sites in London and on oak (*Quercus*) at one site. It was proposed as part of a wider study covering all thirty-two London Boroughs and the City of London recording corticolous lichens, bryophytes and fungi on five *Fraxinus* at each site and young oak (*Quercus*) at selected sites.

The project investigates both spatial and temporal trends in relation to species diversity, vitality and community structure, making reference to the EU Limit values (Air quality framework Directive, 1996) to protect sensitive species from the effects of sulphur dioxide and oxides of nitrogen. The annual mean of 16 ppb (30 $\mu g/m^3$) determined for oxides of nitrogen (NOx) is widely exceeded in London. In contrast the sulphur dioxide annual mean and winter mean (1 Oct to 31 Mar) of 8 ppb (20 $\mu g/m^3$) are rarely exceeded. These values have been incorporated into the Air Quality (England) Regulations 2000 (DETR, 2000). They do not apply in urban environments but nevertheless are critical levels for the protection of sensitive species. Lichens and bryophytes are less well protected from atmospheric pollutants than higher plants because they do not possess cuticles or stomata. Some species, however, are better adapted and demonstrate greater tolerance than others. Only one corticolous species, *Lecanora dispersa* was recorded in central London in 1967 (Laundon, 1970) at a time when the limited diversity in most urban environments was attributed to the impact of sulphur dioxide.

This study applies, for the first time in the UK, the proposed EU Quantitative Lichen Monitoring protocol proposed as a standardised recording technique to assess lichen diversity as an indicator of environmental change.

For this pilot study Westminster, Southwark and Tower Hamlets were selected as the inner London sites, all within 6 km of Charing Cross, and outer London locations were selected in Enfield, Harrow and Bromley. A contour map prepared by the Greater London Authority demonstrating the concentration gradient of nitrogen dioxide decreasing with distance from central London to the suburbs is provided (Appendix B).

2. SITE DESCRIPTIONS

2.1 Inner London

2.1.1 Westminster
Regent's Park (TQ 282 833)
Distance from Charing Cross 3 km

Regent's Park evolved from a 16th Century Royal Hunting Estate into a public park in the early 1800s. Huge areas of trees had been removed to meet timber demands, creating a major challenge in landscaping and design on an area overlying a great depth of London clay. An ambitious tree planting programme, formal gardens and ornamental lakes have transformed the park into a major recreational centre with a wide variety of flora and fauna covering a total area of over 170 hectares.

Ash trees in the vicinity of the York Bridge entrance on the Outer Ring Road, close to the Marylebone Road were selected for the survey, and young oaks adjacent to the Heather Garden. The oaks are situated further from the Inner Road, away from traffic influences and are sheltered by a children's play area. They are closer to London Zoo, and adjacent to the Bird House and lake.

Air pollution data from the kerbside Marylebone monitoring station 0.5 km from York Bridge is provided below but background estimated concentrations calculated by Westminster Local Authority using 1997 data suggest that lowest concentrations of nitrogen dioxide at the present time would be in the region of 31 ppb. The range of total NOx measured at this site is between 50 and 500 ppb (Clapp & Jenkin, 2001).

Annual Average NO_2	1997	Background	31 ppb
Annual Average NO_2	1999	Kerbside	47 ppb
Annual hourly max NO_2	1999	Kerbside	169 ppb
Annual Average SO_2	1999	Kerbside	5 ppb

Nitrogen dioxide measurements for all sites are taken from the SEIPH 1999 Annual Report (SEIPH, 2001), unless otherwise stated, and represent measurements recorded by the London Air Quality Network.

2.1.2 Southwark
Burgess Park (TQ 333 778)
Distance from Charing Cross 4 km

This recreational Park was created in the post war period for the enjoyment of the people of Southwark and includes several ornamental lakes, play areas and landscape features with a wide variety of trees, including lime, plane, and ash, many shrubs and cultivated beds. It covers an area of approximately thirty-five hectares and lies between two major road links. Background concentrations of nitrogen dioxide measured in 1997 with diffusion tubes give an average annual value of 21 ppb in the centre of the park (Southwark, 2001):

Annual Average NO_2	1999 Roadside	39 ppb
Annual hourly Max NO_2	1999 Roadside	112 ppb
Annual Average SO_2	1999 Background	4 ppb

SO_2 values are taken from the DEFRA website maintained by AEA/NETCEN (AEA, 2001).

2.1.3 Tower Hamlets

Victoria Park (TQ 353 835)
Distance from Charing Cross 6 km

This park was created in 1888 in response to the demands of local residents for recreational space in East London. It extends across some 35 hectares and is beautifully landscaped with a variety of mixed broadleaf trees, shrubs, ornamental beds and two lakes. The park is bordered on both sides by major transport links and modelled data (Tower Hamlets, 2002) suggest that background concentrations of nitrogen dioxide remain at approximately 26 ppb in the surveyed areas.

Annual Average NO_2	2002 Background	26 ppb
Annual Average NO_2	1999 Roadside	34 ppb
Annual hourly Max NO_2	1999 Roadside	119 ppb
Annual Average SO_2	1999 Roadside	3 ppb

2.2 Outer London

2.2.1 Bromley

Jubilee Country Park (TQ 436 680)
Distance from Charing Cross 18 km

This country park is a secondary woodland with remnants of ancient woodland, pasture and cultivated areas covering approximately 25 hectares. It has many small mixed areas of oaks, ash and willow supported by open glades with old field boundaries, hedges and ponds enhancing the open aspect. The western end of the park is dominated by hawthorn with elder, pine and birch. Exceedences of the nitrogen dioxide Objectives are not expected in Bromley, unlike most other London boroughs, with background levels modelled at 15ppb. Additional modelled data on NO_2 by the local authority provides the following information:

NO_2 Annual Average Roadside	16 ppb
NO_2 Hourly Maximum	55 ppb
SO_2 Annual Average	3 ppb

2.2.2 Enfield

Covert Way Nature Reserve (TQ 263 974)
Distance from Charing Cross 17 km

This secondary woodland covering approximately 10 hectares, is set within a quiet sheltered suburban location close to Hadley Common. It is rich in mature oak and ash which are quite densely planted in parts, with a small area of grassland in the centre. A return to coppicing within the site has created a more open aspect complementing the denser plantings in this small conservation area, maintaining a good bryophyte flora and a haven for avian populations.

1999 NO_2 Annual Mean Background	18 ppb
1999 NO_2 Hourly Background Max	90 ppb
1999 NO_2 Annual Mean Roadside	24 ppb
1999 NO_2 Hourly Roadside Max	93 ppb
1999 SO_2 Annual Mean	3 ppb

2.2.3 Harrow

Canons Park, Stanmore (TQ 183 915)
Distance from Charing Cross 16 km

This is an 18th Century Estate with some formal features retained, but now managed as open parkland comprising a narrow mixed broadleaf woodland area, open aspect trees, including both ancient and recently planted oaks and a spinney of younger oak, ash, sycamore and elm. Some planted garden areas and ornamental features remain.

1999 NO_2 Annual Mean Suburban	18 ppb
1999 NO_2 Hourly Max Suburban	86 ppb
1999 SO_2 Annual Average	3 ppb

3. METHODS

3.1 Tree selection

Ash was selected as the major phorophyte as it is widely distributed across London, with oak as the second species. Due to time and budget constraints only six sites were selected and surveys of ash carried out at all sites with oak being examined at Regent's park only. Eight bark pH measurements were taken per tree. Trees were selected to meet the following criteria: unbranched below 200 cm, upright, open aspect, without injury or disease with a minimum girth of 50 cm and a maximum girth of 150 cm. They were selected at a minimum distance of 150 metres from the park entrance in order to reduce roadside influence. The first five trees meeting the selection criteria were surveyed.

3.2 Lichen Sampling Strategy

The approach follows that of Asta et al. (2002). A narrow five-laddered quadrat was attached to the tree at a height of 150-cm using stainless steel pins at each intersection. The quadrat comprises five grid squares, each measuring 10 x 10 cm extending to cover 50 cm in depth. It was positioned at four orientations, North, South, East and West. All lichen species in each quadrat were recorded and given a value of 1, allowing a maximum frequency score of 5 per quadrat and 20 per tree for each species. The Lichen Diversity Value (LDV) for each site is then calculated by adding the frequencies of all species in each quadrat segment and dividing by the number of trees examined (5). In addition, all species below 50 cm and all species above 50 cm up to 200 cm were recorded and any special features noted.

3.3 Bark Acidity Measurements

Bark pH readings at positions 1 and 5 in the quadrat were measured using a flathead electrode (HI 8014 pH meter and BDH Gelplas double junction Flat Tip electrode 309/100/09) according to Looney & James (1988) and Farmer et al. (1990). The bark was moistened with a solution of KCl (0.1 mol) and the reading taken after three minutes, immediately following a second application of KCl.

3.4 Bryophytes, Algae, Fungi and Liverworts

All epiphytes were recorded

3.5 Species identification

Three techniques were employed for identification:

Field examination using a x10 hand lens and chemical tests (Orange, 2002)
Microscopical examination of thalli and spores
Thin Layer Chromatography (Orange, 2002)

The nomenclature follows lists maintained by Brian Coppins (British Lichen Society) (see http://www.argonet.co.uk/users/jmgray/syn.htm)

3.6 Statistical methods

Correlations were calculated using Excel. Species composition and frequency was investigated using multivariate ordination analysis that serves to reduce complex species-site data to a form that is visually interpretable. While all multivariate analyses were based on the raw data, additional analyses were performed on transformed (double square root; presence-absence) to detect any improved relationships when effects of dominance were reduced. The methods used were:

Non-Metric Multidimensional Scaling (MDS): data from the 10 stations were subjected to analysis using MDS that is currently widely used in the analysis of spatial and temporal change (e.g. Warwick & Clarke, 1991). The recorded observations from the 6 stations were exposed to computation of triangular matrices of similarities between all pairs of samples. The similarity of every pair of sites was computed using the Bray-Curtis index on the raw and transformed data. Clustering was by a hierarchical agglomerative method using group average sorting, and the results are presented as a dendrogram and as a two-dimensional ordination plot.

SIMPER: the MDS clustering program was used to analyze differences between sites. SIMPER (Plymouth Marine Laboratories PRIMER package) enables those species responsible for differences to be identified by examining the contribution of individual species to the similarity measure.

CONPLOT: this program (Plymouth Marine Laboratories PRIMER package) permits environmental variables to be superimposed on MDS plots, giving a visual indication of correlation between clusters and environmental factors (i.e. pH).

4. RESULTS

4.1. Lichens

Species Diversity

A total of 56 different species were recorded. The data are presented in Table 1. (Appendix A)

- Highest diversity was recorded on ash at the two outer London sites of Harrow and Enfield, with 35 species at each site. The third outer London site, Bromley yielded 29 species.

- The three inner London sites all carried 20 species.

- 32 species were recorded on oak in Regents Park.

Fraxinus : 24 species
Arthonia spadicea, Bacidia arceutina, B. laurocerasi, B. naegelii, Caloplaca phlogina, Candelariella reflexa, C. vitellina, Cliostomum griffithii, Dimerella pineti, Diploicia canescens, Hyperphyscia adglutinata, Hypotrachyna revoluta, Lecanora dispersa, L. muralis, Lecanora saligna, Micarea prasina, Phlyctis argena, Physcia aipolia, P. caesia, P. dubia, Punctelia ulophylla, Rinodina subexigua, R. exigua and *Strangospora pinicola*.

Quercus: 6 species
Lecanora albella, Parmelina tiliacea, P. saxatilis, Physconia grisea, Pleurosticta acetabulum, Rinodina gennarii.

Common to both tree species: 26 species
Amandinea punctata, Bacidia delicata, Candelaria concolor, Evernia prunastri, Flavoparmelia caperata, Flavoparmelia soredians, Hypogymnia physodes, Lecanora carpinea, L. chlarotera, L. conizaeoides, L. expallens, L. symmicta, Lecidella elaeochroma, Lepraria incana, Melanelia subaurifera, Parmelia sulcata, Parmotrema chinense, Phaeophyscia orbicularis, Physcia adscendens, P. tenella, Punctelia subrudecta, Ramalina farinacea, Scoliciosporum chlorococcum, Xanthoria candelaria,
X. parientina and *X. polycarpa.*

Species common to all sites*: Amandinea punctata, Bacidia delicata, Parmelia sulcata, Physcia adscendens* and *P. tenella*.

4.2 Lichen Diversity Values (LDV's)

Highest values were recorded at Harrow, followed by Southwark, and Bromley (Appendix A, Table 2):

4.3. Other corticolous flora on *Fraxinus excelsior*

12 Bryophytes:

Amblystegium serpens, Brachythecium rutabulum, Dicranoweisia cirrata, Dicranum scoparium, Eurhynchium praelongum,Grimmia pulvinata, Hypnum cupressiforme, H.andoi, Orthotrichum affine, O.diaphanum, O.lyellii, Ulota crispa.

2 Parasitic Fungi:

Lachnella alboviolascens, Gloniopsis praelonga

2 Liverworts:

Lophocolea bidentata, Frullania sp.

4 Algae:

Desmococcus viridis, Athelia arachnoidea, Prasiola crispa, Trentepohlia sp.

4.4 Statistical Analysis

4.4.1 Species Numbers

Dendrograms based on species presence on ash at each site (Appendix C, Figure 1) and ash and oak combined demonstrate that similarity percentage contributions lead to a division at the sixty percent level into inner and outer London sites for ash. Flora on the oaks in Regent's Park are more closely aligned to the Outer London sites.

4.4.2. Species Frequency

In all cases quadrat segments (NSWE) cluster together in the 3 principal groups, apart from the north segment quadrat at Tower Hamlets. Bromley trees initially separate at the eighteen percent level (i.e. these are the most distinct) with two further major groups: the first comprising, Southwark, Regents and Tower Hamlets (apart from the north segment) with Enfield, Harrow and the north segment of Tower Hamlets closely aligned (Appendix C, Figure 2).

4.4.3 Species below 50 cm

The dendrogram (Appendix C, Figure 3) suggests a difference between those sites with more dense housing located away from residential areas and those of a more densely populated location where the dog zone would be most influential in creating an eutrophicated substrate in addition to nitrification from anthropogenic emissions. *Xanthoria parientina* and *X. polycarpa* were not recorded in Bromley although they were widespread and frequent at the other sites and are typical species of nitrogen enriched environments. Further details on the origins of the designation 'nitrophyte' and 'acidophyte' can be found in the Section 2: Assessing the role of biological monitoring using lichens to map excessive ammonia (NH_3) deposition in the UK, Appendix 1.

4.5 Girth and pH.

The girth and pH measurements for the sites are given in Table 3 (Appendix A). Ranges on ash were from 3.7 to 5.59 and 2.7 to 5.19 on Oak. The lowest Ash pH was recorded in Bromley. The data presented in Figure 4.5 illustrate that Ash trees at Bromley have a significantly lower bark pH as determined by a Spearman rank correlation coefficient ($p = 0.01$).

4.6 Vitality

Few signs of poor health were recorded except at Canons Park, Stanmore, where some of the macrolichens were dying. However, the largest thalli of *Parmelia sulcata* and *Flavoparmelia caperata* were measured at this site. Thalli of *Hypogymnia physodes, Evernia prunastri* and *Ramalina farinacea* were generally below normal at less than 0.5 cm. Thalli of *Physcia* species at the Enfield site were numerous, but in the region of 0.5 cm, possibly just colonising the recently coppiced woodland area compared with the very well developed populations at the inner London sites.

5. DISCUSSION

This pilot study investigated the distribution of corticolous lichens at six sites in London. As so few sites have been studied results should therefore be interpreted with care. However there are some interesting results that require further consideration. The species number is very high for such a small sample of only thirty-five trees and the species recorded include many not seen for over fifty years, as well as some never recorded in London before (James et al. 2002). In particular, the macrolichen *Pleurosticta acetabulum* and the crustose *Strangospora pinicola* represent a flora uncommon to urban environments in recent decades.

5.1 Temporal and Spatial Trends

Species numbers have increased significantly over the past fifty years and correlate with falling concentrations of sulphur dioxide (all below the critical level of 8 ppb) and increasing concentrations of nitrogen and other transport emitted pollutants as demonstrated in Figs 1 and 2.

Fig. 1

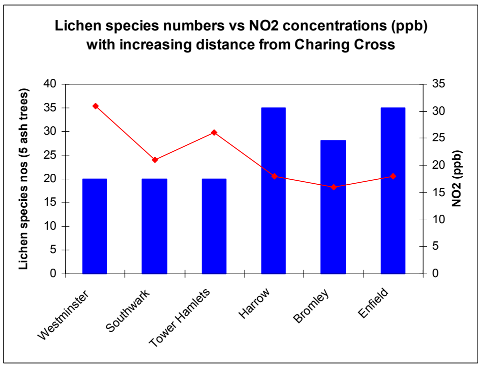
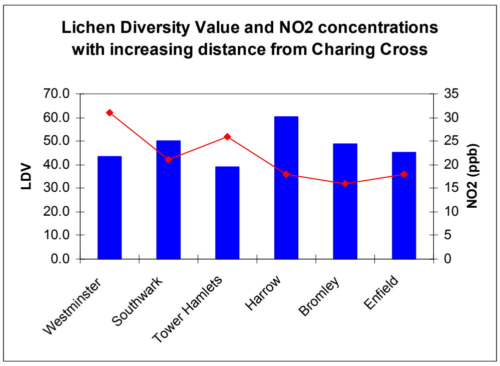


Fig. 2



Laundon (1970) analysed species diversity and distribution in London in the late 1960s. He observed the decline in urban populations to a single species in central London, *Lecanora dispersa*, and a total of nine corticolous species were recorded throughout London (an area defined within a radius of 16 km from Charing Cross).

Significant increases in populations were recorded in 1980 (Rose & Hawksworth 1981) and 1988 (Hawksworth & McManus, 1988) with total corticolous and lignicolous species on mixed tree species across fifty sites recorded as 49, 17 of which were found in Regent's Park. Both these and more recent surveys (Bates et al 1990, 1992, 2001) have referred to the slow reinvasion rates for specific species in London, and noted a change in community structure with the widely distributed species *Hypogymnia physodes* and *Parmelia saxatilis* decreasing and an increase in *Flavoparmelia caperata* and *Physcia aipolia*.

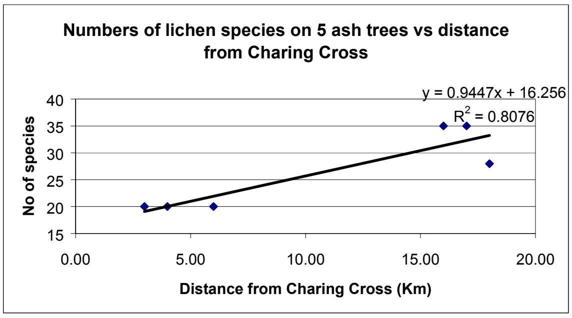


Fig. 3

Spatial distribution suggests that inner London ash carry a significantly lower species range than ash in outer London areas (Fig. 3). The use of a single phorophyte for this study has identified a trend not obvious from other recent studies when lichen diversity on mixed tree species and other substrates are recorded. The young oaks were in a very sheltered position but nevertheless suggest that dispersal mechanisms are bringing a rich diversity of species into the centre of London.

The inner London sites carry many of the characteristics of the *Xanthorion* (James, 1974) community and include a high percentage of species favouring high nitrogen environments. The communities recorded on the Oaks resemble more closely those of the *Parmelia caperata –Pertusaria spp.* community described as characteristic of open woodland*: Lecanora chlarotera, Flavoparmelia caperata, Parmotrema chinense, Parmelia saxatilis* and *P. sulcata*.

5.2 Bark pH and Girth

Lichens are very sensitive to bark pH and many species are limited to specific ranges. Using two phorophytes therefore increases the range of potential diversity.
It is interesting to note the lower bark pH of the ash in Bromley where transport emissions and dog zone influences were at their lowest within the study area

The pH and girth of the oaks demonstrate their young age and may account for the large number of species recorded, contrasting sharply with the small number of species on mature oaks in Regent's Park (communicated, Davies L.) and Kensington Gardens (Bates et al 1990, 2001).

5.3 Air Quality

Annual average concentrations of sulphur dioxide across the area are within the recommended Objectives for sensitive vegetation. Nitrogen dioxide concentrations in Inner London range from 50 at roadside locations and 31 at background locations to a low of 14 ppb at outer London sites. Data for nitric oxide are not included in the study but concentrations in London would add significantly to the total NOx concentration as highlighted by Clapp & Jenkin (2001).

6. CONCLUSIONS

The large number of species recorded in this pilot survey demonstrates a significant increase in lichen diversity in London in recent decades.

However, a comparison of species on ash suggests a major difference in species number and composition between the inner and outer London sites. We have identified a trend towards increasing diversity with distance from Charing Cross. The importance of using the same substrate for comparative purposes is highlighted and further enhanced by the diversity on young oaks in Regents Park (James et al. 2002). This study utilises for the first time the proposed EU Quantitative Lichen monitoring methodology. The approach provides a standardised recording technique to assess lichen diversity as an indicator of environmental change.

Both nitric oxide and nitrogen dioxide are toxic to sensitive species, but very few studies have investigated this effect. Concentrations of NO are higher in central London and frequently exceed nitrogen dioxide concentrations.

The high number of ‘nitrophytes’ suggests that oxidised nitrogen is readily assimilated by many lichen species and is encouraging species considered indicative of eutrophicated areas, particularly on the urban fringe. These results are related to the data collected in other parts of this study.

The disappearance of some species preferring a more acidic substrate was noted during this study.

The survey suggests that species diversity in London is perhaps not as limited by slow dispersal mechanisms as previously considered, although development may be arrested in sensitive species as demonstrated by the frequency of small thalli of several species such as *Evernia prunastri and Ramalina farinacea* and their rarity at other sites The presence of fertile species reproducing by sexual reproduction only (designated F, in Table 1, Appendix A) is interesting and requires further evaluation. An intriguing possibility is that some lichens in London may have existed in forms unrecognisable to lichenologists waiting until conditions are appropriate for development of typical thalli, a phenomenon previously suggested for the lichen *Xanthoria parietina* by Ott (1987). Most lichens identified, however, are able to reproduce asexually using isidia (I) or soredia (S), detachable outgrowths on the thallus containing the photobiont.

