Biomonitoring tools for assessing effects of atmospheric nitrogen deposition on statutory nature conservation sites.

Interim report for contract no. F90-01-535

Carole Pitcairn[a], Ian Leith, Lucy Sheppard[a], Ute Skiba[a], Netty Van Dijk[a], David Fowler[a], Pat Wolseley[b], Peter James[b] and Mark Sutton[a]

a NERC Centre for Ecology and Hydrology Edinburgh

b, Department of Botany, The Natural History Museum, London

29 November 2002

Contents

Introduction

Because of the short time scale for this project and the obvious restraints on any growth related experiments carried out outside the major growing season, work has focussed on the field-work component of the project. Consequently, this progress report concentrates on the field study, including methods used and available results. Comparisons between the methods cannot be made until all results have been received.

The literature review is in progress and initial drafts have already been received on some topics. The workshops attended by staff at the Bradford University and Berne on the setting of critical loads for nitrogen provided useful discussion on several aspects of the literature review.

Intercomparison of N Biomonitoring Methods from Field Study September-November 2002.

In order to compare the different bioindicator methods, and to minimize costs, the experimental work was focussed on a single local gradient in N deposition that has already been well studied. This has the advantage that information on several additional bio-indicators is already available for comparison, so that these methods do not need to be repeated.

A Site description

The site selected for study is a poultry farm situated in southern Scotland at an altitude of 230 m in an agricultural area with background concentrations of ammonia and nitrogen oxides <1 μg m^{-3} and <3 μg m^{-3}, respectively. Impacts of N deposition resulting from ammonia emissions on the surrounding woodland have been studied extensively since 1995 and results have been published in peer-reviewed journals (Pitcairn et al. 1998; Skiba et al. 1998; Fowler et al. 1998; Pitcairn et al In press).