7. RECOMMENDATIONS

- The study area should be extended to cover more sites in London to investigate further the association between lichen diversity and oxides of nitrogen identified by this pilot study. In particular the study should include roadside locations where the impact of NO will be highest, contrasting with the background locations selected in this study.

- Species demonstrating a limited tolerance range should be selected for study at a physiological level.

- This study suggests that sensitive species may respond differently to oxidised nitrogen across a range of concentrations and in different forms. These aspects require further investigation particularly in relation to the Objectives for Sensitive Vegetation and Ecosystems. London provides an ideal laboratory to study the effects of oxidised nitrogen. Ambient concentrations of nitrogen dioxide are the highest in the UK (GLA 2001), nitric oxide is particularly high in inner London and ozone and ammonia generally lower than in rural environments.

- The results of this part of the study support the need for a joint project to investigate further the uptake and effect of oxidised and reduced nitrogen on sensitive plant species in relation to current critical levels for nitrogen dioxide and the impact of ammonia.

- The EU draft protocol for lichen biomonitoring used in this report, whilst offering a valuable protocol for quantifying lichen diversity, requires further evaluation concerning assessment of nitrogen emissions.

- The information gained through the above studies should be used as a precursor to developing practical indicator scales and in the development of their regulatory role to complement physicochemical monitoring and modelling studies.

APPENDIX A

Table 1 Species Diversity Summary. N= nitrophytes; A = acidophytes; F = fertile; S = sorediate; I= isidiate

Summary	Regents park	South-wark	Tower Hamlets	Enfield	Harrow	Bromley	Total	Regents park	Total	N & A	F & S
	Fx	Fx	Fx	Fx	Fx	Fx	Fx		All		
Amandinea punctata	1	1	1	1	1	1	6	1	7		F
Arthonia spadicea				1			1		1		F
Bacidia arceutina			1	1			2		2		S
Bacidia delicata	1	1	1	1	1	1	6	1	7		S
Bacidia laurocerasi				1			1		1		F
Bacidia naegelii				1			1		1		F
Caloplaca phlogina						1	1		1		S
Candelaria concolor				1			1	1	2		S
Candelariella reflexa		1	1	1	1	1	5		5	N	S
Candelariella vitellina	1	1		1	1	1	5		5	N	F
Cliostomum griffithii				1		1	2		2		F
Dimerella pinetii				1		1	2		2		F
Diploicia canescens					1		1		1		S
Evernia prunastri		1	1	1	1	1	5	1	6	A	S
Flavoparmelia caperata		1		1	1	1	4	1	5		S
Flavoparmelia soredians					1		1	1	2		S
Hyperphyscia adglutinata				1	1	1	3		3		S
Hypogymnia physodes					1	1	2	1	3	A	S
Hypotrachyna revoluta				1		1	2		2		F
Lecanora albella							0	1	1		F
Lecanora carpinea					1	1	2	1	3		F
Lecanora chlarotera				1	1	1	3	1	4		F
Lecanora conizaeoides	1	1				1	3	1	4	A	SF
Lecanora dispersa	1	1	1	1	1	1	6		6	N	F
Lecanora expallens	1			1	1	1	4	1	5		S
Lecanora muralis	1						1		1	N	F
Lecanora saligna				1			1		1		F
Lecanora symmicta				1	1		2	1	3		F
Lecidella elaeochroma			1	1	1	1	4	1	5		F
Lepraria incana/lob			1	1	1	1	4	1	5	A	S
Melanelia subaurifera		1	1	1	1	1	5	1	6		S
Micarea prasina			1				1		1		SF
Parmelia saxatilis							0	1	1	A	I
Parmelia sulcata	1	1	1	1	1	1	6	1	7		S
Parmelina tiliacea							0	1	1		I
Parmotrema chinese				1	1	1	3	1	4		S
Phaeophyscia orbicularis	1	1	1	1	1		5	1	6	N	S
Phlyctis argena				1			1		1		S
Physcia adscendens	1	1	1	1	1		4	1	5	N	S
Physcia aipolia	1	1			1		3		3		F
Physcia dubia					1	1	2		2	N	S
*Physcia sp.**	1	1	1	1	1	1	6		7		S
Physcia tenella	1	1	1	1	1	1	6	1	7	N	S
Physconia grisea							0	1	1		S
Physica caesia					1		1		1	N	S
Pleurosticta acetabulum							0	1	1		F
Punctelia ulophylla					1		1		1		S
Punctelia subrudecta	1	1			1	1	4	1	5		S
Ramalina farinacea	1			1	1	1	4	1	5		S
Rinodina gennarii							0	1	1	N	F
Rinodina subexigua	1						1		1		F
Rinodina exigua			1		1		2		2		F
Scoliciosporum chlorococcum	1	1	1	1	1		5	1	6		SF
Strangospora pinicola				1	1	1	3		3		F
Xanthoria candelaria	1	1	1	1	1	1	5	1	6	N	S
Xanthoria parietina	1	1	1	1	1		5	1	6	N	F
Xanthoria polycarpa	1	1	1	1	1		5	1	6	N	F
	20	20	20	35	35	28		32			

Table 2. Frequency Values: Lichen Diversity Values (Asta, 2002)

	North	South	East	West	Totals
Westminster	16.8	9.6	6.3	11.0	43.6
Southwark	12.8	16.0	11.8	9.4	50.0
Tower Hamlets	8.6	11.2	8.4	10.8	39.0
Harrow	11.4	19.0	15.6	14.2	60.2
Bromley	4.5	22.8	8.8	12.8	48.9
Enfield	10.2	15.0	8.8	11.0	45.0

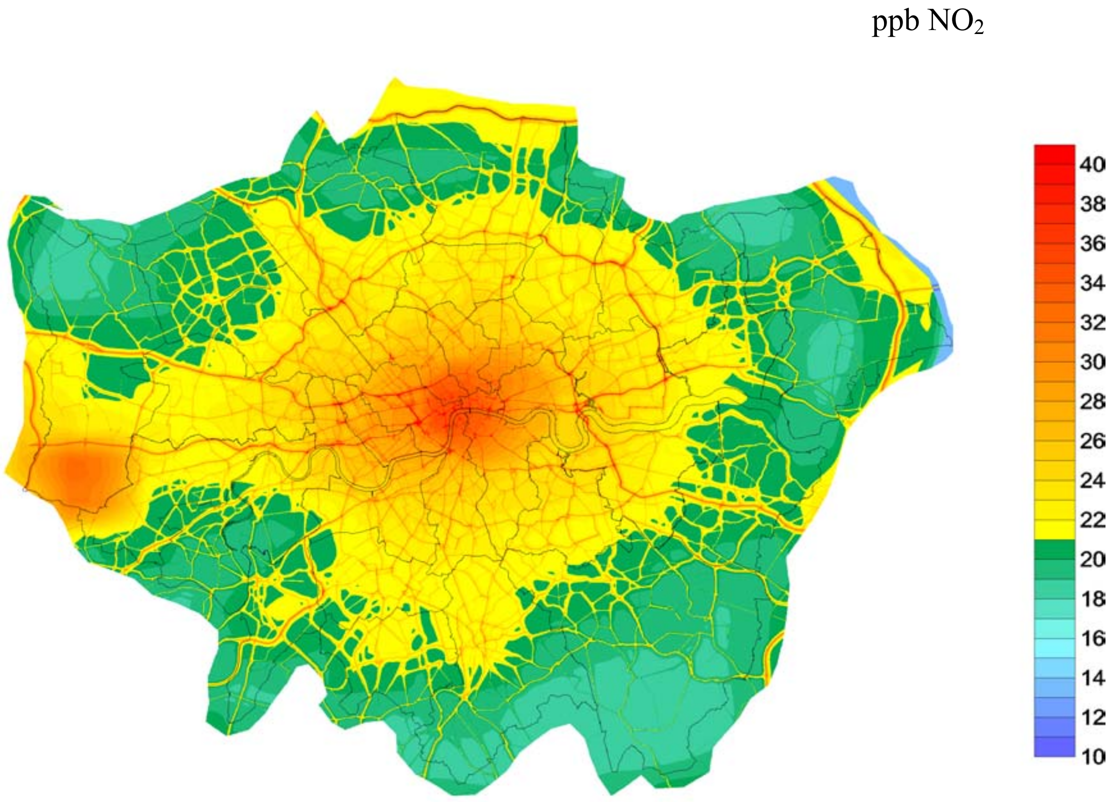
The Lichen Diversity Value is calculated by taking the sum of the frequency of each species as recorded in the total quadrat for the relevant orientation and dividing by the number of trees per site. The totals are then summed to give a final diversity score (lichen diversity value ‘LDV’) per site

	Regents					Tower Hamlets					Southwark					Enfield					Harrow					Bromley				
	R1	R2	R3	R4	R5	T1	T2	T3	T4	T5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	H1	H2	H3	H4	H5	B1	B2	B3	B4	B5
Ash																														
Gir	96	77	107	156	82	65	58	65	52	71	76	79	90	84	92	116	127	70	58	70	50	68	82	91	46	99	108	91	76	133
pH																														
S1	4.5	5	5.1	4.4	4.4	5.5	5.6	4.8	4.7	5.4	4.3	4.5	5	5.3	4.7	4.9	5.6	4.9	5.1	4.9	5	5	5	5.3	5	4.3	4.4	4.5	4.2	4.5
S5	5.2	4.9	5.3	5.2	4.6	5.5	5.2	5.1	4.9	5.4	4.9	5	5	5	4.8	4.5	5.4	5.1	5	5.2	5	4.9	5	5.1	4.9	4.9	4.7	4.6	3.9	4.7
E1	4.7	4.7	5.2	4.7	4.7	5.6	4.9	5.2	5	5.1	4.9	4.5	5.1	5.3	5	5	5.8	5.1	5.1	5.1	5.1	5.1	5	5.3	4.8	3.6	4.3	4.1	3.8	4.9
E5	5	5	5.2	4.2	4.9	5.5	5.2	5.3	5.1	5.3	5	4.9	5.4	5.2	5.1	5.4	5.5	5.1	5	5	4.9	5.2	5.2	5.2	5	4.1	4.4	4.2	3.9	4.9
N1	4.8	4.9	5.2	4.3	4.9	5.8	5.4	5.1	5.1	4.9	4.6	4.5	5.2	5.6	5.3	5.5	5.5	5.2	5.1	5.5	4.9	5	5	5.2	4.2	3.6	4.2	3.7	4.2	3.9
N5	5	4.9	5.1	4.2	4.7	6	5.4	5.1	5.1	5.1	4.8	4.8	4.8	5.4	5.3	5.8	5.6	5.2	5.1	5.6	4.9	5.1	4.9	5	4	4.7	4.2	3.7	3.6	4.3
W1	4.8	5	4.9	4.4	4	5.5	5.5	5.1	5.3	5.5	4.4	4.7	5.1	4.7	5.2	5.3	5.6	5.3	5.3	4.9	5.1	5.2	5.4	5.9	4.7	3.9	4.3	4	3.8	4.6
W5	4.9	5.2	5.3	4.3	4.1	5.8	5.4	5.1	5.4	5.4	4.9	5.5	4.8	4.8	5.3	5.7	5.7	5.3	5.5	4.8	5	5.2	5.4	5.3	4.8	4.5	4.6	4.3	4	4.4
Oak																														
Gir	51	51	62	98	53																									
pH																														
S1	4.2	4.1	4.2	3.2	5																									
S5	4.2	4.3	4.2	3.8	4.9																									
E1	4	4.4	4.7	3.3	4.8																									
E5	4	4.4	4.7	3.9	5.2																									
N1	4.3	4.6	4.4	3.3	4.7																									
N5	4	4.2	4.3	2.7	5.1																									
W1	4.5	4.3	4.5	3.4	4.7																									
W5	4.5	4.1	4.5	3	4.7																									
pH readings - S1 indicates position 1 in quadrat (10x10 cm) at a height of 150 cm(top of quadrat), S5 indicates position 5 at base of quadrat at a height of 60 cm (top of quadrat facing South (then East, North, West)																														
(top of quadrat facing South (then East, North,West)																														

Table 3. Girth and pH measurement

APPENDIX B

Figure 1.1 Modelled Concentrations of Nitrogen Dioxide in London (ppb) 1999 (1997 weather data)
Source: Greater London Authority



APPENDIX C

Figure 1. Species number on Ash by site (PRIMER): A = Outer London Sites. B = Inner London

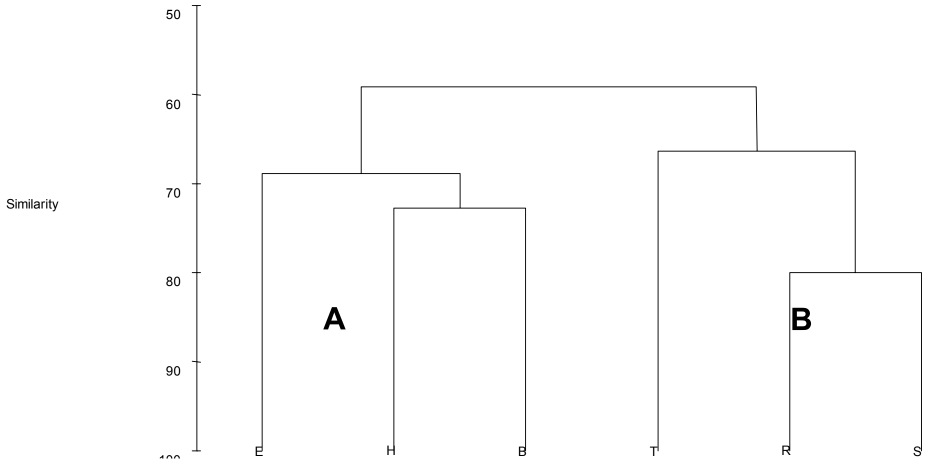


Figure 2. Ash Frequency Data (PRIMER)

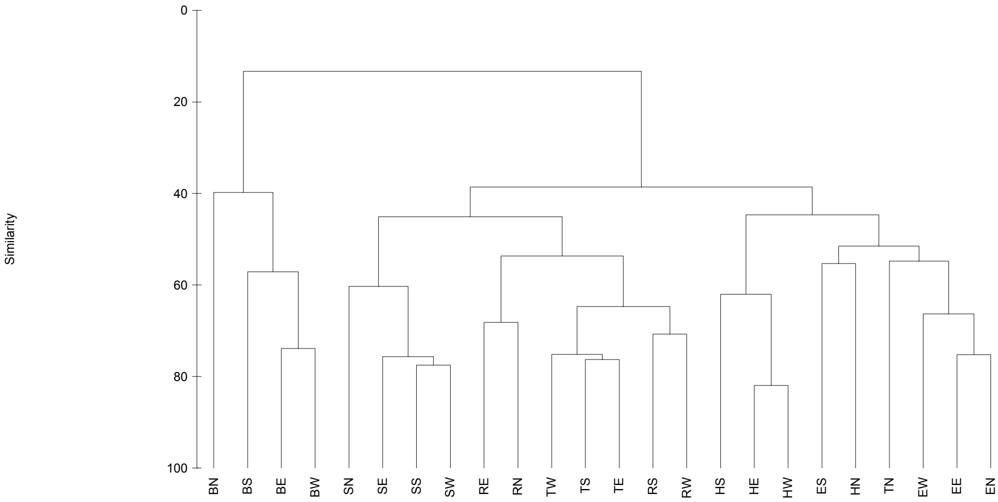


Figure 3. Similarity below 50 cm (Dog Zone) (PRIMER)

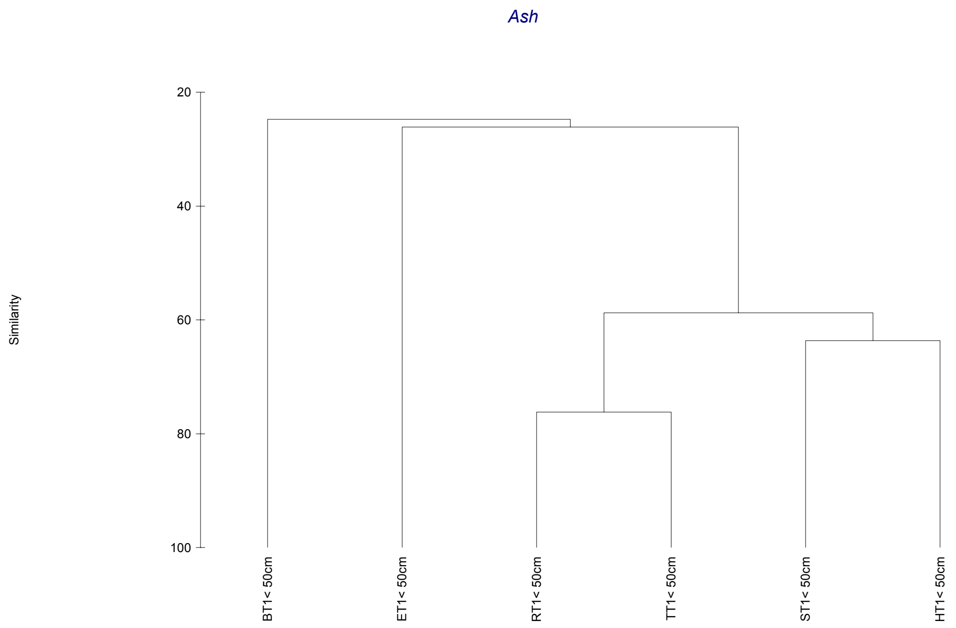
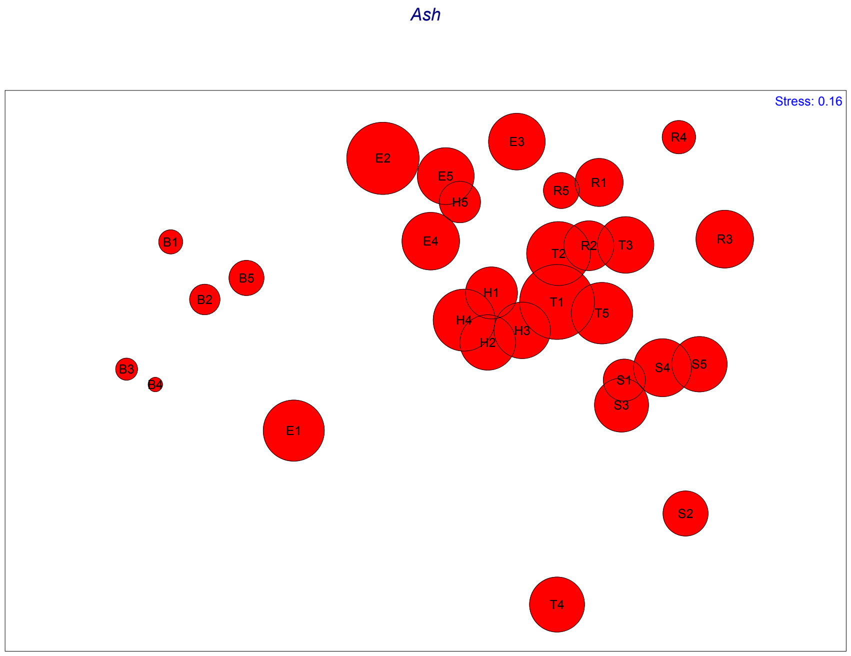


Figure 4. Ash pH ranges (PRIMER)



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PART 2: INVESTIGATING THE IMPACT OF NOx ON TRANSPLANTS

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1. Introduction

Lichen transplants are widely used to assess the impact of pollution in areas where native thalli are rare or absent. Numerous chemical analytical studies have been carried out including monitoring lead from roads, heavy metals from incinerators and large-scale studies investigating spatial and temporal patterns in metal deposition, as well as assessing the impact of pollutants on various physiological parameters (Garty 2001). The national German guideline VDI 3799 relies on a visual assessment of damage to lichen thalli to evaluate phytotoxic effects. As lichens are notoriously sensitive to environmental change, including local climatic factors, recent guidelines emphasise a need to transplant lichens from sites having ecologically similar environments to those of the transplant sites (Mikhailova 2001).

Monitoring studies in London have identified a decrease in SO_2 tolerant species (e.g. Bates *et al.* 2001). At Burnham Beeches, Buckinghamshire, 40 km to the west of London, the appearance of species such as SO_2 sensitive species such as *Xanthoria* and the shrubby lichens (*Evernia prunastri* and *Ramalina*) were noted on oak trees (Purvis *et al.* 2001a,b). Photographic monitoring recorded an impact on lichen growth (*Parmelia sulcata*) during episodic high pollutant emissions, coupled with unusual climatic conditions which suggests that traffic emissions may be responsible for impacts on lichen floras (Purvis et al. 2001a,b). This was supported by chemical analysis of samples demonstrating the accumulation of Zn, N, and other signature elements of traffic pollution. These studies suggested a combination of nitrogen and/or particles may play a role in influencing lichen growth. *P. sulcata* is one of the most widely employed lichen species in Europe to monitor spatial and temporal patterns in metal deposition.

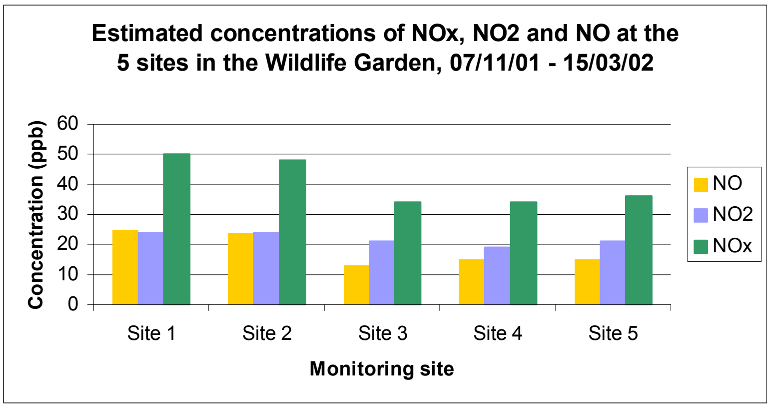


Fig. 1

The Natural History Museum's Wildlife Garden situated next to the junction of Cromwell Road, is infamous for its high traffic density and pollution concentrations (Fig. 1). The DEFRA air pollution monitoring station, part of the automatic pollution recording network is housed in its western corner at the Queen's Gate intersection (*Fig. 2*). Kensington and

Chelsea Environmental Health department has also established weekly dust monitoring guages. Regular exceedances of objectives for human health for NO_2 (21 ppb annual mean) and particles led the Royal Borough of Kensington and Chelsea to declare the borough as an Air Quality Management Area. Imperial College in association with Newcastle, Bradford and Manchester Metropolitan Universities and the Centre for Ecology and Hydrology (CEH) at Bangor is currently conducting a survey to monitor the effect of urban air pollution on vegetation creating an ideal opportunity for the current pilot project.

2. Objectives

To establish lichen transplants in the NHM wildlife garden in the vicinity of existing $N0_x$, 0_3, VOC monitoring gauges in order to evaluate the impact of urban air pollution on epiphytic twig communities over a 3-month period:

- To assess the abundance and vitality of 3 lichen groups: fruticose and SO_2 sensitive (*Ramalina/Evernia*) and the foliose *Parmelia sulcata* (SO_2 tolerant) and *Xanthoria parietina* (N tolerant).

- To sample lichen transplants (*Parmelia sulcata* and *Xanthoria parietina*) along the pollution gradient and retain material for future chemical analysis

- To compare results with physicochemical data and make recommendations for future work.

3. Materials and Methods

Lichens on twigs were selected as transplant materials since:-

- Twigs have been successfully used as transplants both to detect temporal and spatial patterns in metal deposition (e.g. Garty 2001).

- Lichen communities on twigs are particularly sensitive to air pollution (Wolseley 1999)

- The majority of thalli on twigs are young, in active growth and therefore less susceptible to senescence through natural causes

- Twigs established on a pole creates a near natural structure where stem flow effects are minimised

Sample collection was carried out during the first week of December 2001 at Nettlecombe, Somerset. Samples were collected from the outer and lower reachable branches of a single isolated Sycamore tree (*Acer pseudoplatanus*). This species has a neutral/alkaline bark pH and has the closest affinity with the London Plane (*Platanus acerifolio*) surrounding the Wildlife Garden. Approximately 70 twigs aged between 2-8 years and ca. 40-80cm in length were collected and 50 of these mounted on bamboo radial arms supported on five 7 cm diameter pvc pipes. Other more mature twigs (ca.15) aged between 4-18 years from a different sycamore tree were placed on plastic nets at 3 sites at about 5 m from the road along the southern and western boundary of the wildlife garden (*Fig. 2*). The winter period was selected for our experiment owing to shading / barrier effect by trees and increased the microclimatic variation during the Summer period. 20 sticky pads were sited adjacent to the trees at each site according to a method developed by Dr Ben Williamson at the Natural History Museum:
The garden is situated below the Cromwell and Queens Gate roads:

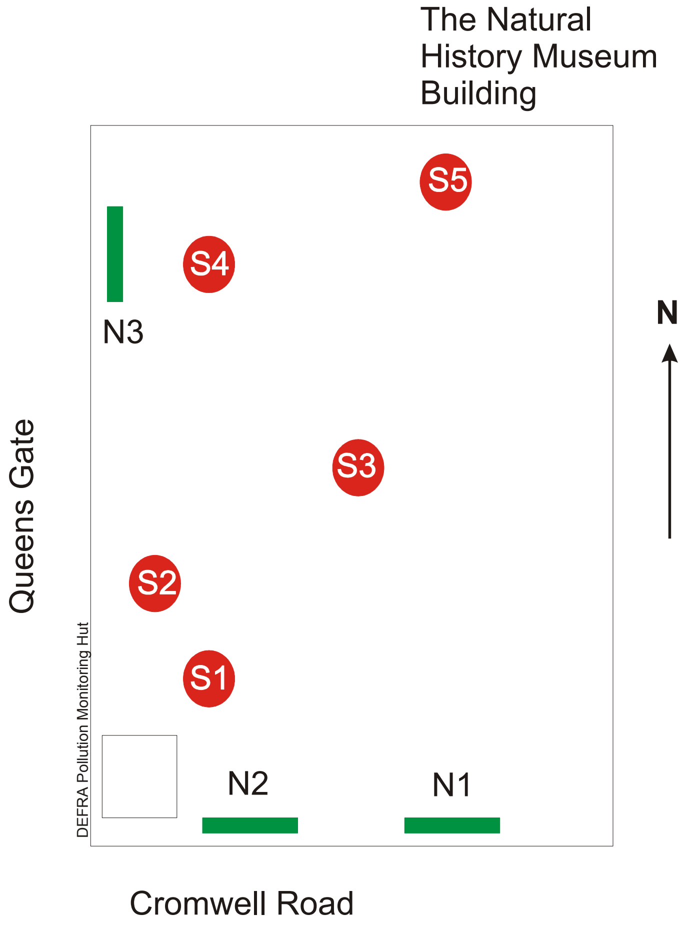


Fig. 2. Sketch showing location of lichen monitoring stations (S1 – S5) and nets (N1 – N3) in the NHM Wildlife Garden

Monitoring Station	**Distance from Cromwell road**	**Aspect**
Site 1	20 m	S-facing, exposed
Site 2	30m	SW, exposed also to Queen's Gate road
Site 3	60m	Sheltered by other vegetation
Site 4	90m	exposed to Queen's Gate road to the west but sheltered on other sides
Site 5	120m	S- and W- facing on the north side lies NHM building and Springhouse

Table 1. Transplant site locality details

Changes in the composition of species groups was assessed at the start and end of the transplant period according to the following indices:

Frequency Index	1 = a single individual	2 = 1-5 thalli	3 = 6-10 thalli	4=11-20 thalli	5 > 20 thalli
Vitality index	1 = healthy (no signs of bleaching or necrotic lesions)	2 = slight signs of necrosis (<30%)	3 = moderate signs of necrosis (>30-50%)	4 = necrotic (100%)	
Class size	< 1 cm = small 's'	1-3 cm = medium 'm'	> 3 cm large 'l'		

Table 2. Indices to quantify lichen transplant frequency, vitality and class size

Chlorophyll fluorescence provides a rapid method to determine lichen vitality in the field (Jensen 2002, Jensen & Kricke 2002). Chlorophyll fluorescence was carried out on 12 & 14 January 2001 at the NHM wildlife Garden by Randolph Kricke using a Walz PAM-210 (teaching PAM) (see http://www.walz.com/pamzhsp.htp. Measurements were repeated using a Hansatech Plant Efficiency Analyser after a 3 month exposure period in the Wildlife Garden (15 March) by Sarah Honour and Annalisa Massara and at the transplant site at Somerset (17 March) by Feliciano Cirimele. Chlorophyll fluorescence was carried out on a minimum of 6 *Parmelia sulcata* thalli at each station with reference to the parameter Fv/Fm on moistened and previously dark adapted thalli. Dark adaptation was carried out in January using black plastic and in March with dark adaptation clips. Measurements were taken on young margins in all cases.

Sample collection for chemical analysis

Samples of *P. sulcata* and *Xanthoria parietina* were collected for analysis. Owing to the major loss of *P. sulcata* towards the centre of the wildlife garden, samples of *Evernia* and *Ramalina* were also taken with a view to performing an inter-species calibration.

Digital photography

Photographs were taken at monthly intervals of 6 thalli of *Xanthoria parietina* and *Parmelia sulcata* at each site using a NIKON Coolpix 995 camera. Images were taken at a fixed distance 7 cm using manual focus.

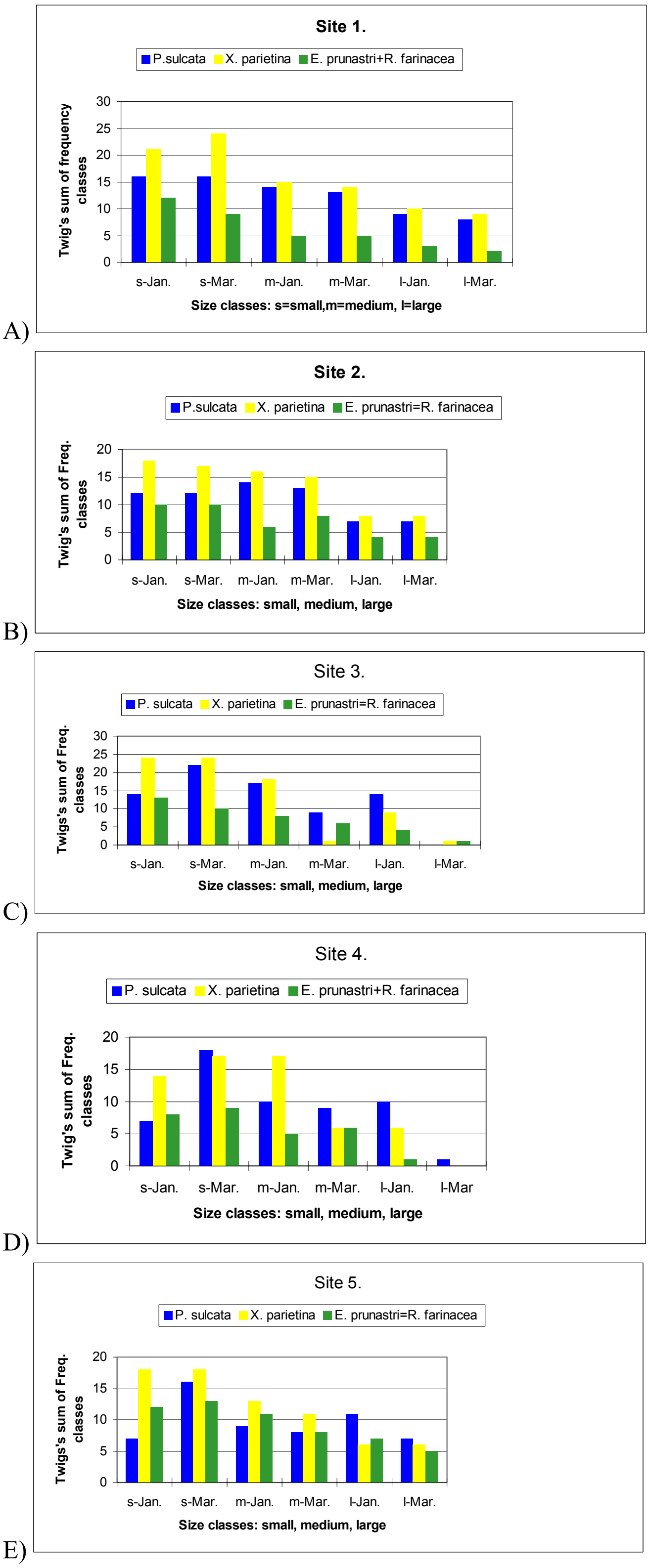
4. Results

Lichens at the control site in Nettlecombe remained healthy throughout the exposure period, both based on photographic recording and measurements of chlorophyll fluorescence. Growth was recorded in both *Parmelia sulcata* and *Xanthoria parietina* demonstrating that the lichens were successfully transplanted at Nettlecombe.

Changes in lichen frequency

In NHM wildlife garden, an assessment of the frequency of different size classes in the 3 species groups revealed different trends across the transect *(Fig. 3)*. Least changes were observed in the most exposed sites (1 and 2) adjacent to road-sites (*Figs 3A & B*) where fewer losses were recorded than 3 and *4 (Figs 3C & D)* (sheltered) and 5 (*Fig. 3E)* (exposed and furthest from roadsides). Maximum losses of all species groups (> occurred in large thalli) at site 3 (*Fig. 3C),* the most sheltered site towards the centre of the garden. Virtually all

Fig. 3. Changes in Lichen Frequency as a function of thallus size over the period Jan – March 2002



Parmelia sulcata disappeared from this site. The increase in numbers of small thalli recorded in sites 4 and 5 reflect the disintegration of larger thalli leaving marginal remnants.

Changes in vitality

Minor signs of thallus bleaching or the development of a characteristic pinkish or brownish discoloration was observed at any site. Least changes in lichen vitality were recorded at the exposed sites 1 and 2 in all species groups as determined by a visual assessment (Fig. 4) and digital photography. Indeed, images of lichens from Site 2 at the level of Queen's Gate road showed the least changes.

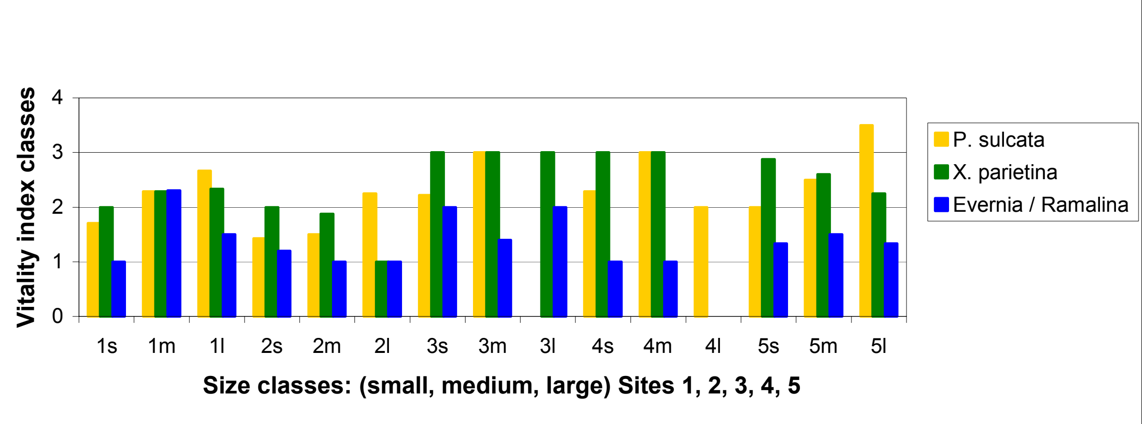


Fig. 4. Average Vitality Index

This contrasted with sheltered sites where major losses occurred. Exposed site 5 furthest from the road suffered the most significant loss of thalli of *Parmelia sulcata*, particularly towards the inner thallus centre and the site appeared to be deteriorating in a similar fashion to sites 3 and 4. *Xanthoria parietina* showed the opposite behaviour to *Parmelia sulcata* with younger thalli suffering the major loss, especially from sites 3 and 4.

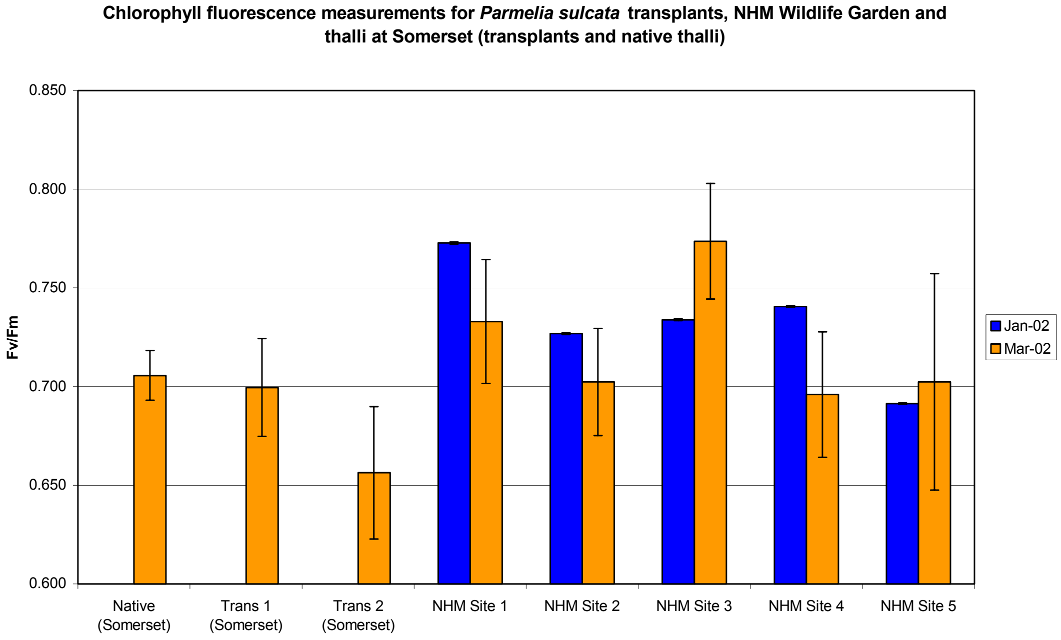


Fig. 5

Chlorophyll fluorescence

Considerable variation in chlorophyll fluorescence as measured by Fv/Fm was noted (*Fig. 5)*. Most striking is the observation that the Somerset specimens have much lower values (March 2002) than those transplanted to NHM wildlife garden – including measurements made in January 2002 one month after transplantation. The standard error is also considerably larger for March measurements. Measurements confirm that all lichens are physiologically active and alive.

Digital photography

Photographic recording confirms that growth has occurred in transplants at the Somerset control site. Little change was noted in thalli at exposed sites at the NHM wildlife garden (1 and 2). Major loss was noted at sites 3 and 4. Older thalli of *Parmelia sulcata* after 6 weeks of exposure showed a major loss of inner parts at sites 3, 4 and 5. In contrast, larger *Xanthoria* thalli tend to loose the outer part at the same sites (*Appendix E).*

5. Discussion

A combination of a visual assessment of abundance, vitality, digital photography and chlorophyll fluorescence confirms that the healthiest lichens are those transplanted at sites 1 and 2 adjacent to the Cromwell and Queen's Gate roads. Maximum $N0$, $N0_2$, N0x and VOC air concentrations were recorded at these sites suggesting that NOx concentrations which exceed the health standards for human health are not having a major impact on lichen health over the short term (3 months). This contrasts with previous studies where major impacts were recorded on lichens exposed to pollution. Data on the effects of NOx on lichen vitality in the field are lacking although Gilbert (1968) recorded major reductions in chlorophyll content (84%) and respiration (80%) in transplants of the lichen *Ramalina farinacea* at mean SO_2 concentrations of 264 µg/m^3 (Gilbert 1968) during the survey. The loss of lichens in the sheltered sites might be due to a number of reasons quite apart from pollution including:

- Microclimate: Increased shelter will result in prolonged hydration (and hence metabolic activity) during which various pollutants may be in solution. Unfavourable microclimatic factors would result in degeneration in the absence of pollution. December remained dry and relatively warm. Under these conditions lichens would remain physiologically less active. However, rainfall increased during January and February when lichens would be metabolically active and most sensitive to damage from pollution.
- Other biological factors including Long-tailed tits and other birds for use in nesting materials, grazing by invertebrates.

Chlorophyll fluorescence

Caution is advisable over the interpretation of fluorescence data owing to the different equipment used with potentially different sensitivities, the different operators and methods used to dark adapt thalli. If there is a relationship there appears to be a tendency towards higher levels within the wildlife garden. However, levels above 0.75 are rare in lichens (Jensen 1992) and this upward trend contradicts previous studies mainly examining impacts of SO_2 resulting in a decrease in Fv/Fm (see Jensen 2001). SO_2 is well known to result in chlorophyll pigment degradation in lichen photobionts. However, Von Arb & Brunold (1990) identify higher chlorophyll contents in lichens exposed to traffic pollution. There is no significant difference between Fv/Fm values obtained for NHM wildlife garden site 2 (exposed to traffic) and transplant site 1 in Somerset.

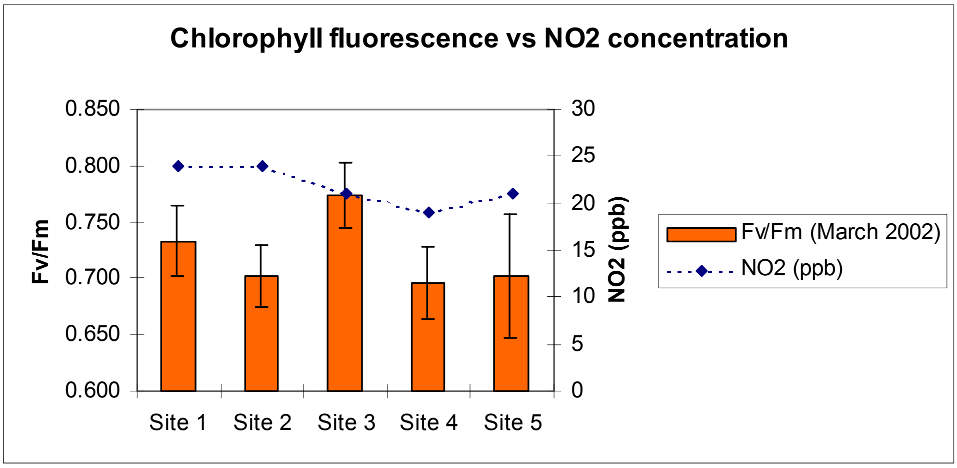

Chlorophyll fluorescence vs NO2 concentration
Fv/Fm
0.850
0.800
0.750
0.700
0.650
0.600
NO2 (ppb)
30
25
20
15
10
5
0
Site 1
Site 2
Site 3
Site 4
Site 5
Fv/Fm (March 2002)
NO2 (ppb)

Fig. 6

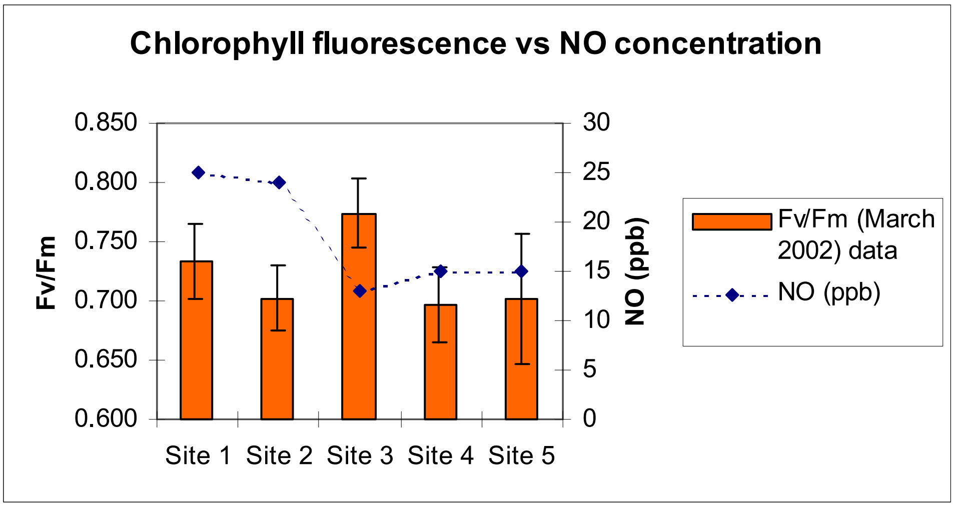

Chlorophyll fluorescence vs NO concentration
Fv/Fm
0.850
0.800
0.750
0.700
0.650
0.600
NO (ppb)
30
25
20
15
10
5
0
Site 1
Site 2
Site 3
Site 4
Site 5
Fv/Fm (March 2002) data
NO (ppb)

Fig. 7

6. Conclusion

The pilot project confirms that epiphytic lichens on twigs can be successfully introduced into London to study pollution effects even where NO and NO_2 levels are high. The absence of a major direct impact on the lichens at road-sites adjacent to Cromwell Rd and Queen's Gate suggests that NOx is not having a major impact on lichen growth over this time period. This suggests that N may not, at least at these concentrations and meteorological conditions, have an effect on the vitality of *P. sulcata* although, a longer exposure period would certainly be required to fully test this hypothesis. The possible effect of VOC's and particulates also needs to be considered. It is interesting to note that the shrubby lichens *Evernia* and *Ramalina* remained largely unaffected in this region. There was clearly a varied response amongst the different species and thalli of different ages. We suggest that in addition to pollution, microclimatic factors may have played a role in determining the vitality and loss of particular species. A larger study over a longer time period is required.