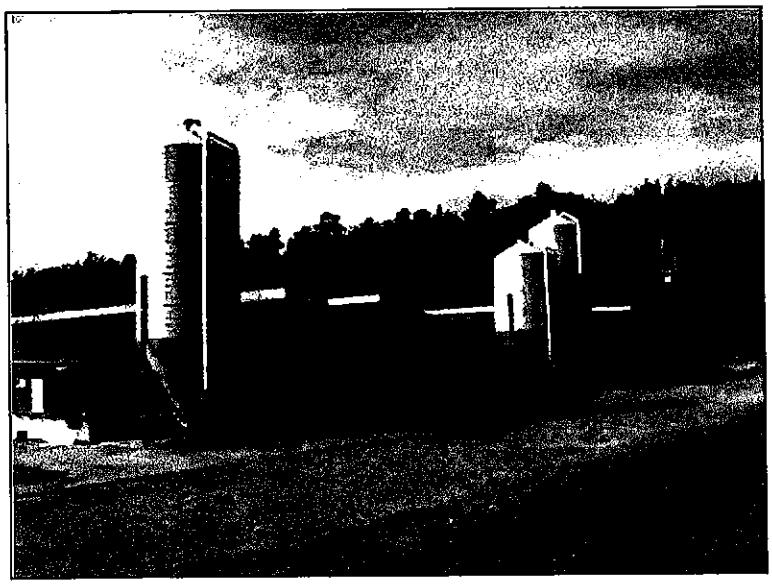


Figure A.1. Poultry unit at Earlston, southern Scotland

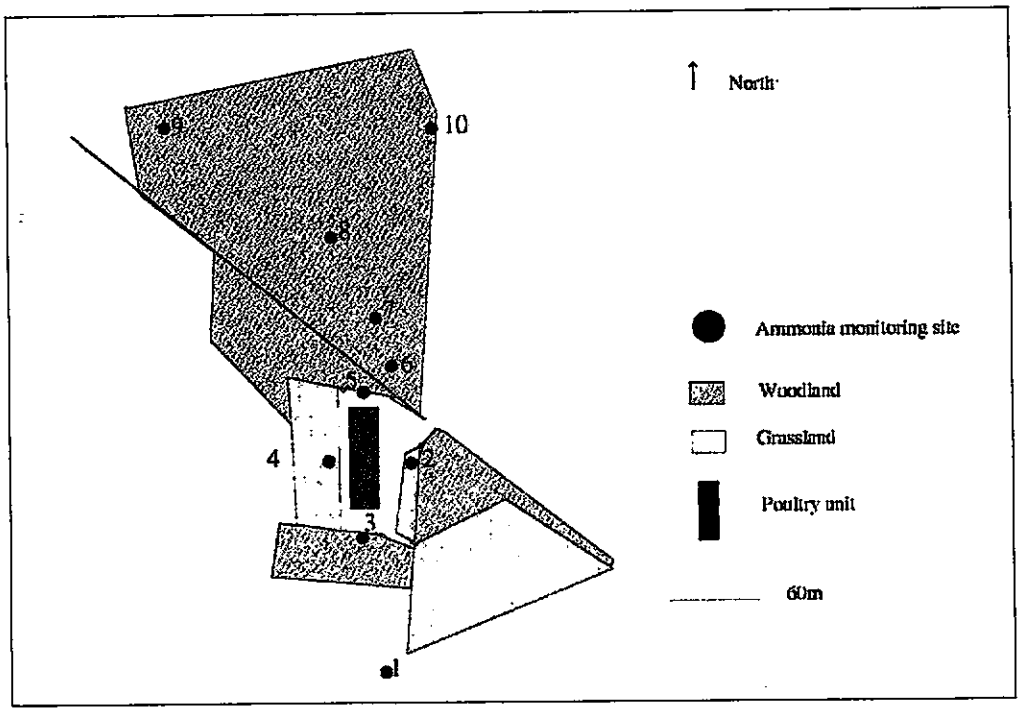
The poultry farm has been operating for 21 years and contains approximately 120,000 birds. The birds are farmed on a roughly 54-day cycle, 60 % of the birds being removed after around 40 days. There are several other units in the area and some litter spreading occurs on surrounding arable land.

The east of the site was bounded by dense coniferous plantation of mainly *Pinus sylvestris* with some *Picea sichensis* and little or no ground flora. However this area has recently been felled leaving a thin fringe of trees. The most extensive stretch of woodland which can be said to be downwind of the unit is to the north. This area of fairly open, largely coniferous woodland is composed of 30-40 year-old *Pinus sylvestris*, with some *Betula pubescens* and *Sorbus aucuparia*. The soil is a poorly drained non-calcareous gley and the ground flora is a mosaic of shade tolerant species of fern, herbs and moss. Past and present studies have been concentrated in this area.

B Summary of previous work at this site

Ammonia monitoring

Ammonia was measured continuously for 12 months from February 1995, using open-ended passive diffusion tube samplers at 5 sites around the farm and 5 sites along a transect through woodland north of the farm, at 16, 46, 76, 126 and 276 m from the poultry buildings.



Site plan of Earlston Poultry Farm Field Site 1995/9

Annual mean ammonia concentrations ranged from 29 μg m^{-3} close to the livestock buildings to <1.0 μg m^{-3}, 300 m downwind of the buildings and exceeded critical levels for NH_3 (8 μg m^{-3} annual mean). Concentrations for some individual sampling periods covering the latter half of the poultry cycles reached values in excess of 300 μg m^{-3}. This ammonia concentration gradient also provide a major gradient in nitrogen deposition, which has been estimated by Fowler *et al.* (1998) to be approximately 50 kg N ha^{-1} $year^{-1}$, at the woodland boundary exceeding critical loads for acidic coniferous forest (i.e. 15-20 kg N ha^{-1} $year^{-1}$ to protect ground flora).

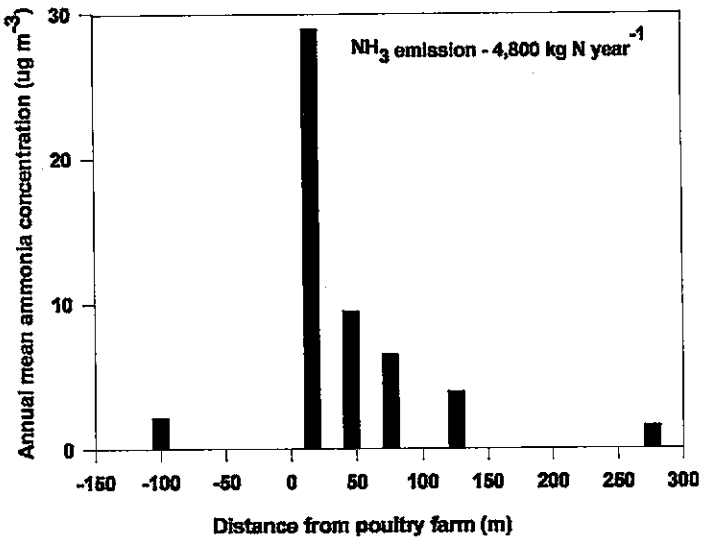


Fig B.1: Annual mean ammonia concentrations ($\mu g\ m^{-3}$) downwind of the poultry farm (1995-96)

Vegetation surveys

The aim of the surveys was to quantify the frequency and cover of the higher plant species present and the major bryophyte species along a transect from livestock buildings out to a distance of ~ 0.5 km. Complete species lists of bryophytes and lichens were not determined at that stage, although this work is now in progress under the present project.

The vegetation was surveyed in strips 50 m long and 2 m wide at different distances tangential to the farms and at right angles to the ammonia monitoring transect. In each survey strip, percentage species cover was recorded in 2 m x 2 m squares, and a mean percentage cover per 50 m strip was obtained for each species. Approximately 7 strips were surveyed at each farm corresponding, where possible, with ammonia monitoring stations. Results indicated an abundance of nitrogen loving species such as *Holcus lanatus* and *Chamaenerion angustifolium*, close to the houses. 'More sensitive species' such as *Oxalis acetosella, Galium odoratum, Potentilla erecta* and the mosses such as *Polytrichum commune, Plagiothecium undulatum* and *Pseudoscleropodium purum* increased in abundance with distance from the houses.

Ellenberg Index

Ellenberg nitrogen indicator values modified for British conditions by Hill *et al.* (1999), were determined for each transect. Indicator values from Siebel (1992) were used for bryophytes. Unweighted and abundance (% cover) weighted mean indicator values for all species (vascular plants and bryophytes) at different distances from the farm were calculated.

The N index was able to distinguish differences in composition along the transect downwind of the farm (Fig B2a). Mean Ellenberg N indicator values for transects close to the livestock buildings were larger than those for the more distant transects, but the decline with distance from the buildings was small and the standard deviations were very large. A mean Ellenberg Index of >4.5 may indicate a change in species composition of woodland groundflora but error bars are large. The mean abundance-weighted Ellenberg indicator values for transects close to Poultry Farm E also showed a trend with distance from the livestock buildings (Figure B2b).