7. Recommendations

- It is now important to carry out chemical analysis of the lichen transplants and bark samples we collected during the experiment for N and a range of emission relevant elements. It will be interesting to analyse the lead levels following reductions in the use of leaded petrol. Characterisation of the chemical and physical form of particulates trapped on the sticky pads by SEM and comparison with metal accumulation by lichens at the same sites.

- Further transplant experiments should be established across a wider NOx gradient in London to complement biodiversity studies in areas where insufficient native thalli are available for analysis to determine possible critical levels under different meteorological conditions.

- Further research needs to be carried out to test and develop chlorophyll fluorescence as an indicator of NOx air pollution both in relation to transplants and natural thalli.

- Detailed growth measurements (e.g. Hill 2002) can be carried out on lichens provided sufficient thalli are available for measurements and growth coefficients calculated. In addition to transplants, this can usefully be extended to the study of natural populations as little is understood of the dynamics of natural populations in London.

Appendix A

Estimated Air Pollution Data for the Wildlife Garden, 7th November 2001 to 15th March 2002

Nitrogen Oxides
Concentrations of nitric oxide (NO) and nitrogen dioxide (NO_2) at the 5 sites in the Wildlife Garden were estimated by calculating the relationship between measured site concentrations and the NOx levels recorded by the DEFRA monitoring station at the corner of the garden. This relationship was then applied to data from the monitoring site for the time period when the lichen were exposed.

The relationship between NOx concentrations at the sites in the Wildlife Garden and those measured by the DETR monitoring site were calculated from existing data (See Appendix 1). The NO_2 relationship was calculated from 4 sets of measurements made with Palmes type diffusion tubes and 3 sets of measurements made with Ogawa NO/NOx passive samplers. The NO relationship was calculated from 2 sets of measurements made with Ogawa NO/NOx passive samplers. The relationship from the 3rd set of measurements made with these samplers were not used in the calculations since they were taken during a period of high NO concentrations and gave a different relationship to the other measurements. The NO levels recorded at the DEFRA monitoring site during the experiment were similar to those used to estimate the relationship.

	DEFRA station	Site 1	Site 2	Site 3	Site 4	Site 5
NO %	100	34	32	18	20	20
NO conc.(ppb)	75	**25**	**24**	**13**	**15**	**15**
NO_2 %	100	67	66	57	52	57
NO_2 conc.(ppb)	36	**24**	**24**	**21**	**19**	**21**
NOx conc.(ppb)	111	**50**	**48**	**34**	**34**	**36**

Figure 1: Estimated NO and NO_2 concentrations at the 5 sites in the Wildlife garden, 07/11/01 – 15/03/02

Appendix B

Measurements used to estimate the NO and NO_2 relationship for the 5 Sites in the Wildlife Garden

	Sampling Dates	DEFRA	Site 1		Site 2		Site 3		Site 4		Site 5	
			Conc. (ppb)	% DEFRA	Conc. (ppb)	% DETRA	Conc. (ppb)	% DETRA	Conc. (ppb)	% DETRA	Conc. (ppb)	% DETRA
Nitrogen Dioxide												
Palmes	12-26/07/00	44	27	61	26	59	23	52	20	45	36	
type	26/07-23/08	42	33	79	32	76	32	76	27	64	20	48
diffusion	23/08-22/09	44	32	73	34	77	22	50	18	41		
tubes	22/09-20/10	47	32	68	26	55	26	55	24	51	29	62
Ogawa	18/10-01/11	37	24	65	24	65	18	49	19	51	18	49
passive	01/11-16/11	39	27	69	27	69	26	67	24	62	27	69
samplers	19/11-3/12	41	23	56	24	59	20	49	20	49	23	56
	Relationship			**67**		**66**		**57**		**52**		**57**
Nitric Oxide												
Ogawa	18/10-01/11	82	28	34	27	33	14	17	13	16	16	20
passive	01/11-16/11	103	45	44	39	38	41	40	30	29	33	32
samplers	19/11-3/12	83	28	34	26	31	16	19	20	24	16	19
	Relationship			**34**		**32**		**18**		**20**		**20**

Appendix C

SOMERSET TRANSPLANT SITE

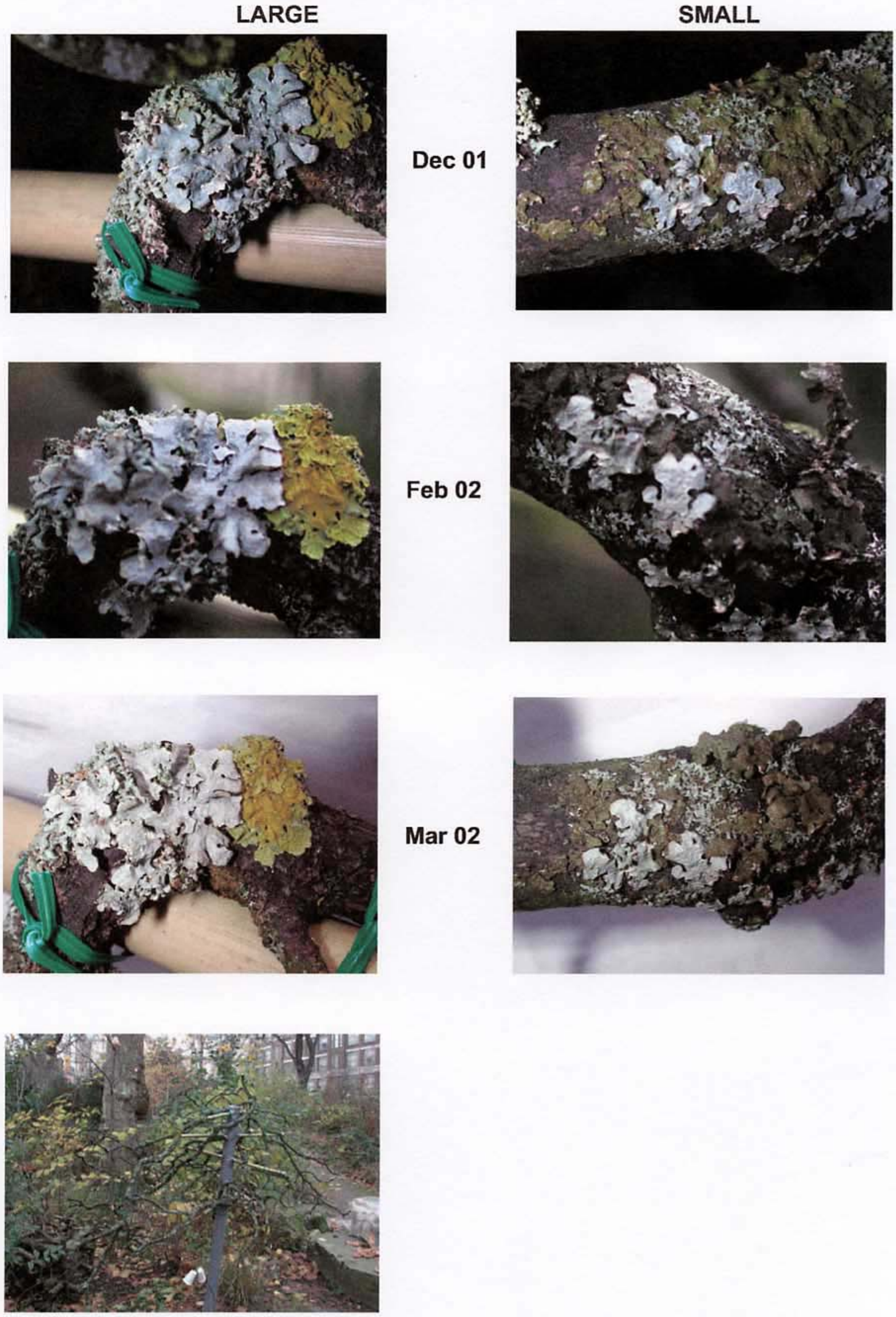
January 2002 March 2002

Parmelia sulcata

Xanthoria parietina

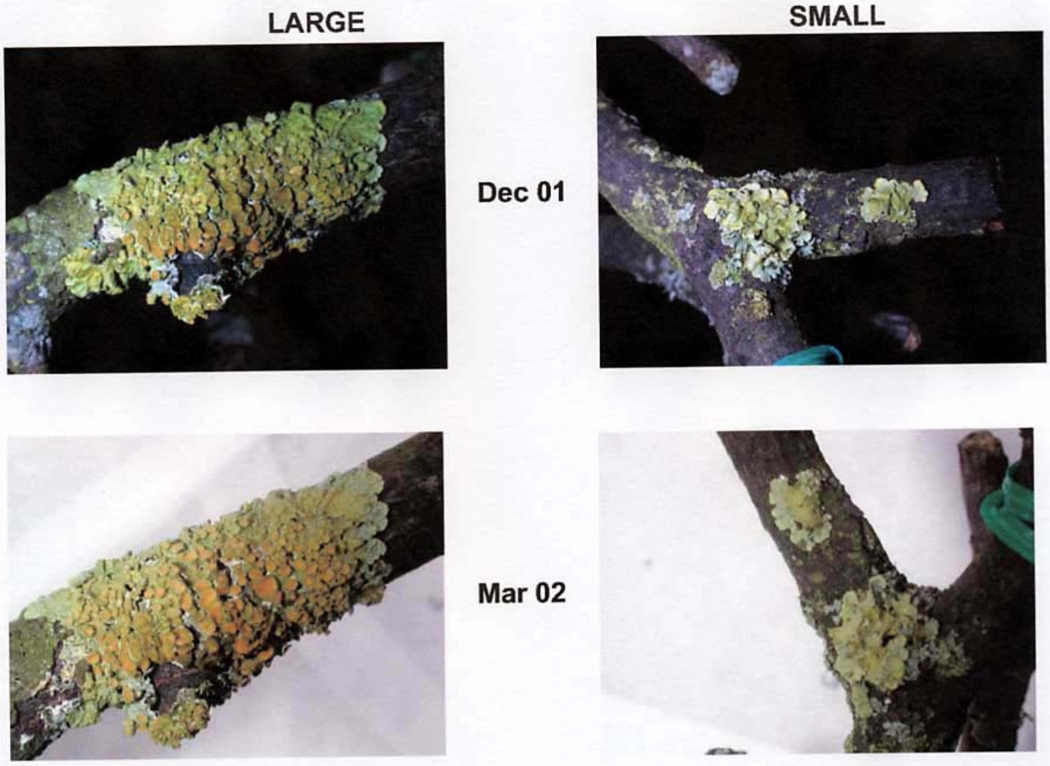
Appendix D

NHM WILDLIFE GARDEN TRANSPLANT SITE 2 (Parmelia sulcata)



Appendix E

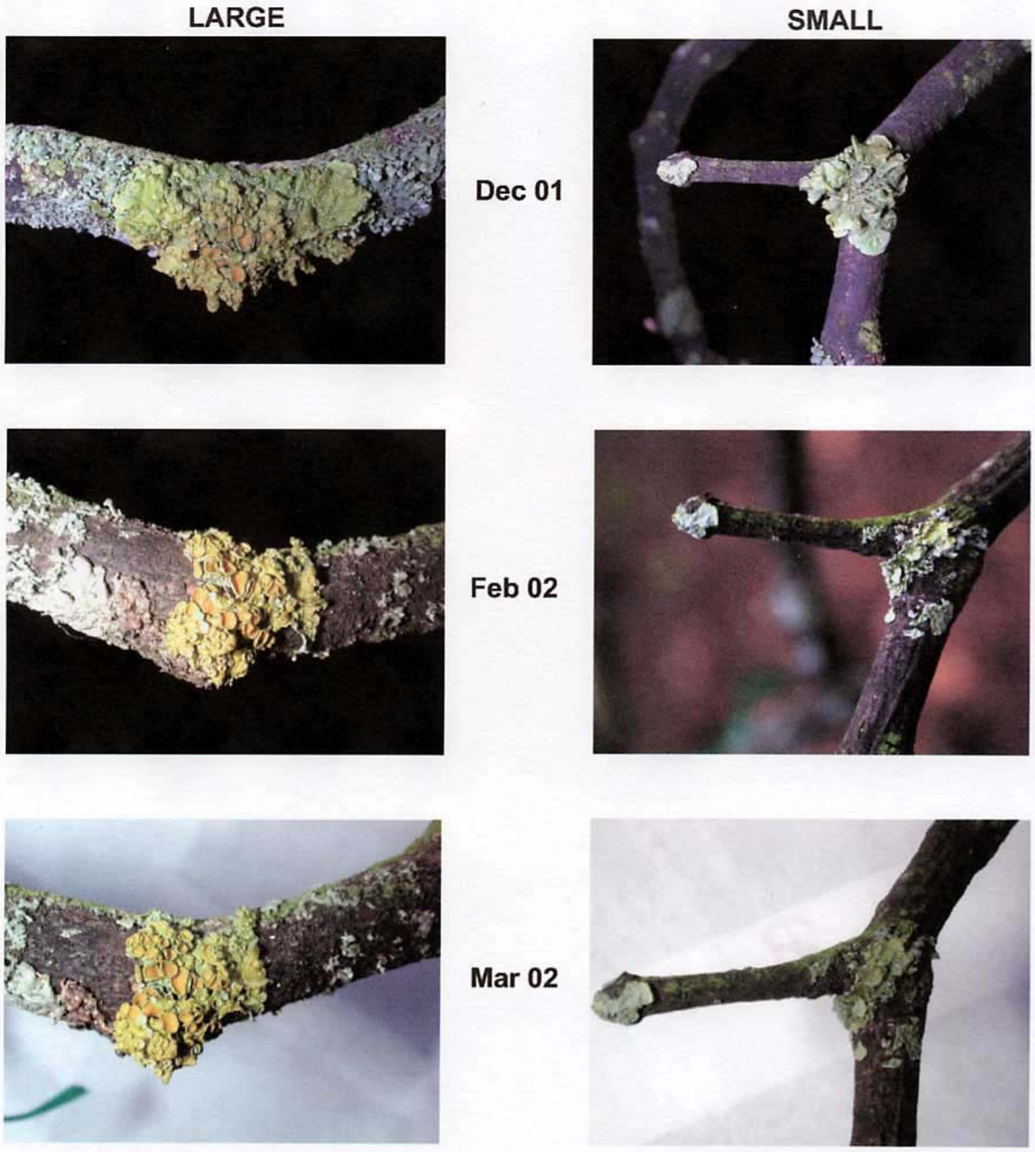
NHM WLDLIFE GARDEN TRANSPLANT SITE 2 (*Xanthoria parietina*)



Appendix F


NHM WLDLIFE GARDEN TRANSPLANT SITE 4 (Parmelia sulcata)

Appendix G



NHM WLDLIFE GARDEN TRANSPLANT SITE 4 (Xanthoria parietina)

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Purvis, O.W., Chimonides, J, Din, V., Erotokritou, L., Jeffries, T. Jones, G.C., Louwhoff, S., Read, H., Spiro, B. Which factors are responsible for the changing lichen floras of London? *Science and the Total Environment* [in press]

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SECTION 2

Impacts of urban NO_x pollution on vegetation in London

Authors

Sally Power and Tilly Collins
Imperial College, London

Table of contents

Summary

A transect study was carried out during winter 2001/2, to assess changes in shoot chemistry of *Calluna* (heather) along a gradient of decreasing NO_x pollution from central London to rural Surrey. Shoots were analysed for concentrations of nitrogen, carbon, phosphorus, lead, zinc and stable isotopes of nitrogen.

Foliar nitrogen concentrations were positively related to NO_2 concentrations and also decreased significantly with increasing distance from central London. Carbon:nitrogen ratios showed the opposite relationship with both NO_2 and distance, with linear regressions again statistically significant. High phosphorus concentrations in central London were responsible for a significant increase in foliar N:P ratios with distance from the city centre and suggested that phosphorus availability would not limit plant response to nitrogen at any of the study sites.

NO_2 data from the nearest National Diffusion Tube Network sites may not provide the most accurate representation of vegetation exposure to NO_x in urban green spaces. Unlike studies elsewhere using mosses, foliar lead and zinc concentrations do not appear to be a useful index of exposure to traffic-derived pollution in *Calluna.* This may be because the sites used in this study were typically located at distances of more than 150 m from major roads, or because higher plants are more effective at excluding heavy metals than mosses or lichens.

Delta ^{15}N measurements were positively related to NO_2 concentrations and declined significantly with increasing distance from central London and increasing distance from major roads. This may suggest a decreasing contribution of traffic-derived NO_x to the nitrogen nutrition of higher plants away from busy roads and in less urban areas. However, changes in isotopic signature associated with soil nitrogen transformations and root uptake of nitrate/ammonium make it difficult to interpret this relationship without further investigation of isotopic signatures of nitrogen sources.

Foliar nitrogen concentrations and nutrient ratios suggest that many sites in urban areas experience high deposition inputs of nitrogen and as such are at risk from changes in species interactions and ecosystem function. Further work is required to establish the sensitivity of urban vegetation to realistic levels of both NO and NO_2, and to determine the implications of current and future emissions of traffic-derived pollution on urban ecology.

1. Introduction

National air quality standards for gaseous pollutants are concerned primarily with the protection of human health and there are currently only limited data detailing the effects of ambient levels of urban air pollution on sensitive vegetation. Whilst national objectives for NO_x and SO_2 have been proposed to afford protection to vegetation and ecosystems (DETR, 2001), these are currently excluded from local air quality management plans. There is therefore an urgent need to establish the effects of realistic levels of the principal urban pollutants, NO and NO_2, on vegetation in urban areas and, if possible, the contribution that oxidised nitrogen makes to the total deposition load for urban ecosystems. London has the highest levels of traffic-derived pollution in the UK and thus provides a useful starting point from which to assess the potential impacts of NO_x on urban ecosystems. Furthermore, the comprehensive network of air quality measurement sites located throughout the capital and home counties provide pollution data in areas of varying traffic density throughout the south-east, and thus a framework within which to assess the effects of NO_x on urban ecology.

At low concentrations, plants are able to detoxify and assimilate gaseous nitrogen compounds. However, at higher concentrations, foliar damage occurs, often accompanied by changes in physiology, shoot chemistry and detrimental effects on growth. The toxic effects of NO_2 are largely associated with its breakdown products, soluble nitrate and nitrite ions (Wellburn, 1990). If concentrations of these ions exceed the cell's capacity to reduce and assimilate these products, cell damage and/or a disruption in physiology will occur. Effects on photosynthesis, stomatal conductance, foliar chemistry and growth have all been reported following exposure to high concentrations of this pollutant (e.g. Ashenden, 1979; Caporn & Mansfield, 1976; Saxe, 1994).

Whilst NO_2 is believed to be considerably more phytotoxic than NO, it has been suggested that NO may also be converted to nitrate and nitrite ions (Wellburn, 1990) and that it may enter the cytoplasm directly (Mansfield, 2001). There is certainly experimental evidence that NO is toxic to plants and that it may have a different phytotoxic mechanism to NO_2 (Morgan *et al.,* 1992; Saxe, 1994). Recently it has been shown that NO plays an important role in plant signalling, in particular in relation to plant defence against pathogens (Beligni & Lamattina, 2001; Durner & Klessig, 1999). It is possible, therefore, that high levels of this pollutant may interfere with normal signalling mechanisms.

In addition to the direct toxic effects of NO and NO_2, these pollutants also contribute to the total deposition load of nitrogen in urban ecosystems. Since most non-agricultural ecosystems are nitrogen-limited, this may result in a stimulation in growth. Furthermore, increased foliar nitrogen concentrations, changes in phenology and patterns of assimilate distribution have been shown in response to elevated nitrogen availability (See reviews by Bobbink *et al*., 1998; Green *et al.,* 1997). These changes are associated with increased sensitivity to abiotic and biotic stresses such as drought, frost and herbivore/pathogen attack. Genotypic and phenotypic differences in plant response to both NO_x and total nitrogen availability will have an effect on intra- and inter-specific competition and thus, ultimately, on community composition.