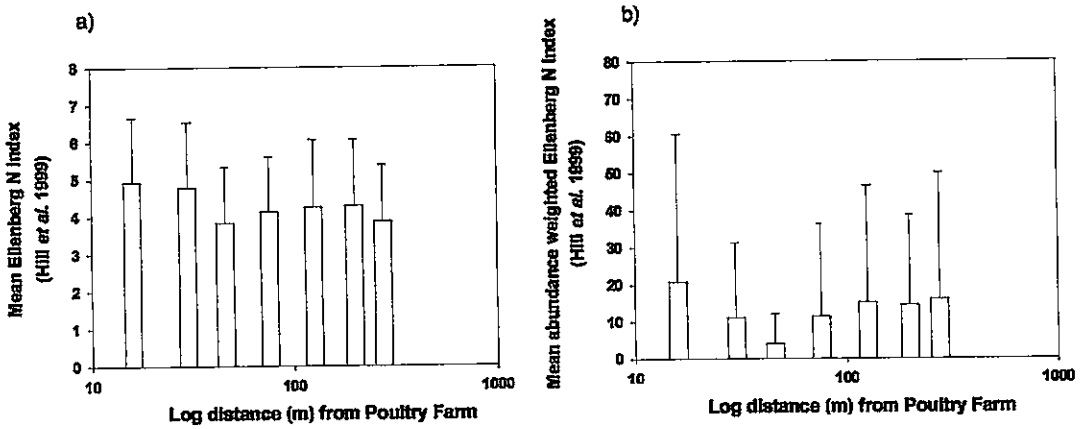


Fig B2: Ellenberg Nitrogen indicies of woodland ground flora in the vicinity of the poultry farm. a) Mean unweighted nitrogen index, b) Mean abundance weighted nitrogen index. Indicator scales from Siebel (1992) were used for bryophytes throughout. For higher plants and ferns, indicator scales are from Hill *et al.*, (1999). Error bars are standard deviations. (From Pitcairn et al. In press)

Soil trace gas emissions

The gases NO and N_2O are produced in soils by nitrifying and denitrifying bacteria and the magnitude of the emissions is controlled by the availability of N as NH_4 or NO_3 and also by certain climatic and soil properties which promote nitrification or denitrification, e.g. temperature, rainfall, organic matter content. Studies of a range of semi-natural ecosystems which have received various forms of N deposition suggest that measurements of soil NO and N_2O emissions may be useful indicators of soils where N supply exceeds demand of vegetation (Skiba *et al.* 1998). N_2O emissions were measured at 4 distances downwind of the farm on 5 occasions during summer 1997 and N_2O and NO emissions were measured on 2 occasions during autumn 1997. Nitrous oxide fluxes were measured using static chambers (3 per site) which remained in situ throughout the measurement period (May to November). Chambers were sealed for 1 hour when gas samples were withdrawn, stored in PTFE bags and analysed by ECD gas chromatography. The measurement of nitric oxide fluxes requires more complex equipment and must be measured on site. Methods are described in Skiba *et al.*, (1993). Soil emissions of N_2O and NO were large close to the poultry houses and decreased with increasing distance from the poultry houses (Fig B3)

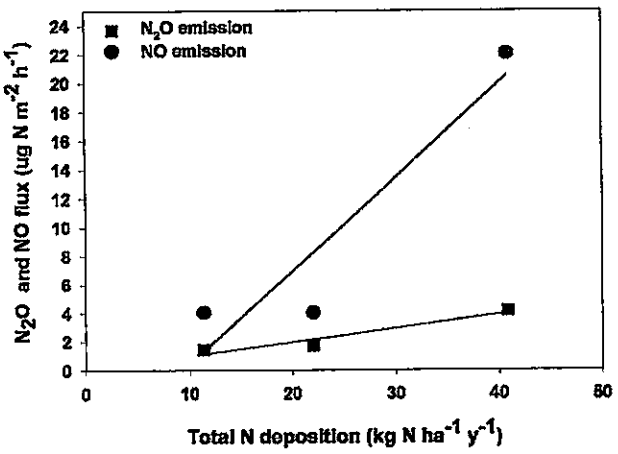


Fig. B3 Nitrous oxide and nitric oxide fluxes downwind of the poultry farm

Stable isotope δ $^{15}N/^{14}N$ content of leaves

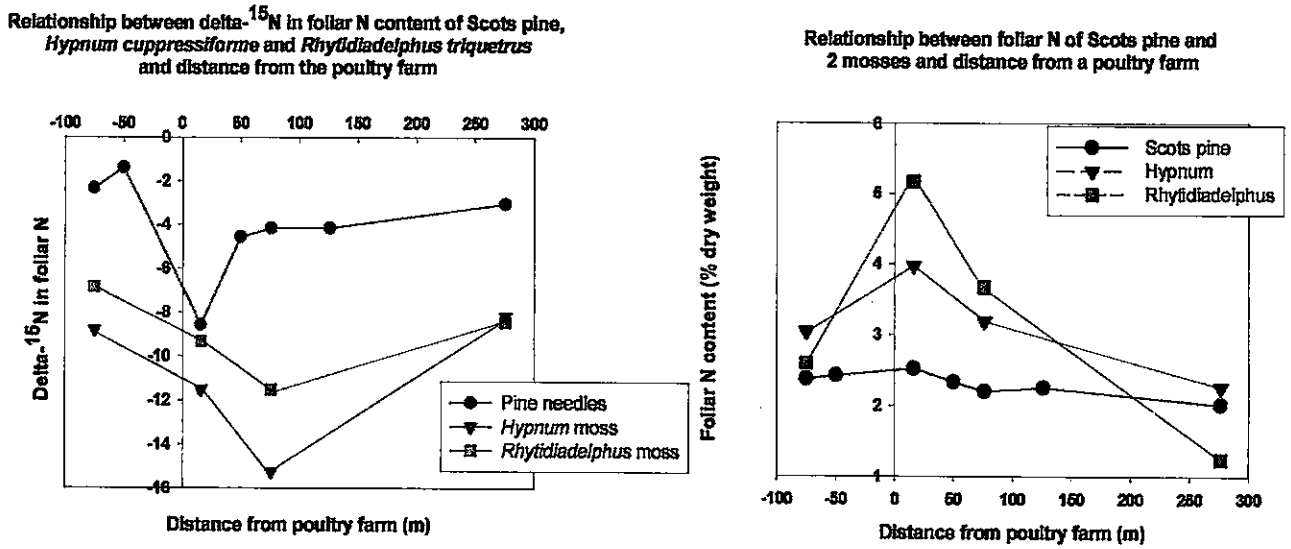
Stable isotope studies can help to identify the source and fate of N added to the environment by anthropogenic activities. Isotopic composition is expressed in terms of δ values – parts per thousand (per mil ‰) differences from a standard:

$$\delta\ ^{15}N = [(R\ sample/R\ standard)-1]*10^3$$

δ values are measures of the amounts of heavy and light isotopes in a sample ($^{15}N/^{14}N$), δ+ means an enhancement of ^{15}N and *vice versa.*

Changes in (or fractionation of) the ratio can result from physico-chemical reactions such as gaseous loss of NH_3-N where the lighter isotope is more readily lost and also from biochemical processes such as de-nitrification. Direct uptake of atmospheric N by mosses without possible fractionation occurring in the soil means that δ ^{15}N in mosses would be more closely related to the source atmospheric signal. Ammonia produced by livestock farms has very negative δ ^{15}N values as the ammonia volatilised is preferentially enriched with the lighter ^{14}N.

Fig. B4 Comparison of $\delta^{15}N$ and total N measurements, February 1999 (J. Pearson pers com, 1999)



$\delta^{15}N$ values of vegetation sampled upwind and downwind of the poultry farm were shown to reflect the deposition of negative ammonia and its uptake into plant tissue (Pers comm. J Pearson). $\delta^{15}N$ values decreased from –6.8 ‰ and –8.8‰ for *Rhytididelphus triquetrus* and *Hypnum cuppressiforme* respectively, upwind of the poultry buildings to –9.3‰ and –11.5‰ respectively, downwind of the poultry buildings. Values increased to the upwind values at a distance of 276 m downwind (Fig B4).