Foliar nitrogen concentrations of *Calluna vulgaris* (heather) have been shown to be related to deposition rates of nitrogen, along gradients of both latitude and altitude (Hicks *et al*., 1999; Pitcairn *et al*., 1998, 2001)˙ As such, it *Calluna* represents a useful indicator species to quantify the magnitude of traffic-derived nitrogen pollution in urban ecosystems. Furthermore, since effects of nitrogenous pollution on the growth and physiology of this species are reasonably well understood, changes in foliar chemistry along a gradient of decreasing pollution will allow a wider evaluation of the potential impacts of NO_x on vegetation in urban areas throughout the UK.

It has recently been suggested that oxidised and reduced forms of nitrogen pollution have different natural isotopic signatures; oxidised forms being enriched in ^{15}N, relative to reduced forms (Heaton *et al.,* 1997). The use of $\delta^{15}N$ signatures to distinguish between nitrogen sources has been have been pioneered by Pearson *et al*. (2001), using epilithic mosses which receive most of their nitrogen from the atmosphere. Experiments in The Netherlands have indicated that direct foliar uptake by *Calluna* shoots can account for up to 60-65% of deposited nitrogen (Heil & Bobbink, 1993). Whilst soil nitrogen transformations and associated changes in isotopic signature may mask any relationship between deposition and foliar $\delta^{15}N$ signature, foliar nitrogen concentrations and $\delta^{15}N$ signatures of *Calluna* may nevertheless provide an indication of the contribution of atmospheric pollution to the nitrogen load in urban ecosystems.

The objectives of this pilot study were as follows:

1. To determine whether a relationship exists between foliar nitrogen concentrations of *Calluna* and ambient NO_x concentrations, using a gradient of decreasing pollution away from central London.

2. To evaluate whether isotopic ^{15}N signatures can provide a useful index of the contribution of traffic-derived pollution to the nitrogen nutrition of *Calluna*.

3. To establish whether plant foliar indicators can provide an estimate of the magnitude of the urban nitrogen footprint.

2. Materials and Methods

2.1. Transect design and site selection

A 50 km south-westerly transect was established along a gradient of decreasing atmospheric pollution, from central London to Surrey. The transect thus included 14 sites in either urban, suburban or semi-rural locations; sites ranged from the centrally located Natural History Museum (NHM) Wildlife Gardens to Thursley Common National Nature Reserve, 50 km away in Surrey. Three additional sites in north London, were also sampled. These additional sites were not intended for inclusion in the primary transect, but rather to consider the importance of wind direction (i.e. downwind *versus* upwind of the metropolitan area) in determining the relationship between location, NO_2 data and vegetation response. All statistical analyses presented in the results section are based on the 14 main transect sites and exclude the three north London sites.

Site selection was based on three main criteria: 1) Location along an approximate south-west line from central London; 2) Permission to sample granted by site owners and (where appropriate) English Nature; 3) Sufficient *Calluna* on site to sample. A map of the transect and details of all sites can be found in Appendix I.

2.2. Calluna sampling

At each site (except Barnes Common) three samples of *Calluna* were taken from distinct, but representative, heathland patches. At Barnes Common, the small patch size of *Calluna* meant that it was possible to obtain only 2 replicate samples. Sample collection was restricted to current year shoots and was completed during a two week period in winter 2001/2. At the time of collection, shoot growth had ceased for the season. Based on data from The Netherlands (Heil & Bobbink, 1993) and the UK (Power, unpublished data), foliar nutrient concentrations can be expected to have stabilised, at a seasonal low, at this time. At each site, two replicate soil samples were collected, although it was not possible to carry out any analysis on these samples within the scope of this pilot study.

Following collection, plant samples were immediately placed in a chilled environment (cool box) in the field. On return to the laboratory, samples were divided into two; half of the material was frozen at –80°C, while the other half was dried in an oven at 80°C, for three days. Frozen material has been stored for amino acid analysis at a later date and is beyond the scope of the pilot study. Oven dried material was ground to a fine powder using a ball mill.

2.3. Foliar analyses

Nitrogen concentration, C:N and N:P ratios. Foliar nitrogen concentrations were determined on dried shoot material. Samples were analysed using a Gas Chromatography Mass Spectrometer (GC-MS), following combustion and oxidation of plant material. Nitrogen concentrations were determined in the Department of Agricultural Sciences at Imperial College (Wye). Carbon concentrations were also determined at this time, allowing an assessment of changes in C:N ratio along the transect.

Phosphorus concentrations were determined commercially on dry plant material, by Natural Resource Management Limited, Bracknell, Berkshire (www.nrm.uk.com). Replicate samples were pooled prior to analysis, resulting in a single value per site. Phosphorus is an essential plant nutrient and limits plant growth in many natural ecosystems. Nitrogen:phosphorus ratios have been used as an indicator of plant nutritional imbalance.

Delta ^{15}N. Isotopic enrichment of the heavy (^{15}N) isotope of nitrogen, relative to its lighter (^{14}N), more abundant isotope is increasingly recognised as an indicator of the form of nitrogen which has been assimilated. Isotopic discrimination by many biological processes means that an enrichment of the heavier isotope, relative to its natural abundance in the atmosphere (0.3663 atom %), is indicative of nitrogen uptake predominantly in the oxidised form. This results in a δ^{15}N signature which is positive, with respect to atmospheric N_2. Conversely a negative δ^{15}N indicates nitrogen uptake predominantly in the reduced form. Isotopic enrichment was analysed, for three replicate samples per site, using a GC-MS. Analyses were carried out in the Department of Agricultural Sciences at Imperial College (Wye). Isotopic enrichment is expressed in parts per thousand, according to the following equation:

$$\delta^{15}\text{N (‰)} = ((^{15}\text{N}:^{14}\text{N}_{\text{sample}} / ^{15}\text{N}:^{14}\text{N}_{\text{standard}})-1) \times 1000$$

Pb and Zn concentrations: **Although lead has not been added to petrol for many years, it has accumulated close to roadsides during past decades. As such, it may provide an useful index of past traffic flow in an area. Zinc on the other hand is a consistent constituent of motor vehicle components and, as such may provide an integrating index of past and current levels of motor vehicle activity in an area. It was thus envisaged that Pb and Zn concentrations might provide information about historical and recent/current traffic flow in the vicinity of each sample site, and thus provide a check on the representativeness of data from the NO_2 diffusion tube network to traffic-derived NO_x pollution at each of the sites. Foliar Pb and Zn concentrations were measured using atomic absorption spectrophotometry, following digestion of dry plant material in sulphuric acid.**

2.4. Calculation of distance values for transect sites

Two distance terms for each sample site have been used in the analysis. The first is the straight-line distance from Hyde Park Corner, representing central London; sites were located between 2km and 50 km from the city centre. The second distance term used in the analysis is the distance of the site from the nearest major road (defined as ‘A’ road). Where several A roads were adjacent to a site, the replicate sample locations within a site were measured individually and a mean value obtained.

2.5. Estimation of NO_x concentrations

Nitrogen dioxide concentrations at the individual *Calluna* sampling sites were estimated using diffusion tube data from the UK Nitrogen Dioxide Network. Only sites defined as "background" i.e. >50m from any busy road and typically in a residential area, were used to estimate concentrations at the sample sites. This was to ensure that pollution data came from stations which best reflected the *Calluna* sampling locations. Since "background" network sites were not always located close to sample sites, consideration was given to averaging data from the nearest roadside and background network sites for those sample sites located within 150 m of the nearest A road. However, since relatively few sample sites were located within this distance, and statistical analysis revealed that this modification did not increase the explanatory power of this variable, only data from background network sites were included in subsequent analyses.

Diffusion tube data were used, rather than data from the DEFRA automatic network, because they provide far more detailed spatial coverage of London and the South East, particularly for non-kerb/roadside sites.

Averages of 2000 and 2001 data from the nearest NO_2 network site were obtained for each *Calluna* sampling site. Where a sample site lay between two network sites an average of the concentrations from the two sites was taken. The exception to this was in fact the site at the NHM where measurements of NO_2 at the actual sampling location, undertaken as part of a separate study under the NERC-URGENT programme, were used. This provided an accurate reflection of NO_2 exposure of *Calluna* at this site.

Whilst NO_2 data from the National Diffusion Tube Network are comprehensive, few data exist for NO concentrations at the same locations. Data from DEFRA monitoring stations around London indicate that in 2000, the ratio of NO to NO_2 ranged from 4-5 at the most polluted roadside site (Marylebone Road) to 0.5-1.5 at an urban background site (North Kensington). It seems probable, therefore, that the data obtained from NO_2 diffusion tubes at the urban background sites used in this study underestimate NO_x concentrations by something in the order of 100%. Additional (unpublished) data, collected by Sarah Honour using paired NO and NO_2 diffusion tubes at the NHM Wildlife Garden are summarised in Table 1. These data support the assumption that NO_2 data represent only approximately half of the NO_x burden in urban areas. This proportion would be expected to decrease with increasing distance from both major roads and the city centre.

Sample date		Site 1	Site 2	Site 3	Site 4	Site 5
18/10/01 – 01/11/01	NO conc. (ppb)	28	27	14	13	16
	NO_2 conc. (ppb)	24	24	18	19	18
	NO as a % of NO_2 conc.	117%	113%	78%	68%	89%
01/11/01 – 16/11/01	NO conc. (ppb)	45	39	41	30	33
	NO_2 conc. (ppb)	27	27	26	24	27
	NO as a % of NO_2 conc.	167%	144%	158%	125%	122%
19/11/01 – 03/12/01	NO conc. (ppb)	28	26	16	20	16
	NO_2 conc. (ppb)	23	24	20	20	23
	NO as a % of NO_2 conc.	122%	108%	80%	100%	70%

Table 1. *Summary of NO_x data for sites in the NHM Wildlife Garden, 2001. Site 1 is closest to Cromwell Road, while site 5 is furthest away.*

2.6. The importance of prevailing wind direction

Concentrations and deposition rates of NO_x at a particular site are likely to be influenced by whether the site is upwind or downwind of a given source of pollution (i.e. a major road). In the south of England the prevailing winds are Atlantic south-westerlies. It was therefore considered informative to include a 'distance moderator' in the analysis. Such a moderator is based on the assumption that a site downwind of the road in question is effectively closer to it and that one upwind is effectively further from it. This assumption is substantiated by data from the three (downwind) north London sites, which indicated higher deposition loads of nitrogen in relation to distance from central London, than upwind sites. No directional modifier was used for sites parallel to the prevailing wind direction.

2.7. Statistical analyses

Data manipulation and graph generation were carried out in Microsoft Excel. Linear and multiple regression analyses were performed in Statistica. For all statistical analyses, the 14 sites in the main transect have been used (except where stated). North London sites have been added to graphs for comparison, but are not included in the analysis of main transect data.

3. Results and discussion

In the following analyses, plant parameters have been considered in relation to both NO_2 concentrations and distance from central London. This is because NO_2 data represent only a proportion of total NO_x deposition, whereas distance from the city centre may provide a alternative indicator of the contribution of traffic-derived NO_x to the nitrogen load at a given site.

3.1. Relationship between NO_2 concentrations and distance

Background concentrations of nitrogen dioxide decline significantly with increasing distance from central London (Figure 1, Table 2).

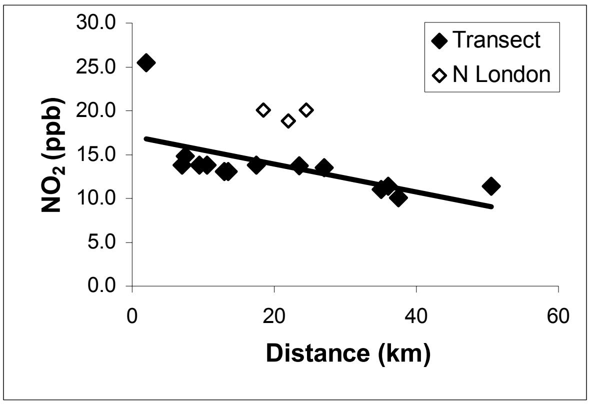

Figure 1. *The relationship between concentrations of NO_2 (ppb) and distance from central London. The north London (downwind) sites are also shown for comparison.*

Values range from 25.5 $\mu g\ m^{-3}$ in the NHM Wildlife Gardens to 11.4 $\mu g\ m^{-3}$ at Chobham Common in Surrey. The Natural History Museum in South Kensington is located at the junction of the A4 Cromwell Road and Queens Gate, both busy roads with frequent stationary traffic. Due to the fact that NO_2 data for this site were obtained within the Wildlife Garden itself, they are considered to accurately reflect the exposure of the sampled vegetation to exhaust emissions and as such, have been retained in subsequent analyses. Interestingly, a statistically significant relationship between distance from the city centre and NO_2 concentration is apparent even if this data point is excluded from the regression analysis (Table 2)

The three north London sites all lie well above the regression line for the main transect data, with proportionately higher NO_2 concentrations for a given distance than the 14 principle sites. Since these three sites are downwind of the urban area, they serve to emphasise the influence of prevailing wind direction. On average the north London sites experience NO_2 concentrations 57% higher than would be predicted from the regression equation for the main transect describing the relationship between concentrations and distance from Hyde Park Corner.

	Relationship	d.f.	F	P		r^2
Full transect	Y= -0.14x + 16.92	1,12	6.45	0.026	*	0.35
Without NHM	Y= -0.07x + 14.63	1,11	19.55	0.0013	**	0.66

***Table 2.** Summary of the linear relationship between background nitrogen dioxide concentration and distance from central London. The first regression includes all sites along the sampled transect (n=14), the second excludes the Natural History Museum site (n=13).*

3.2. Foliar nitrogen concentration

Foliar nitrogen concentrations of *Calluna* decreased significantly with increasing distance from central London (Figure 2a) and with decreasing background NO_2 concentrations (Figure 2b). Values ranged from 1.50% to 0.91%.

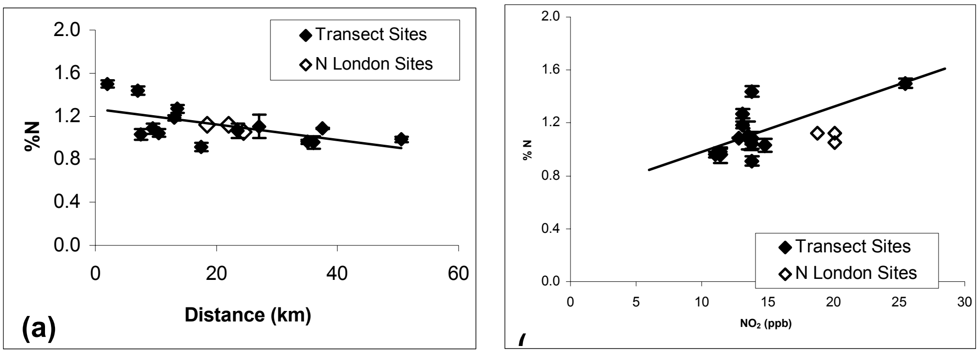

***Figure 2:** The relationship between foliar nitrogen concentration and (a) distance from Central London (Hyde Park Corner, London W1) and (b) estimated background NO_2 concentration. Error bars represent within-site standard error.*

Table 3 summarises the regression analyses for foliar nutrients against distance and NO_2 concentrations. It appears that NO_2 concentration explains more of the variance in foliar nitrogen than distance, although the relationship was highly influenced by the high NO_2 data point from the NHM. Foliar nitrogen concentrations of *Calluna* do appear to reflect nitrogen deposition in London and the surrounding areas, supporting similar reports in the literature from transect studies across larger geographical and altitudinal gradients (Hicks *et al.*, 1999; Pitcairn *et al.*,2001).

	Relationship with distance from London (km)					
		d.f.	F	P		r^2
%N	y=-0.0072x+1.26	1,12	6.5	0.025	*	0.35
C:N	y=0.28x+40.65	1,12	6.08	0.03	*	0.33
N:P	y=0.0059x+10.58	1,12	6.82	0.023	*	0.36
	Relationship with background NO_2 concentration					
%N	y=0.034x+0.64	1,12	10.12	0.008	**	0.46
C:N	y=-1.23x+63.79	1,12	7.02	0.021	*	0.37
N:P	-	1,12	0.24	0.63	n.s.	0.019

***Table 3.** Summary of regression analyses for plant nutrient variables against both distance from London and background NO_2 concentration.*

3.3. Carbon:Nitrogen ratios in foliage

As would be expected in view of the relationship with foliar nitrogen concentration described above, carbon:nitrogen ratios in *Calluna* shoots increased significantly with increasing distance from London (Figure 3, Table 3). Measured ratios ranged from 32 to 56. C:N ratios were also significantly related to NO_2 concentrations (Table 3), with the latter explaining a similar proportion of the sample variance as distance.

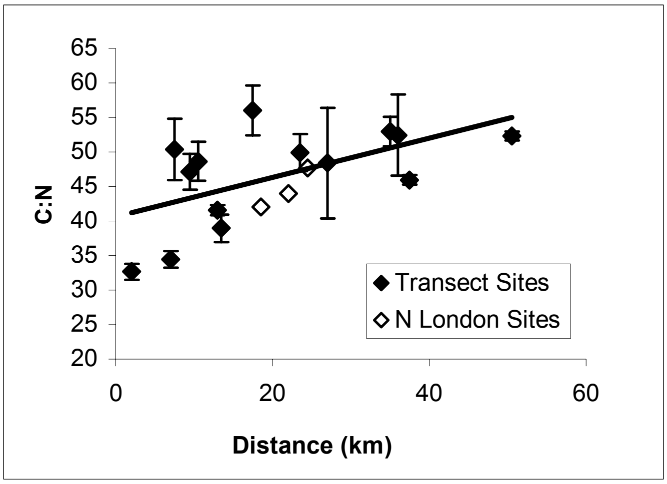

Figure 3. *Variation in the carbon to nitrogen ratio in the foliage of Calluna with distance from central London (Hyde Park Corner, London W1). Error bars (SE_{mean}) graphically represent within-site variation.*

3.4. Nitrogen : phosphorus ratios

Foliar N:P ratios increased significantly with increasing distance from London (Figure 4, Table 3), contrary to expectations. The relationship between N:P ratios and NO_2 concentration was, however, not significant. It appears that phosphorus availability is higher in urban than in rural areas. Using the N:P ratio as an index of relative nutrient limitation (Koerselman & Mueleman, 1996), the data suggest that none of the sites are currently phosphorus-limited and thus may be expected to continue to exhibit growth and other responses to nitrogen inputs in the future.

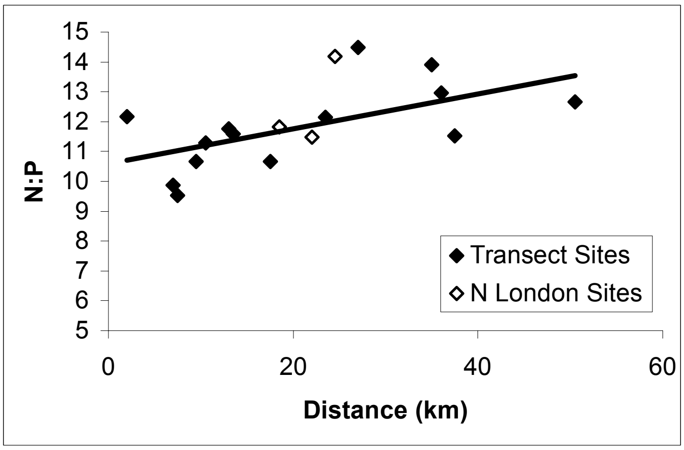

Figure 4. *Variation in the nitrogen to phosphorus ratio in the foliage of Calluna with distance from central London.*

3.5. Stable isotope signatures

The relationship between $\delta^{15}N$ and background NO_2 concentration was statistically significant, indicating a decline in the relative proportion of the heavy (^{15}N) isotope with decreasing NO_2 concentrations (Figure 5, Table 4). The relationship was, however, driven by the high (but accurately measured) value for NO_2 at the NHM, and explained a relatively small proportion of the data variance.

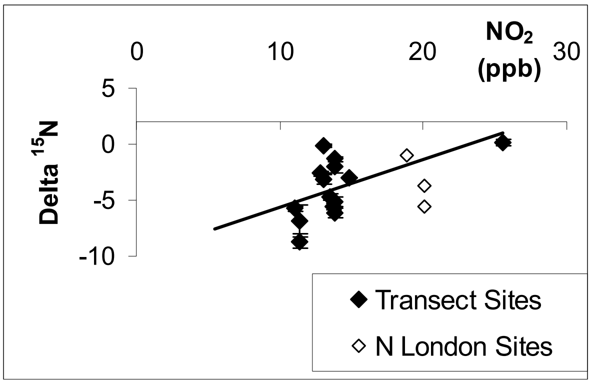

Figure 5. Relationship between $\delta^{15}N$ signature and background NO_2 concentration. Error bars represent within-site standard error.

Since NO_2 concentrations represent a smaller proportion of total oxidised nitrogen in urban areas, particularly close to roads, than in rural areas, the relationship between isotopic signature and distance from both central London and the nearest major road was also investigated. Table 4 summarises the linear regressions for both parameters.

		d.f.	F	P		r^2
NO_2 concentration	y=0.43x-9.91	1,12	5.92	0.031	*	0..33
Distance from London	y=-0.12x-1.44	1,12	9.62	0.009	**	0.44

Table 4. Summary of linear regressions for relationship between $\delta^{15}N$ and either NO_2 concentration or distance from London

A highly significant negative relationship was seen between $\delta^{15}N$ and distance from central London (Figure 6a). A similar, relationship, albeit with more scatter, was also seen for increasing distance from major roads (Figure 6b).