Foliar nutrient concentrations

Plant samples were collected for foliar N analysis in the vicinity of each NH_3 monitoring site in July 1995. Collections were made (where present) of *Pinus sylvestris, Picea sitchensis, Betula pubescens, Fagus sylvatica, Dryopteris dilatata, Oxalis acetosella,* and mixed ectohydric moss species. Further samples have been collected on numerous occasions between 1995 and 2002.

triquetrus was present at all sites, data from the other species provided additional information about species specific amino acids and the composition of the amino acid pool.

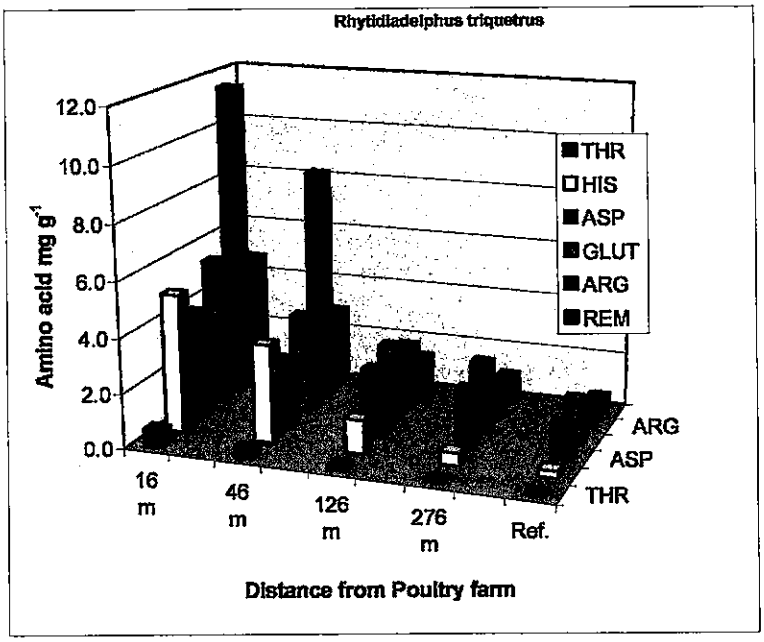


Fig. B6 Free amino acid concentrations in *Rhytidiadelphus triquetrus* at 4 distances downwind of the poultry farm and a 'clean'reference site (Ref.), November 2001.(THR-threonine, HIS-histidine, ASP-aspartic acid, GLUT-glutamic acid, ARG-arginine, REM- remaining amino acids)

Free amino acid concentration in all 3 moss species showed a strong relationship with N deposition Pitcairn et al (In press). Arginine was the dominant amino acid at high N deposition close to the buildings in the 3 species but was especially dominant in *R. triquetrus* representing 30% of total free amino acids. Concentrations close to the poultry buildings (except in *R. triquetrus*) were similar to those reported by Nasholm et al (1994) and Nordin et al (1998) following N fertilizer additions to mosses of understorey vegetation in boreal coniferous forest. Note also that histidine concentrations decrease with distance from the poultry houses. Histidine concentrations are very small under clean conditions which may make it a good candidate for bioindication.

Changes in the composition of the amino acid pool in response to changes in ammonia concentrations downwind of the poultry farm are shown in Table B1. The composition of the amino acid pool at 300 m downwind of the farm, where NH_3 concentrations are around background level, showed a co-dominance of aspartic acid and glutamic acid in the 3 species, arginine comprising < 8%. Closer to the farm buildings (46 m), the composition of the amino acid pool changed from aspartic acid and glutamic acid dominance to arginine dominance in *R. triquetrus* and *P. purum,* whereas in *B. rutabulum,* aspartic acid was still dominant. At a distance of 16 m from the poultry buildings, where measured NH_3 concentrations were largest, the amino acid pool was dominated by arginine, especially in *R. triquetrus.* Clearly a change in dominance from aspartic acid and glutamic acid to arginine would denote a change from low to high N deposition. The different uptake strategy of *B. rutabulum,* compared with those of *P. purum* and *R. triquetrus,* and the arginine dominance of the amino acid pool only close to the poultry buildings, may partly explain its frequency close to sources of N and its ability to accumulate tissue N.

Table B1: Relative percentage of 3 key amino acids in the amino acid pool of 3 mosses at 3 distances downwind of a poultry farm. (Hist – histidine; Asp-aspartic acid; Glu – glutamic acid; Arg – arginine) From Pitcairn et al (In press)

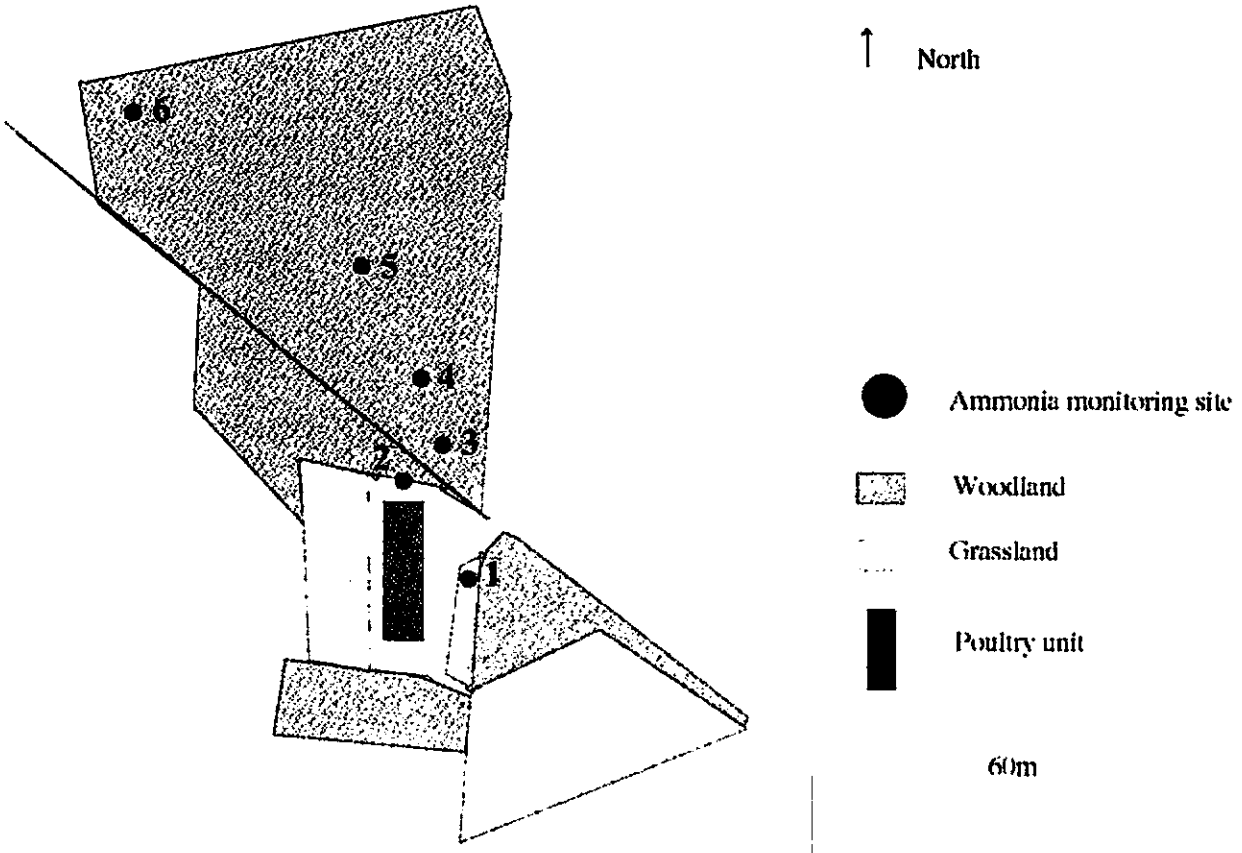
Moss species	Relative % of amino acids downwind of the poultry farm											
	276 m				46 m				16 m			
	Asp	Glu	Arg	Hist	Asp	Glu	Arg	Hist	Asp	Glu	Arg	Hist
Rhytidiadelphus triquetrus	31.6	37.7	7.1	7.0	11.6	16.8	40.0	16.0	12.5	17.3	37.1	16.1
Pseudoscleropodium purum	36.9	34.7	4.5	5.9	14.8	15.3	29.6	9.0	14.5	17.3	23.7	12.2
Brachythecium rutabulum	34.5	23.5	7.3	11.2	23.0	14.6	16.0	15.9	13.8	13.5	23.6	16.9