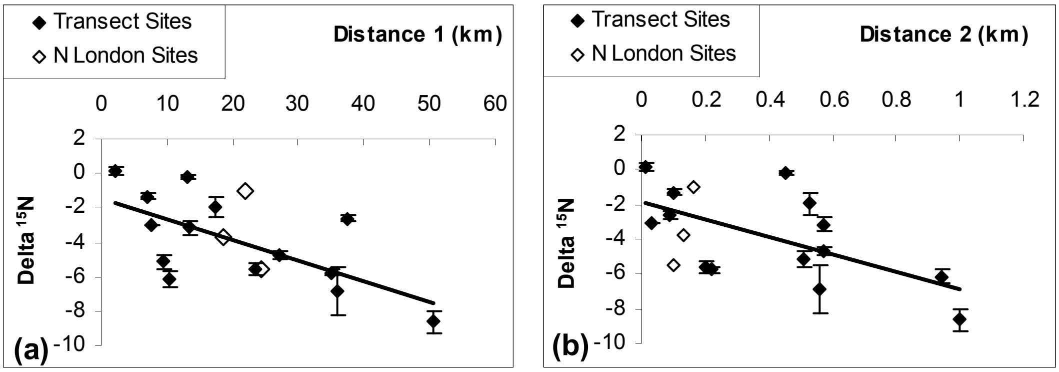

***Figure 6.** The relationship between $\delta^{15}N$ signature and (a) Distance 1(km from Hyde Park Corner) and (b) Distance 2 (km from the nearest A road, moderated by -50% if downwind and +50% if upwind)*

The contribution of both distance parameters to the variation in foliar $\delta^{15}N$ values for *Calluna* was also investigated using multiple regression analysis. This revealed that both distance variables contributed significantly to an explanation of the variance in foliar $\delta^{15}N$ (Table 4).

	Parameter estimate	SE	t	p	
Intercept	-0.51	0.94	-0.55	0.59	n.s.
Distance 1	-0.093	0.036	-2.60	0.025	*
Distance 2	-3.59	1.62	-2.21	0.049	*
	Full model:	$r^2=0.62$	$F_{2,11}=8.82$	P=0.0052	**

Table 4. *Multiple regression summary table of $\delta^{15}N$ signature with Distance 1 (distance in km from Hyde Park Corner) and Distance 2 (distance in km from the nearest A road, moderated by -50% if downwind and +50% if upwind).*

The relationship with distance from central London was in the direction expected from earlier work by Pearson *et al*. (2000). The latter study reported a positive $\delta^{15}N$ signature in epilithic mosses at kerbside sites, falling to a negative signature with an increasing contribution of reduced nitrogen to total deposition. $\delta^{15}N$ values for *Calluna* growing close to the roadside at the Natural History Museum were positive, although, none of the other sites were within 100 m of a major road.

However, is difficult to interpret these data without investigation of the isotopic signature of pollutant sources in central London. Ammonia emissions associated with catalytic converters can be expected to be relatively high at kerbside sites. Since NH_3 has a higher deposition velocity than NO_2, reduced nitrogen may in fact make a substantial contribution to nitrogen inputs in kerbside areas. There is clearly a great need to investigate the isotopic signatures of vehicle-derived NO_x and NH_3, and the effect of soil nitrogen transformations on $\delta^{15}N$ signatures, before any conclusions can be reached concerning the use of this parameter as an index of traffic-derived pollution. Nevertheless, a significant negative relationship between $\delta^{15}N$ and distance from central London was found in this investgation and suggests that this relationship may be worth pursuing in a more detailed follow on study.

The $\delta^{15}N$ data from the current study is unable to reveal information about the proportion of foliar (as opposed to root) uptake of nitrogenous pollution for *Calluna* in urban areas. A variety of soil, plant and environmental factors affect the $\delta^{15}N$ signal of foliage in higher plants. Högberg (1997) lists these factors as (1) the source of nitrogen (soil, precipitation, NO_x, NH_3, N_2 fixation), (2) the depth(s) in soil from which nitrogen is taken up, (3) the forms of soil-N used (organic N, NH_4^+, NO_3^-), (4) influences of mycorrhizal symbioses and fractionations during and after nitrogen uptake by plants, and (5) interactions between these factors and plant phenology.

3.6. Trace metals: Lead and Zinc

The study by Pearson *et al.* (2000) mentioned above also suggested that lead and zinc concentrations in moss tissue may provide a reasonable index of traffic density and thus, by extrapolation, of exhaust emissions in an area. Although lead is no longer added to petrol, high concentrations in the soil adjacent to formerly busy roads, together with a re-circulation of Pb-contaminated dust and soil particles near roadsides might be expected to lead to a legacy of enhanced foliar concentrations. Elevated concentrations could therefore reflect past and possibly present (in proportional terms) traffic flow in a given area. A similar pattern can also be expected for zinc, since in urban areas, away from smelters and heavy industry, the main sources of zinc are likely to be wear of vehicle parts, particularly engines and tyres (Freedman & Hutchinson, 1981).

The highest foliar concentrations of lead were, somewhat surprisingly, found at Chobham Common in Surrey, whereas zinc was highest on Hampstead Heath. Whilst the latter site is relatively close to a major road, the locations from which shoots were collected at Chobham Common were not. No significant relationship was found between either of the heavy metals and distance from nearest A road (Figure 7), distance from central London, or background NO_2 concentrations.

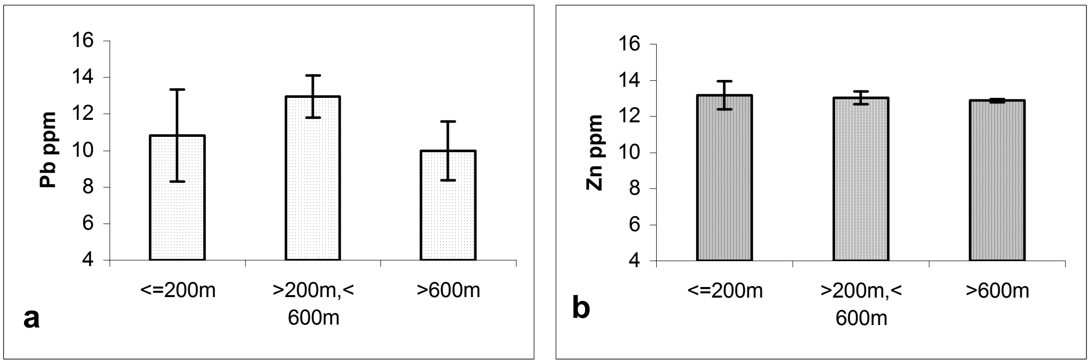

Figure 7. *Shoot lead (a) and zinc (b) concentrations in Calluna averaged over three distance categories from the nearest A road. Error bars show the SE.*

Overall, measured concentrations were very low (5-15 ppm Pb, 12-15 ppm Zn); more than an order of magnitude lower than those reported for moss in Pearson *et al*. (2000). Several reasons may exist for this. Firstly, Pearson *et al.* (2000) reported that heavy metal concentrations fall off rapidly at distances of greater than 40m from roadsides. With the exception of the Natural History Museum, all of the sites used in this study were further than this from major roads. Secondly, a greater degree of discrimination against uptake of heavy metals would be expected for mycorrhizal plants such as *Calluna*, compared to mosses. Mycorrhizal fungi have been suggested to reduce heavy metal uptake by acting both as physical barriers to uptake and by precipitation/complexation in the hyphal network (e.g. Wilkins, 1991). It would appear that neither Pb nor Zn are useful surrogates for NO_x exposure in higher plants.

3.7. Implications for urban vegetation.

Nitrogen dioxide diffusion tube data suggest that, amongst the sites used in this study, critical levels for NO_x are exceeded only at the NHM Wildlife Gardens in South Kensington. This, however, contrasts with maps showing modelled NO_2 data across greater London, which suggest that large areas of the capital exceed the 16 ppb threshold for vegetation effects (GLA map reproduced in Figure 1.1 in the previous section of this report). Consideration must also be given to the fact that a lack of appropriate data for NO led to its exclusion from this study. Whilst the contribution of NO to NO_x declines with increasing distance from roadsides, measurements in the NHM Wildlife Gardens (Honour, unpublished data) indicate that NO concentrations approximately equal those for NO_2. Data from the DEFRA automatic monitoring station at Harwell suggest NO:NO_2 ratios in the region of 0.23 (2001) to 0.47 (2000). NO_x levels are therefore undoubtedly higher in urban areas than indicated by NO_2 diffusion tube data alone, suggesting that NO_x standards might be exceeded at many more of the study sites.

Plant responses to nitrogen deposition are varied, but include a number of potentially detrimental effects on physiology, growth and phenology. A reduction in fine root biomass, root:shoot ratios and an increased sensitivity to drought have been shown for many ecosystems (e.g. De Visser *et al.,* 1996; Bardgett *et al.,* 1999; Power *et al.,* 1998; Gorden *et al.,* 1999). This may be particularly important for vegetation in urban environments which typically experience higher air temperatures and lower humidities than rural areas.

Changes in plant phenology and associated effects on sensitivity to low temperature stress have also been linked to enhanced nitrogen availability. For example, earlier spring growth of *Calluna* and an associated increase in sensitivity to frost stress has been demonstrated in both field and laboratory studies (Gorden *et al.,* 1999, Power *et al.,* 1998, Van der Eerden *et al*., 1991). Increased foliar nitrogen concentrations and decreased C:N ratios are frequently reported in response to elevated nitrogen availability. This study showed a clear relationship between these variables and both distance from central London and NO_2 concentration. Furthermore, stable isotope signatures indicate that vehicle-derived pollution constitutes a greater proportion of the nitrogen budget of plants in inner city, compared with rural areas. Elevated foliar nitrogen concentrations are typically associated with increased attack by insects such as aphids (Fluckiger & Braun, 1998) or by fungal pathogens (Strengbom, 2002).

Species differences in growth response to elevated nitrogen inputs and sensitivity to biotic and abiotic stresses will affect resource competition at a community level, and there is a strong indication from this study that urban ecosystems may be more at risk from nitrogen-induced changes in species composition and associated effects on ecosystem function and biodiversity than vegetation in more rural areas. Changes in plant growth and community composition have been reported with increasing proximity to a major trunk road in the UK (Angold, 1997). Angold (1997) suggested that changes in the growth and vitality of lichens and higher plants in the New Forest could be related to vehicle emissions, providing field evidence of the potential for effects of NO_x on sensitive plant communities.

3.8. Estimating total nitrogen deposition inputs

As mentioned previously, foliar nitrogen concentrations in *Calluna* have been shown to have a distinct relationship with nitrogen deposition load across wide geographical ranges and across a variety of soil types (Hicks *et al*., 1999; Pitcairn *et al*., 1998, 2001). In their recent study involving data from both the UK and NW Europe, Pitcairn *et al.* (2001) reported that foliar nitrogen concentrations increased at a rate of 0.036% for every 1 kg ha^{-1} yr^{-1} increase in nitrogen deposition rate, with an intercept of 0.865 %N. If this relationship is applied in reverse, total nitrogen deposition loads can be estimated from foliar concentrations.

The lowest foliar concentrations found in this study was 0.91% N, at Thursley Common, 50 km away from central London. The equation derived by Pitcairn *et al*. (2001) indicates that this represents a deposition load of approximately 1.25 kg N ha^{-1} yr^{-1}. This is in fact considerably lower than calculated total nitrogen deposition for this site (10-15 kg ha^{-1} yr^{-1}; Power, unpublished data) and may be in part be explained by the fact that sample collection for this study took place at a time when foliar nitrogen concentrations are at a seasonal low (Heil & Bobbink, 1993) whereas the relationship established by Pitcairn *et al*. (2001) used concentration data obtained during the summer/autumn, when levels are typically higher. Foliar nitrogen concentrations have been measured at different times of the year at Thursley Common, as part of an ongoing study, and show that summer concentrations are typically 25% higher than those measured in autumn/winter (S. Power, unpublished data). If a seasonal correction is applied to data for this site, winter values of 0.91% N could be considered equivalent to summer values of 1.14 %. Using the Pitcairn *et al.* (2001) relationship, this would equate to a deposition load of 7.6 kg N ha^{-1} yr^{-1}.

The highest foliar concentrations measured in the present study were 1.5% N, at the Natural History Museum. Using the same relationship, this represents a deposition load of 17.6 kg N ha^{-1} yr^{-1}. When corrected for seasonality, this rises to values of 1.88% and 28.0 kg N ha^{-1} yr^{-1} respectively. Another way of looking at the relationship between foliar nitrogen concentration and deposition is to establish a threshold concentration which represents exceedance of a particular deposition load. For *Calluna*-dominated heathland the critical load for nitrogen is 15-20 kg ha^{-1} yr^{-1}. Using the Pitcairn *et al.* (2001)

relationship, this equates to summer/autumn foliar concentrations of 1.33-1.58%. Given the seasonal differences in foliar concentrations, it seems reasonable to suggest that sites with winter foliar concentrations in excess of 1.06-1.26% N (equivalent to 15-20 kg ha^{-1} yr^{-1}, after applying a 25% seasonal correction to the winter foliar %N values) receive nitrogen inputs in excess of current critical loads for this nutrient.

The Natural History Museum, Barnes Common and Coombe Hill had (winter) foliar nitrogen concentrations of 1.5%, 1.44% and 1.27% (respectively), suggesting that these sites receive nitrogen deposition loads in excess of 20kg ha^{-1} yr^{-1}. Critical nitrogen loads have been suggested for only a limited number of ecosystem types (Bobbink & Roelofs, 1995). However, with the exception of particularly sensitive ecosystems which would not occur in UK urban areas (e.g. ombrotrophic fens, arctic/alpine heaths), most suggested critical loads fall within the 15-30 kg N ha^{-1} yr^{-1} range. It seems likely, therefore, that in addition to exceeding critical levels for NO_x, many urban ecosystems are also receiving deposition inputs close to or in excess of the nitrogen critical load.

One of the aims of this study was to try to assess the magnitude of the urban nitrogen footprint. However, this has not been possible for a number of reasons. Of the possible indicators of urban nitrogen deposition (%N, C:N, $\delta^{15}N$), none showed a clear cut off at a particular distance from the city centre. There was an indication that %N was higher and C:N ratio lower in inner city than in suburban areas, but there are insufficient data points to fit a non-linear slope to the graph with distance for these variables. $\delta^{15}N$ also had the potential to be an indicator of the size of the urban nitrogen influence, but was clearly affected by site location in relation to major roads, and possibly also root uptake of nitrogen. An increase in the number of sites surveyed and the inclusion of other species such as mosses (which were initially proposed, but outside of the scope of this pilot study), could provide a clearer estimate of the geographic extent of traffic-derived pollution in urban/suburban areas.

4. Conclusions and recommendations

Whilst this study was intended as only a small scale pilot project, it has nevertheless generated some important data which indicate that urban ecosystems are experiencing considerably higher deposition inputs of nitrogen than those in more rural locations. Foliar nitrogen concentrations are particularly high in central London and, whilst NO_2 represents only a proportion of total oxidised deposition in urban areas, these parameters were significantly related. $\delta^{15}N$ values for *Calluna* shoots were related to distance from central London, although it is not currently possible to interpret this as an index of traffic-derived pollution without further study. **Investigation of the isotopic signature of traffic-derived NO_x and NH_3, together with more detailed assessment of changes associated with soil N transformations and root uptake in urban areas would enable an evaluation of the use of isotopic signatures in higher plants as biological indicators of traffic pollution**.

Although the pilot project was not designed to test whether plants exposed to higher concentrations of NO_x exhibit changes in growth or phenology, the implications are that nitrogen deposition loads are high enough to have effects on species composition and ecosystem stability. **However, an assessment of the sensitivity of urban vegetation to ambient levels of NO_2 and, perhaps even more importantly, NO is urgently required.** Large cities are subject to a variety of environmental stresses, such as higher temperatures and wind speeds. These, in combination with other factors unique to built up areas (e.g. night time illumination from street lights) may make urban vegetation particularly sensitive to atmospheric pollution. **Interactions between atmospheric pollution and these urban stresses therefore clearly require investigation.**

The current study demonstrated the value of *Calluna* as an indicator species but, of necessity, was restricted to remnants of acidic heathland habitat within the London area. This naturally limited the choice of sites and resulted in the use of samples at a variety of distances from major roads. **An expansion of this pilot study, to include further sites and other key species, would allow a more accurate assessment of the contribution of local, as opposed to diffuse, sources of NO_x and the applicability of the results obtained for *Calluna* to other vegetation types and habitats.**

5. Acknowledgements

The contribution Sarah Honour made to this report, particularly in terms of pollution data from the National Diffusion Tube Network and from her own studies at the Natural History Museum's Wildlife Gardens, was invaluable. Her help is greatly appreciated and gratefully acknowledged. Cathy Shields, Jon Fear and Melanie Hardie all helped with sample preparation and chemical analyses at speeds beyond the call of duty, making it possible to produce this report in the available timeframe, so my thanks also to them. Thanks are also due to the many site owners, wardens and managers who not only gave permission to sample, but who often enthusiastically accompanied me on sample visits.

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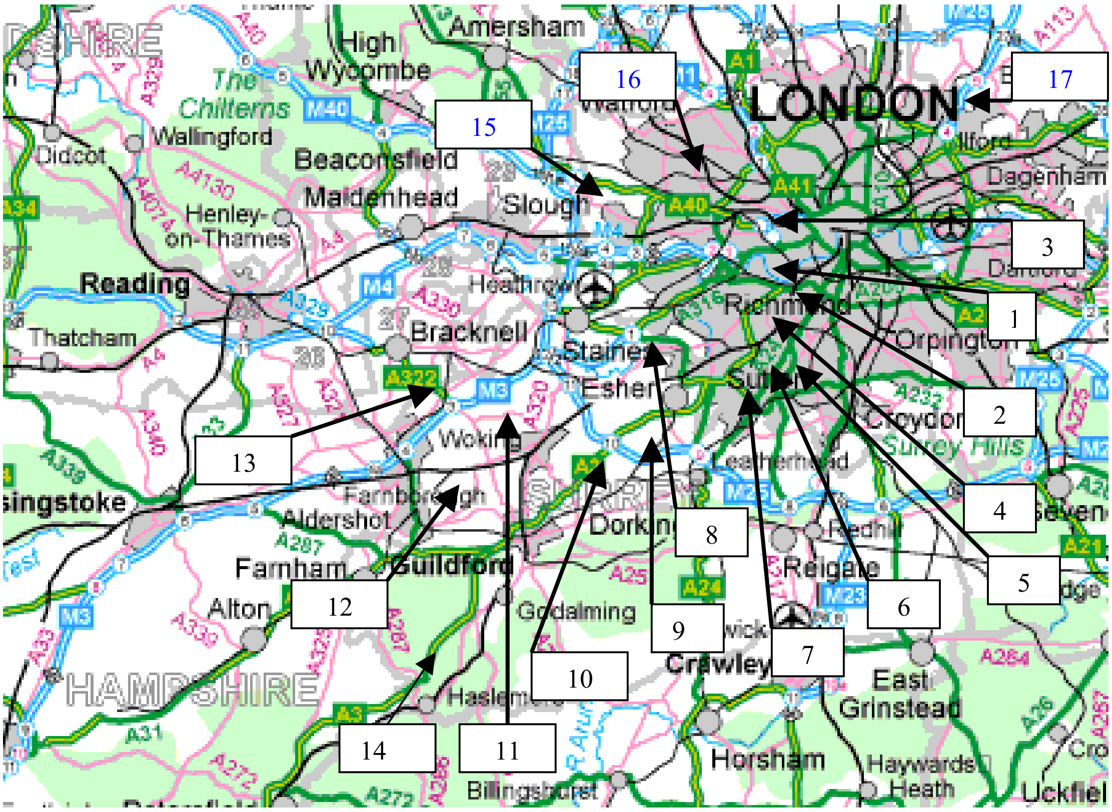

Appendix 1. Map of the sample transect (1-14) and North London sites (15-18)

Sample transect sites, their distances from Hyde Park Corner and the nearest 'A' road

No	Site name	Distance from Hyde Park Corner (km)	Distance from nearest 'A' road (km)
1:	Natural History Museum	2	0.02
2:	Barnes Common	7	0.10
3:	Hampstead Heath	7.5	0.03
4:	Putney Heath	9.5	0.34
5:	Wimbledon Common	10.5	0.63
6:	Richmond Park	13	0.90
7:	Coombe Hill Golf Course	13.5	0.57
8:	Hounslow Heath	17.5	0.53
9:	Esher Common	23.5	0.20
10:	St Georges Golf Course, Byfleet	27	0.57
11:	Chobham Common	35	0.44
12:	Horsell Common	36	0.56
13:	Silwood Park	37.5	0.17
14:	Thursley Common	50.5	1.00
<u>North London Sites</u>			
15:	Ruislip	22	0.33
16:	Stanmore Common	18.5	0.26
17:	Epping Forest	24.5	0.20

Appendix 2: Summary of estimated annual mean NO_2 concentrations for Calluna sites

Calluna sampling site	National Diffusion Tube Monitoring Network site	Distance from *Calluna* site (km)	2000 (ppb)	2001 (ppb)	Average (ppb)
Natural History Museum	**Natural History Museum**	**0**	**30**	**21**	**25.5**
Barnes Common	KENSINGTON 4N	4.3	16	12.7	
Barnes Common	RICHMOND UPON THAMES 3N	2.3	13.3	13.2	
Barnes Common	**Average**		**14.7**	**13.0**	**13.8**
Hampstead Heath	**CAMDEN 6N**	**1.3**	**14.5**	**15.2**	**14.5**
Putney Heath	KENSINGTON 4N	6.9	16	12.7	
Putney Heath	RICHMOND UPON THAMES 3N	4.2	13.3	13.2	
Putney Heath	**Average**		**14.7**	**13.0**	**13.8**
Wimbledon Common	KENSINGTON 4N	8.1	16	12.7	
Wimbledon Common	RICHMOND UPON THAMES 3N	4.9	13.3	13.2	
Wimbledon Common			**14.7**	**13.0**	**13.8**
Richmond Park	**RICHMOND UPON THAMES 3N**	**4.0**	**13**	**13.2**	**13.1**
Coombe Hill	**RICHMOND UPON THAMES 3N**	**5.3**	**13**	**13.2**	**13.1**
Hounslow Heath	SUNBURY ON THAMES 1N	4.8	14.1	14.2	
Hounslow Heath	RICHMOND UPON THAMES 4N	5.0	13.7	13.2	
Hounslow Heath	**Average**		**13.8**	**13.8**	**13.8**
Esher Common	**ESHER 3N**	**2.3**	**12.6**	**14.8**	**13.7**
Byfleet	**ADDLESTONE 5N**	**3.5**	**13.1**	**13.8**	**13.5**
Chobham	WOKING 7N	6.3	11.5		
Chobham	ADDLESTONE 3N	3.6	13.1	9.6	
Chobham	**Average**		**12.3**	**9.6**	**11.0**
Horsell Common	**ADDLESTONE 3N**	**3.6**	**13.1**	**9.6**	**11.4**
Silwood Park	WINDLESHAM 1N	4.5		14.0	
Silwood Park	WOKING 7N	9.9	11.5		
Silwood Park	**Average**		**11.5**	**14.0**	**12.8**
Thursley Common	FARNHAM 4N	8.0	11.8	8.7	
Thursley Common	GUILDFORD 3N	13.4	11.2	14.0	
Thursley Common	**Average**		**11.5**	**11.3**	**11.4**
Ruislip	**RICKMANSWORTH 3N**	**5.2**	**18.8**	**18.8**	**18.8**
Stanmore Common	**BOREHAMWOOD 4N**	**4.5**	**23.3**	**16.8**	**20.1**
Epping Forest	**BOREHAMWOOD 4N**	**5.3**	**23.3**	**16.8**	**20.1**

SECTION 3

Assessing the Role of Biological Monitoring Using Lichens to Map Excessive Ammonia (NH_3) Deposition in the UK

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Appendices

1. BACKGROUND

Research on isolated oak trees in the vicinity of intensive livestock units in the Netherlands has shown a strong correlation between the composition of lichen communities and atmospheric ammonia levels (van Herk 1999, 2002). The recording technique identified species that were obligate nitrophytes (nitrogen-loving species) or acidophytes (SO_2 tolerant species) enabling the construction of indices of nitrophyte 'NIW' (Nitrofiele Indicatie Waarde) and acidophytes 'AIW' (Acidofiele Indicatie Waarde).

In Britain, the Ammonia monitoring network at the Centre for Ecology and Hydrology, Edinburgh (http://www.nbu.ac.uk/cara/UKNAMN/uknamn.htm), monitored ammonia levels at 95 sites. Recent research in the vicinity of intensive poultry units in Britain has shown high levels of ammonia deposited locally along a deposition gradient of up to c. 300m from source (Fowler *et al.,* 1998). The residence time of ammonia in the atmosphere is rather brief so that the effects on vegetation are often localised around the source (NEGTAP 2001).

Although lichens have long been shown to be indicators of atmospheric acidification (Nimis *et al*., 2002), recent observations on epiphytic lichen communities in rural areas in the Netherlands have shown a widespread change in lichen communities with an increase in nitrophytes and a corresponding decrease in acidophytes (van Herk, 1999). Whereas there is little variation in the climate of the Netherlands, there is a marked climatic gradient across Britain ranging from drier conditions in the east to wetter oceanic conditions in the west which have a pronounced influence on the species composition of lichen communities. The continental method developed by van Herk was applied during the present survey in the contrasting climatic regions of Norfolk and Devon, in the vicinity of ammonia monitoring stations in order to test the potential application of the system in Britain.

2. OBJECTIVES

- To collect data from 2 sites in Britain with a history of intensive livestock management where NH_3 emissions have been monitored over a period of time.
- To test the results against scales developed in the Netherlands.
- To evaluate the method in order to develop a biomonitoring procedure in Britain.

3. METHOD

3.1. Site selection

Sites were selected using aerial photographs, taking into account: the availability of ammonia monitoring stations; the distribution of lichens in Britain including both continental and oceanic components; the presence of exposed oak trees on acid soils; and the presence of a site of conservation importance in the vicinity.

A site with a continental climate was selected in the Thetford region, Norfolk, where extensive ammonia monitoring has been taking place in the vicinity of intensive poultry and pig rearing units and in unimproved grassland utilised by the MOD. A site with an oceanic climate at North Wyke in Devon was chosen where ammonia is recorded in the vicinity of intensive cattle rearing sheds at IGER (Institute of Grassland and Environmental Research).

3.2. Station selection

Stations were selected according to availability of *Quercus* trees and prevailing wind direction. The distance to source of pollution was estimated from ordnance survey maps. Managed woodland and woodland edge trees were avoided where possible, as lichen communities varied considerably in these sites.

Stations were selected in Norfolk to coincide with existing ammonia monitoring stations. At North Wyke stations were selected within zones of up to 1000m radius of the farm units, as the ammonia station is located south and upwind of the cattle sheds and slurry tank.

Given the restrictions of time and the availability of trees, 3-5 upright standard *Quercus* trees >50cm girth were selected in exposed well-lit situations. Leaning trees were avoided or those with low epicormic growth or ivy on the trunk. Trees on the edge of managed woodland or intensively used roads were also avoided. Stations and tree position were marked on a map. Bark samples were collected for pH determination.

3.3. Lichen recording.

Corticolous species on each tree trunk up to 2m were listed and assigned a frequency value according to van Herk; 1 thallus on the trunk, 2 or more thalli, and species with >10cm^2 present. Where identification was not possible in the field specimens were collected for identification in the laboratory.

The data was subsequently converted for each station according to van Herk.

a) Only one thallus present
b) 2 or more thalli on one tree
c) Present on 30-50% of trees, < 10cm^2/tree
d) Present on 30-50% of trees, > 10cm^2/tree
e) Present on 50-100% of trees, <10cm^2/tree
f) Present on 50-100% of trees, >10cm^2/tree
g) NIW and AIW species were distinguished and scored according to van Herk and the mean AIW and NIW scores for each station were calculated (Appendix 1).

3.4. Lab techniques

Bark pH.

3 samples of bark were collected from each tree, placed in paper packets and dried. pH was measured using a GELPLAS flathead electrode after moistening bark with 25% KCl for c. 5 minutes (Bates *et al*. 1990).

Lichen species were identified using light microscopy and thin layer chromatography

3.5. Data analysis

Ammonia deposition was supplied as monthly averages for all stations in the Thetford area by the Breckland Council for Environmental Health and by CEH Edinburgh ammonia for the period February to October 2001 and as monthly averages for North Wyke by CEH Edinburgh between May 1997 and September 2001. Where present, mean ammonia values for lichen sampling stations in the Thetford area were plotted against NIW and AIW indices. As ammonia deposition was only recorded at one station at North Wyke, the distance from the farm units and slurry tank was plotted against NIW and AIW indices as a surrogate measure for ammonia deposition.

4. RESULTS

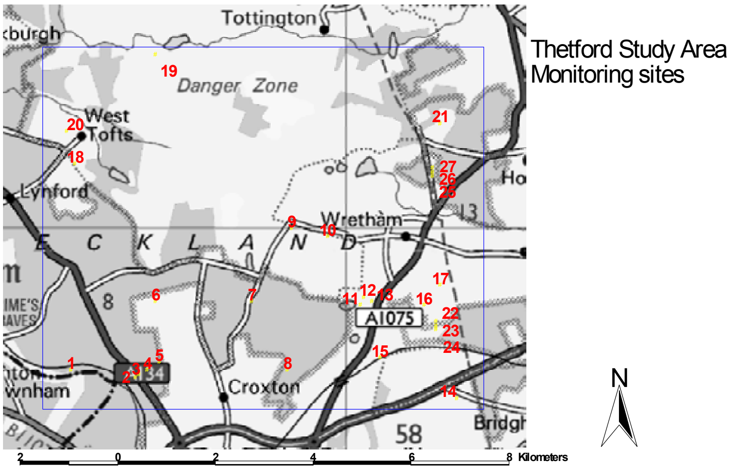
Site 1: Thetford area, Norfolk

In Norfolk 12 stations were sampled in the Thetford area coinciding where possible with ammonia monitoring stations (Fig1, table 1).

Table 1. Stations in the Thetford area.

			NH_3 deposition				Lichen diversity			
Stat	Station location	NH_3 no.	High	Mean	Low	no. T's	Trunk	Twig	Distance from source	Direction from source
1	Norfolk Wildlife Reserve 1	11	2.64	2.88	0.8	5	7.4	5	500	SW
2	Abrey field	c.13	3.13	2.9	1.86	3	10	2.7	300	All round
3	West Tofts	20	2.7	1.9	0.99	4	8.5	6.6	8000	W
4	Thorpe Gt heath	c.9	2.95	2.2	1.32	3	7.7	7.7	2600	W
6	Military camp	10	3.91	2.8	1.44	3	7	3	2-400	SW & NE
7	Hockham	25	124.7	43	27.27	4	7	4	2-300	SW &NE
8	Peddars way adj. Poultry units	16	16.46	9.2	5.63	3	5.3	0.5	50	E
9	Peddars way adj. pigs	c.22	9.81	4.54	1.4	3	10.3	2	50	All round
10a	Norfolk Wildlife Reserve 2	12	4.45	1.6	1.92	2	7.2	3.5	800	SW
10b	Norfolk Wildlife Reserve 3	15	2.91	1.9	1.12	2	7.2	5.5	1300	N & E
11	Stanford area	c.19	2.03	1.4	0.86	3	10	6.1	7000	NW
12	Santon Downham FC	1	3.32	2.05	1.2	3	7.3	8	8000	W

Fig. 1. Map of Thetford area showing NH_3 recording stations (see Appendix 1 for map of site with lichen stations).



Lichen diversity

A total of 45 lichen species were recorded in the Thetford site with mean lichen numbers varying from 4.7 to 10.7 species per station (Appendix 1). The lowest diversity coincided with proximity to intensive poultry units where ammonia levels were highest (32-124 µg/m^3 in October 2001) (fig. 2.). Overall the highest diversity of lichens occurred on oaks on MOD land west of the poultry units in unimproved grassland where stocking rates were low (12,000 sheep on 27,000 acres (*pers. comm.*)). Lichen diversity on twigs showed a better correlation with ammonia deposition than lichen diversity on trunks (figs. 2 & 3.). Contrary to expectations, diversity was often highest on younger tree trunks and lowest on trees of great girth and age in all stations (fig. 4.), even on MOD land where there has been no intensive agriculture for more than 50 years. This suggests previous damage to older trunks probably from a long period of sustained acidification. Within the MOD area foliose species of the Parmelietum were present and often abundant in the canopy, but on the trunks they were absent or restricted to the lowest parts of the tree trunk.

Diversity was also high in arable areas, although the community was dominated by nitrophyte species of the Xanthorion.

Distribution of nitrophyte and acidophyte species.

Only 9 NIW and 4 AIW species were recorded in the Thetford site in Norfolk from 20 possible nitrophyte and 20 acidophyte species characterised by van Herk. Mean ammonia deposition in all stations adjacent to lichen sampling was plotted against indices of AIW and NIW for these stations (Figs. 5 & 6). The NIW index showed a strong correlation with ammonia levels (Fig. 5), and the AIW showed little correlation with ammonia levels (Fig. 6). However in stations adjacent to the highest ammonia deposition lichen diversity was 0 -1

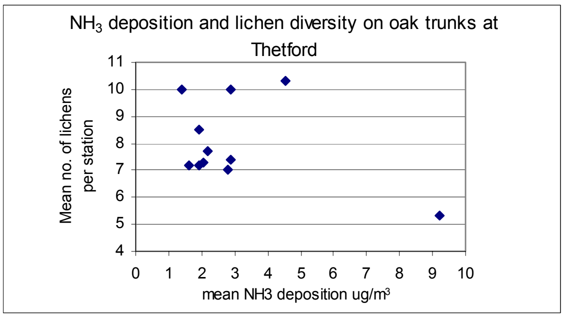


Fig. 2

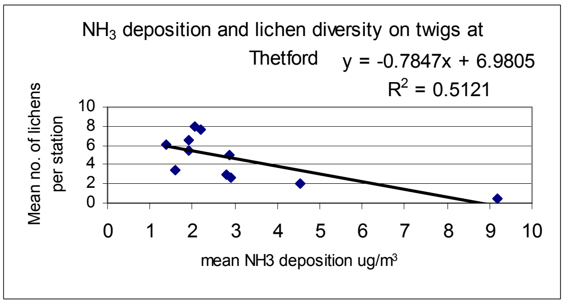


Fig. 3

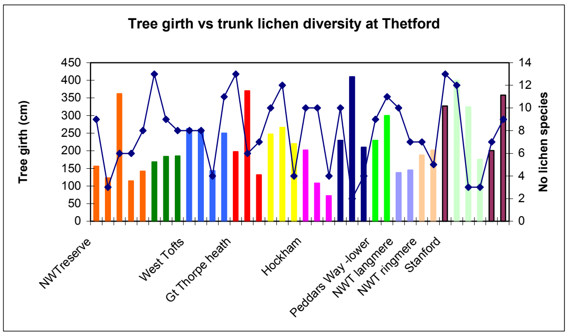


Fig. 4

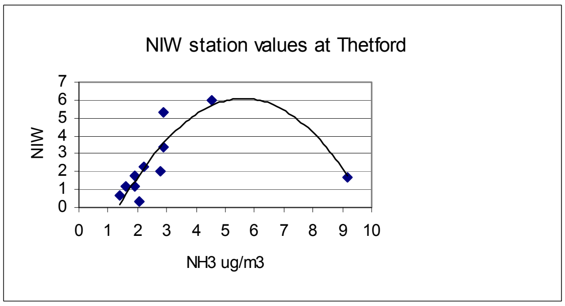


Fig. 5

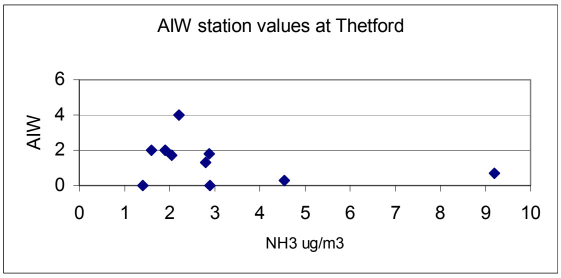


Fig. 6

on both trunks and twigs suggesting that there are critical loads of ammonia at which nitrophytes are absent.

Bark pH was also strongly affected in those areas where NH_3 levels exceeded 10µg/m^3 (Fig. 8). The stations in unimproved grassland in MOD or Forestry Commission sites with mean bark pH's between 3 and 4.50 coincided with ammonia deposition below 3 (except site 12 where lichen sampling was in unimproved pasture upriver from the ammonia recording site which was by a horse-riding centre without oak trees). In contrast mean bark pH's >5 were associated with stations where ammonia levels were above 10 µg/m^3 (fig. 7). The local ammonia deposition gradient was confirmed by bark pH measurements at the Norfolk Wildlife's Reserve (NWT), where bark pH was up to 6.2 in the vicinity of the poultry units and fell to c. 4 in the vicinity of Langmere 400m away. The directional nature of deposition was also indicated by variation in pH around the trunk on one young *Quercus* in the vicinity of the poultry unit, where a pH of 6.2 on the N. side of the trunk contrasted with a pH of 3.9 on the S side.

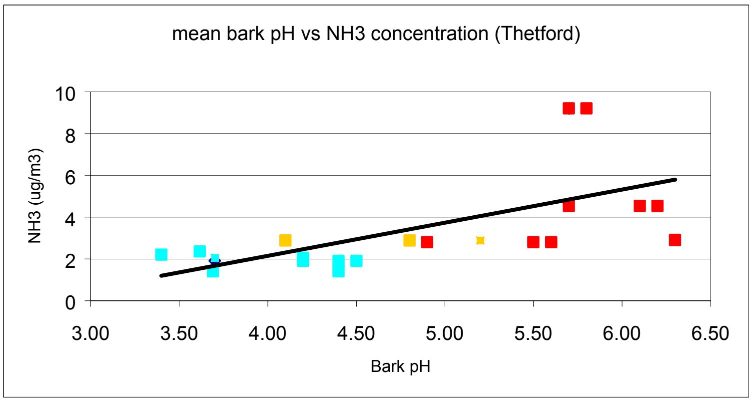


Fig. 7

blue – trees in unimproved grassland stations 5,11 & 12 with low ammonia levels.

red – trees at stations 7,8 & 9 in the vicinity of high ammonia levels.

yellow – trees on NWT nature reserve.

Site 2. North Wyke IGER, Devon.

In Devon 7 sampling stations were studied at North Wyke (Fig. 8, Table 2.) and 77 lichen species recorded (see Appendix 2.).

Table 2. Stations sampled at North Wyke, Devon

				Lichen diversity			
Station no	No. trees	Distance from source	Direction from source	trunk	twig	NIW	AIW
2	5	-20	E	18.2	5	0.8	1
1	5	30	W	18	4.4	0.4	1.4
3	4	-300	E	15.7	9.3	0	0
4	4	300	W	20.7	11.5	1.25	3
5	3	750	NW	18.6	12.3	0	5
6	2	150	SW	16.5		0	1
7	4	700	W	18	11.2	0	3.5

Distribution of stations sampled at North Wyke shown in Appendix 2

Lichen diversity

Lichen diversity per tree was high in all stations, averaging 17.6 on trees east of the cattle sheds and slurry tank to 20.7 on trees in pasture west of the sheds. Ammonia data was only available for one site to the west of the farm unit and levels were low throughout the year the highest being 3.54 µg/m^3 during August and September in each year. As the main concentration of ammonia is from the overwintering cattle sheds and slurry tank east of the ammonia monitoring station, distance from source was used as a measure of likely ammonia distribution along an east to west transect. The prevailing wind is from the southwest (figs. 8 & 9).

Mean lichen diversity on *Quercus* trunks per station plotted against distance from source on an east west axis shows little positive correlation (Fig. 8). Lichen diversity of twigs was considerably lower in the vicinity of the cattle sheds and slurry tank and was positively correlated with distance from source in both eastern and western directions (Fig. 9).

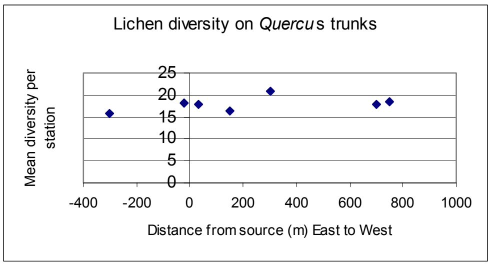


Fig. 8

This site also supports 10 lichen species that are indicators of long ecological continuity (NIEC species) as defined by Rose (1992), including the highly pollution-sensitive *Lobaria pulmonaria*, Teloschistes flavicans** and *Usnea ceratina*. These species occur in well-lit sites with high relative humidity and absence of either acidification or eutrophication (Table 3.). Other indicators of long ecological continuity include *Cresponea premnea* and *Lecanographa lyncea,* both species characteristic of dry bark. These species were fragmented and in poor condition restricted to the densely

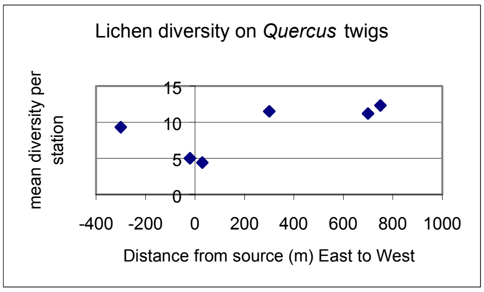


Fig. 9

shaded drier E. side of the trunk. These ancient woodland indicators are now almost all restricted to a single tree on the boundary of the settlement (S6) and as such are relicts of a former wood pasture regime.

Table 3. Distribution of NIEC species in stations at North Wyke
stations in order of distance from source

No. of trees at station	5	4	5	4	4	3	1
	S2	S3	S1	S4	S7	S5	S6
Arthonia vinosa						2	
Dimerella lutea			2				
Cresponea lyncea							2
Lecanographa premnea							1
Lecanora jamesii	2			2	1		
Lobaria pulmonaria							2
Punctelia reddenda			6		1		
Phaeographis dendritica	1	1					
Teloschistes flavicans							2
Usnea ceratina				3	2	3	
No of NIEC species	2	1	2	2	3	2	4

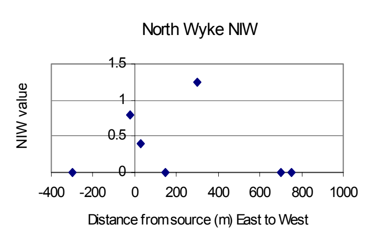


Fig. 10

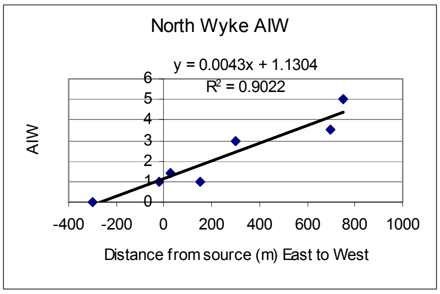


Fig. 11

NIW and AIW distribution.

12 AIW and 6 NIW species were recorded at North Wyke from a total of 20 AIW 20 NIW species as defined by van Herk. However species of the Xanthorion in the NIW were rare or absent on trunks but present at low to high levels on twigs at all sites, except in one sampling station on an acid wetland reserve where acidophytic species were dominant and the nitrophyte species of the Xanthorion absent. There is little correlation between distance from the farm units and NIW species (Fig. 11) while AIW species show a strong correlation – (R^2=0.9) with distance from source suggesting that the farm unit has an effect on these species.

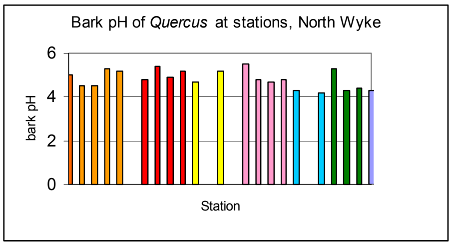


Fig. 12 Stations 1- orange, 2 - red, 3 - yellow, 4- pink, 5- blue, 7 - green

Bark pH.

Bark pH for all trees sampled showed little variation between trees at each station at North Wyke (Fig. 12). The lowest bark pH occurred at station 5 on the edge of a *Molinia* moor. If bark pH is plotted against distance from source, there is a trend towards higher pH in the vicinity of the farm unit and a rapid fall off within 400m in both an easterly and westerly direction (Fig. 13).This suggests that the farm unit is responsible for the observed changes in bark pH.

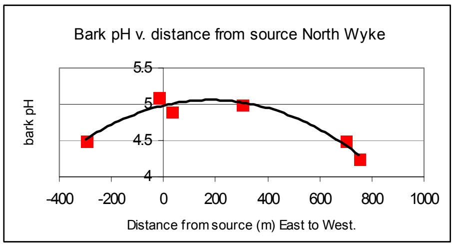


Fig. 13

5. DISCUSSION

Variation between sites.

Both sampling sites were on acid substrata, one on sand (Thetford) and the other on heavy riverine clays and loams (North Wyke), and both sites contained mature oak trees well distributed across the site. The sites are climatically very different. Rainfall in East Anglia averages 55 mm per year with considerable dry periods whereas in Devon the average is c. 176 mm per year with high relative humidity in all months. This allows a wide variety of oceanic lichen species to flourish and lichen cover and diversity tends to be higher in this region.

Previous acidification effects are conspicuous in the Thetford region on the ancient oaks where lichen diversity was very low even in areas of unimproved wood pasture in the MOD area where there has been long ecological continuity. At North Wyke, where there is no evidence of former acidification, large old *Quercus* tree trunks support a highly diverse lichen community even in the vicinity of the farmstead including many oceanic species, pollution sensitive species and indicators of long ecological continuity (Table 1). In this site there was little impact of ammonia on long established natural lichen communities. Although both Thetford and North Wyke are areas with a tradition of wood pasture since Norman times with potentially long ecological continuity, the absence of former acidification and the higher rainfall at North Wyke must contribute to the survival of pollution sensitive species in this site.

Changes in temperature regimes may also contribute to changes in the distribution of lichen species. Many nitrophytes are predominantly warm temperate species and are benefiting from the trend of increasing mean annual temperatures associated with global warming (van Herk *et al*., 2002).

Nitrophyte and acidophyte species.

Only 9 out of a possible 20 NIW species, and 8 out of 20 possible AIW species listed in van Herk were recorded in the surveys. This is partly due to the bias towards continental species in the Netherlands, and that the UK flora is more oceanic even in the eastern regions. Nitrophytes that are abundant in the Netherlands are absent or rare in Britain and others that may be considered as nitrophytes are excluded (see James et al. 1976). Alternative species considered to be nitrophytes in Britain that are present at the sites visited are indicated by a* in the table of species in the Appendices. Further testing in order to derive an appropriate species list for both nitrophytes and acidophytes is essential in regions where atmospheric conditions are monitored. In the Netherlands several new species have been described that are now appearing in Britain.

Ammonia deposition and lichens.

Ammonia concentrations at stations in the area around Thetford were positively correlated with NIW indices and distance from source and showed little correlation between AIW indices and distance from source.

There was no correlation of NIW species at North Wyke on trunks with distance from the farm unit as very few of the NIW species of the Xanthorion were present in the closed communities on the oak trunks. However, NIW species were present on twigs, indicating that these species were colonising recently established lichen communities. At North Wyke ammonia levels were low, but the recording site was situated on the western side of the farm unit and would have only received higher levels during the distribution of slurry. The negative correlation of AIW species with distance from the farm unit, indicates that these acidophyte species are affected by the farm unit. The corresponding increase in bark pH in the vicinity of the farm unit correlates with the loss of acidophytic species characteristic of undisturbed lichen communities in this region. Bark pH correlated with lichen communities recorded at both Thetford and North Wyke and to the ammonia levels (Thetford) or distance from source (North Wyke). In areas where there is little environmental change, bark pH's are relatively stable on the tree and at the site. Whereas in areas where changes are occurring as in the Thetford region, there are wide variations in bark pH even on one tree suggesting that there is a directional element in the pollutant causing an increase in bark pH. Increased bark pH is associated with an increase in ammonia deposition. Preliminary results on 3 trees at site 1 & 10 in the NWT reserve show a rapid change in bark pH suggesting that there is an environmental gradient on this site which agrees with Fowler et al. (1998) in falling off at c. 700m from the source. This is not supported by the ammonia deposition tubes which record highest levels of ammonia as $4.2\mu g/m^3$ in a recording station in the vicinity of station 10a.

The positive correlation between ammonia deposition and NIW species at Thetford indicates a similarity between lowland sites in the east of Britain and the Netherlands. It also suggests that there are critically high levels of ammonia in this region where lichens including NIW species are absent (Figs 3 & 5).

Lichen diversity on trunks and twigs

Preliminary observations from a basic inventory of lichen diversity on twigs at both sites suggest that primary colonisers of the newer bark surfaces are the most sensitive to changes in atmospheric conditions. In contrast, trunk species may reflect longer-term environmental conditions of ecological continuity (as at North Wyke) and former acidification through SO_2 air pollution (Thetford). Although there was very little correlation between lichen diversity on trunks and proximity to sources of ammonia at North Wyke and Thetford, there was a strong correlation between lichen diversity on twigs and ammonia sources at both sites (figs. 3 & 9). Nitrophyte species were present in this community. At North Wyke nitrophyte Xanthorion species were conspicuously absent from the trunks but present on the twigs in the vicinity of the farmstead.

6. CONCLUSIONS

This pilot survey using techniques and indicator species defined as nitrophytes and acidophytes during research in the Netherlands (van Herk, 2002) suggests that epiphytic lichen communities on oak are sensitive to increased levels of atmospheric ammonia in Britain.

However many NIW and AIW species recorded for the Netherlands were not found in sites sampled in either Norfolk or in Devon, suggesting that further work is needed to define appropriate nitrophyte and acidophyte indicator species in Britain. The research should define indicator species in relation to the wide climatic gradient across Britain, and in relation to other widespread nitrogen sources such as from fertilisers, car exhausts and fossil fuels, whose effect on lichen assemblages is poorly understood. Lichen species identified as possible candidates for these lists are indicated by a * in Appendices 3 & 4. As with the research in the Netherlands it is to be expected that species new to science or to Britain may be identified during surveys.

The results of a rapid survey of lichens on twigs made during the pilot study is particularly interesting and suggests that there is a strong correlation between species colonising the new bark surface and distance from ammonia sources. Further research is needed together with NH_3 monitoring to test the capacity for twig communities to respond to rapid fluctuations in NH_3 concentrations over short periods of time, compared with communities and NIW/AIW indicators on trunks and twigs. The existence of a sampling method (Wolseley & Pryor, 1999; Wolseley, 2002) will allow a quantitative assessment of change in lichen communities to be tested against ammonia levels and other parameters.

7. RECOMMENDATIONS

- Define nitrophyte and acidophyte species appropriate for the UK based on multivariate analysis.

- Extend quantitative sampling to include twigs on oak trees across a gradient of NH_3 deposition.

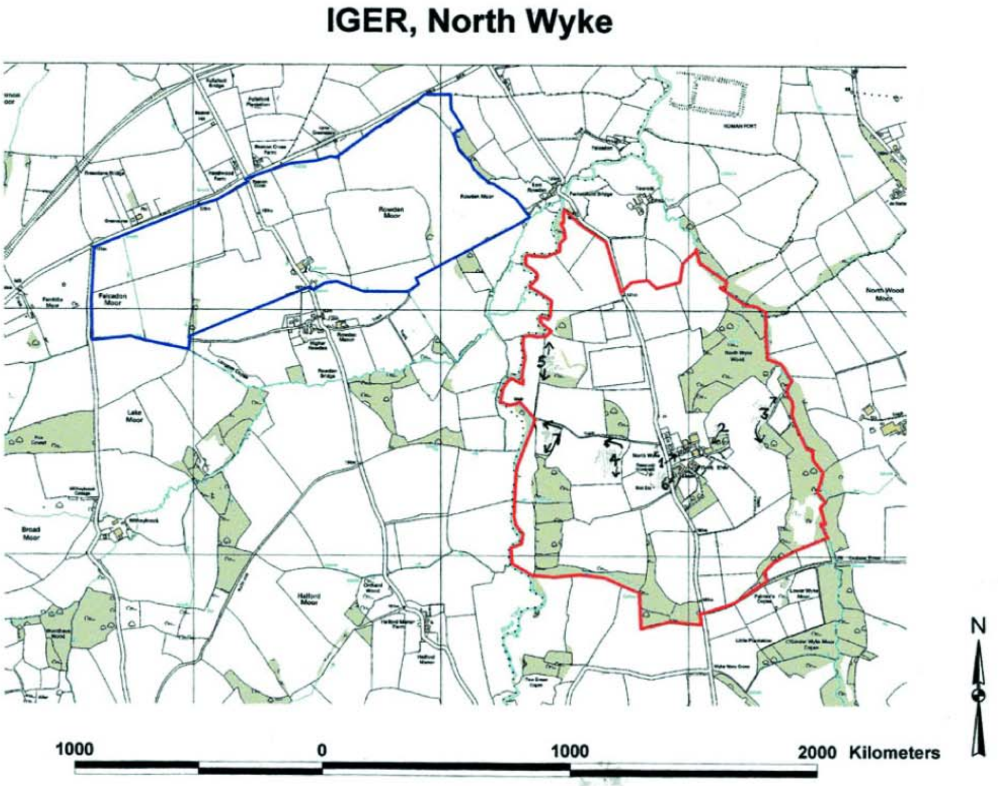
- Devise a practical recording method using lichens to detect changes in atmospheric nitrogen appropriate for Britain

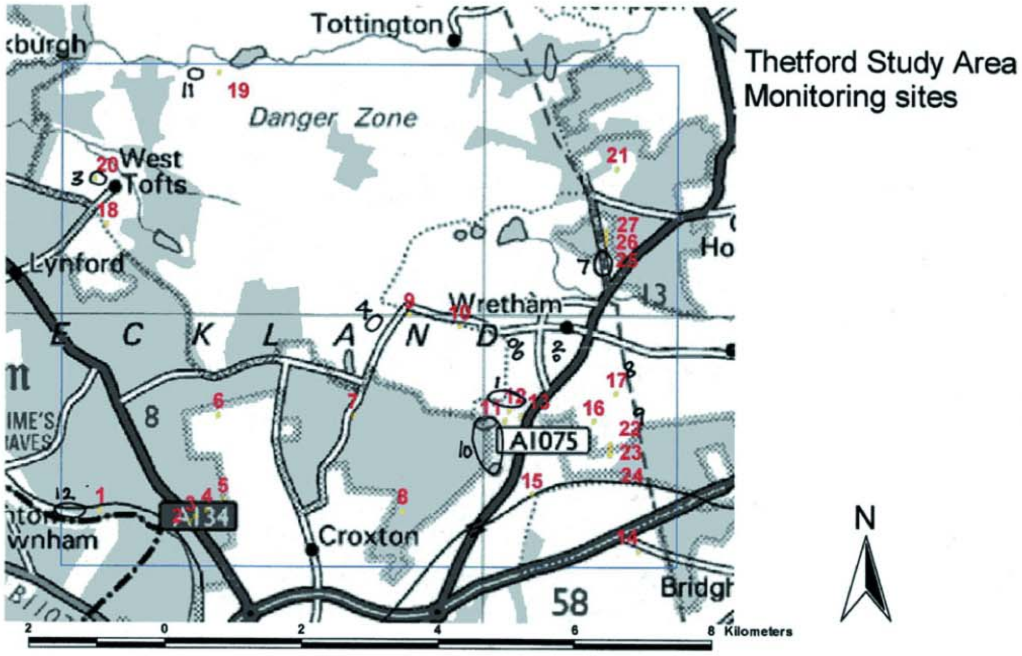
- Include recording of bark pH together with ammonia and other nitrogen products monitoring across Britain, with particular reference to areas that were formerly acidified.

- Undertake further research on the effects of agricultural management on lichen communities including research stations where agricultural methods and eutrophication factors are being researched. The high lichen diversity at North Wyke makes this an ideal situation in which to extend ammonia monitoring and establish lichen monitoring.

APPENDIX A

Maps of lichen stations at Thetford and North Wyke.





APPENDIX B

Species identified by van Herk as nitrophytes and acidophytes (1999, 2002). Species highlighted were found during the survey.

Nitrophytes	**Acidophytes**
Caloplaca citrina	*Cetraria chlorophylla*
C. holocarpa	*Chaenotheca ferruginea*
Candelaria aurella	*Cladonia sp.*
C. reflexa	*Evernia prunastri*
C. vitellina	*Hypocenomyce scalaris*
C. xanthostigma	*Hypogymnia physodes*
Lecanora muralis	*H. tubulosa*
L. dispersa grp.	*Lecanora aitema*
(incl. *L. hagenii)*	*L. conizaeoides*
Phaeophyscia orbicularis	*L. pulicaris*
P. nigricans	*Lepraria incana*
Physcia adscendens	*Ochrolechia microstictoides*
P. caesia	*Parmelia saxatilis*
P. dubia	*Parmeliopsis ambigua*
P. tenella	*Placynthiella icmalea*
Rinodina genarii	*Platismatia glauca*
Xanthoria candelaria	*Pseudevernia fufuracea*
X. calcicola	*Trapeliopsis flexuosa*
X. parietina	*T. granulosa*
X. polycarpa	*Usnea spp.*

APPENDIX C.

Table of species found on *Quercus* trunks in vicinity of Thetford with van Herk frequency values for each species at each station. Acidophytes in blue, nitrophytes in yellow. *Additional species to be considered as nitrophytes in the UK.

Order of stations by NH$_3$ dep.		1	2	3	4	5	6	7	8	9	10	11	12
Mean diversity per trunk		7	5.3	10.3	10	7.4	7	7.7	6.1	7.5	8.5	10	7.3
	Stations	S7	S8	S9	S2	S1	S6	S4	S12	S10	S3	S11	S12
Species on trunk													
*Amandinea punctata		2		5	6	1	6			3	3	3	
Arthonia spadicea									1				1
Arthonia sp						1							
Athelia sp						1							
*Bacidia delicata		1				2		2		2		2	
Bryophytes		6		1	6				6	6	5	5	6
Candelariella reflexa		1											
Candelariella vitellina					3								
*Chrysothrix candelaris											2		
Cladonia sp.									2				2
Cliostomum griffithii			2	3	1		6		3	2		3	3
Desmococcus			6	6	6	6	6	6				6	
Dimerella pineti									1				1
Diploicia canescens		3			3		2				3	5	
Evernia prunastri		2				1	6	6		3	2		
*Hyperphyscia adglutinata					1								
Hypogymnia physodes								2					
cf Lecanora compallens		3		5	5	2			6	6	6		6
Lecanora dispersa		2		5									
*Lecanora expallens		1	3		2		2			3		3	
*Lecidella elaeochroma		2		1									
Lepraria incana			5	2		6		6	6	6	6		6
Melanelia subaurifera			1		1	3	2	3	2	3			2
Micarea prasina										2		1	
Haematomma caesium					2								
Opegrapha atra				1									
Flavoparmelia caperata									2	2	3	5	2
Hypotrachyna revoluta												2	
Parmelia sulcata					1			6		3	2	2	
Pertusaria amara											2		
Pertusaria hymenea												2	
Phaeophyscia orbicularis						2							
Phlyctis argena			2						2		2		2
Physcia adscendens		2	1	5									
Physcia tenella			2	5	6	6	5	3		2	3		
*Physconia grisea					5								
Punctelia borreri												1	
Punctelia subrudecta						3	3	2	2			3	2
Punctelia ulophylla													
Pyrrhospora quernea												3	
Ramalina farinacea							3			3	1	1	
Ramalina fastigiata								3					
Schismatomma decolorans				3								3	
*Scoliciosporum chlorococcum		3				6				5			
Xanthoria candelaria		3	2	1		3		2		3		3	
Xanthoria parietina		6	2	6	6	3	3	2			2		
Xanthoria polycarpa		3	2	3	5	2	1		2				2
Total 48 species													

APPENDIX D

Table of species found on *Quercus* trunks at North Wyke, Devon with van Herk frequency values for each species at each station. Acidophytes in blue, nitrophytes in yellow. *Additional species to be considered as nitrophytes in the UK.

Stations sampled	S1	S2	S3	S4	S5	S6	S7
Av. Species no. per station	18	17.6	18	20.7	18	18	16.5
Acrocordia conoidea		3					
Acrocordia gemmata							2
*Amandinea punctata	5	4	3	3			
Arthonia impolita					3		
Arthonia radiata	2						2
Arthonia vinosa					2		
*Bacidia delicata			2	3			
Bacidia rubella	1						
Candelariella reflexa		1					
Candelariella vitellina				2			
*Chrysothrix candelaris		5	2	3	3		4
Cladonia coniocreae					1		3
Cladonia pyxidata					2		
Cladonia ramulosa					1		
Cladonia sp.	2				3	1	
Cliostomum griffithii	2		3	5	3		2
Dimerella lutea	2						
Dimerella pineti			2				
*Diploicia canescens		4					
Enterographa crassa	1	4				2	
Evernia prunastri		3		3			3
Fuscidea lightfootii				2			
Hypogymnia physodes		1			2		
Hypotrachyna revoluta	6	5	4			2	3
Cresponea lyncea						2	
Lecanographa premnea						2	
Lecanora chlarotera	5	3	4	5	3	2	3
Lecanora dispersa		1					
*Lecanora expallens	6	6	6	6	6	2	6
Lecanora jamesii		2		2			1
*Lecidella elaeochroma	5		5	5		1	2
Lepraria sp	2		4	3	3		
Lobaria pulmonaria						2	
Melanelia fuliginosa							2
Melanelia subaurifera	5	3		3			3
Normandina pulchella						2	
Ochrolechia subviridis	5	2	3	3		2	2
Ochrolechia turneri			1				
Opegrapha atra	5	6	1	1		2	
Opegrapha herbarum		3					
Opegrapha multipuncta					1		
Opegrapha vulgata							
Flavoparmelia caperata	6	4	1	5	3		6
Parmelia saxatilis	1	2		3			3

Parmelia sulcata	2	3	3	3	2		2
Parmotrema chinense	6	4	4	6	6	2	6
Pertusaria albescens v. corallina	4	3	6		6	2	6
Pertusaria albescens			2	6	2		1
Pertusaria amara	3	3	4	3	3		6
Pertusaria hemispherica					2		
Pertusaria hymenea	6	6	6	6	6	2	5
Pertusaria pertusa			3	5	4		6
Phaeographis dendritica		1	1				
Phaeophyscia orbicularis		1					
Phlyctis argena		3		5	4		3
*Hyperphyscia adglutinata		3		1			
*Physcia pulverea		3					
Physcia tenella				2			
Placynthiella icmalea	1						
Punctelia borreri				1			
Punctelia reddenda	5						1
Punctelia subrudecta		3	3			2	
Punctelia ulophylla			3				
Pyrrhospora quernea	5	5	1	3	6		6
Ramalina calicaris							1
Ramalina canariensis	2						
Ramalina farinacea	2	1	4	3			4
Ramalina fastigiata	2	1	3	3			2
Rinodina roboris		1					
Schismatomma cretaceum		3					
Schismatomma decolorans	6	6	4	5	6		6
Teloschistes flavicans						2	
Usnea ceratina				3	3		2
Usnea cornuta	3				2		2
Usnea flammea				2	3		
Usnea rubicunda				1	1		
Xanthoria parietina	2	2		1			
Total diversity = 78							
Total AIW = 12	3	3	0	5	9	1	5
Total NIW = 6	1	4	0	3	0	0	0

APPENDIX E

Table of species recorded on *Quercus* twigs Acidophytes in blue, nitrophytes in yellow.

North Wyke	**Thetford**
Acrocordia conoidea	Amandinea punctata
Amandinea punctata	Arthonia punctiformis
Arthonia punctiformis	Arthonia sp
Arthonia radiata	Arthopyrenia sp.
Arthopyrenia fallax	Athelia sp
Arthopyrenia punctiformis	Bacidia delicata
Evernia prunastri	Candelariella vitellina
Fuscidea lightfootii	Chrysothrix candelaris
Graphina anguina	Cliostomum griffithii
Graphis scripta	Desmococcus
Hyperphyscia adglutinata	Diploicia canescens
Hypogymnia tubulosa	Evernia prunastri
Hypotrachyna revoluta	Hyperphyscia adglutinata
Lecanora albella	Hypogymnia physodes
Lecanora chlarotera	Hypogymnia tubulosa
Lecanora expallens	Lecanora carpinea
Lecanora intumescens	Lecanora chlarotera
Lecanora persimilis	cf. Lecanora compallens
Lecanora symmicta	Lecanora expallens
Lecidella elaeochroma	Lecanora dispersa grp
Lecidella elaeochroma f. soralifera	Lecanora expallens
Melanelia subaurifera	Lecidea symmicta
Micarea prasina	Lecidella elaeochroma
Opegrapha atra	Lepraria incana
Opegrapha herbarum	Melanelia subaurifera
Flavoparmelia caperata	Mycoporum quercus
Parmelia sulcata	Flavoparmelia caperata
Pertusaria amara	Parmelia sulcata
Phaeographis dendritica	Pertusaria amara
Physcia aipolea	Phaeophyscia orbicularis
Physcia tenella	Physcia adscendens
Punctelia borreri	Physcia aipolea
Punctelia subrudecta	Physcia tenella
Ramalina canariensis	Phlyctis argena
Ramalina farinacea	Physconia grisea
Ramalina fastigiata	Punctelia borreri
Scoliciosporum chlorococcum	Punctelia subrudecta
Usnea subfloridana	Punctelia ulophylla
Xanthoria parietina	Ramalina farinacea
Xanthoria polymorpha	Scoliciosporum chlorococcum
	Xanthoria candelaria
	Xanthoria parietina
	Xanthoria polycarpa

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