The histidine component is small at 300 m in *R. triquetrus* and *P. purum* but larger in *B. rutabulum.* It increases to around 15% close to the houses. Arginine + histidine comprise 10-18% of the amino acid pool at 300 m, but 30->50% at 46 m from the houses. A change in the composition of the amino acid pool may prove to be early indicator of enhanced N deposition

C Field Survey 2002

CEH Bioindicator tests

Procedures for testing *Lolium perenne* as a standard plant bioindicator and soluble N as a biochemical indicator were coordinated with the poultry farm bird cycle to maximise exposure to ammonia. One-day old chicks were placed into the houses on 30 September 2002 and 60% of the birds were removed on 7 November 2002. Plants were thus exposed from 1 October 2002 until 7 November 2002, and ambient NH_3 concentrations were continuously monitored throughout this period. A new site map has been produced for the 2002 field survey with revised numbering to cover the 6 monitoring sites used as shown below.



Ammonia Monitoring

Ammonia concentrations were continuously monitored at 1.5 m above ground level at each site using either passive ammonia diffusion samplers (site 1) or Alpha samplers (Adapted low cost passive high absorption samplers: sites 1- 6:) see Tang *et al* (2001) for specifications of samplers and chemical analysis. Due to the potentially high NH_3 concentrations at site 1, both diffusion tubes with their longer path length (allowing absorption of higher NH_3 concentrations) and Alpha samplers were placed at site 1. There were 3 replicate samplers at each of the 6 sites. The diffusion and Alpha samplers were exposed from 1 October 2002 until 7 November 2002, with 2 sampling periods (1 - 24 October 2002 and 24 October – 7 November 2002).

Results

The mean ambient concentrations (1 October –7 November 2002) of NH_3 decrease approximately 100 fold over the length of the transect (Table C1) The NH_3 concentrations range from 69.6 μg m^{-3} at 30 m to 0.58 μg m^{-3} at 276 m from the units. These values are very similar to the annual mean concentrations for 1995/96 shown in Figure A.1

Table C1 Mean ambient NH_3 concentrations along 300 m transect from intensive Poultry units

Site No.	**1**	**2**	**3**	**4**	**5**	**6**
Distance from Poultry units (m)	30	16	46	76	126	276
Mean NH_3 Concentration (μg m^{-3})	69.60	29.13	12.82	8.01	3.37	0.58

Site 1 is east of the poultry farms. The other 5 sites are north of the farm in woodland (see site map)

Lolium perenne – Standard Plant Indicator

Plant material

Lolium perenne (L.) seed (supplied by Herbiseed, The Nurseries, Billingbear Park, wokingham, Berkshire, RG40 5RY.) was sown at a rate of 1.22 g per pot on 22 August 2002 into round black pots (volume 3.6 litres) with 4 drainage holes in the bottom. The compost was a peat-loam and grit (4:1:1) mixture with no added fertilizer. After sowing a thin layer of gravel was placed over the seeds in each pot.

During the 38 days NH_3 exposure period, the sites would only be visited twice. Therefore it was necessary to use a wicking system to provide irrigation (see EurobBioNet instruction manual for details). Four pieces of glass-fibre cord (300 mm) were placed in each pot, with the wicks running from just below the soil surface to the water reserve tray.

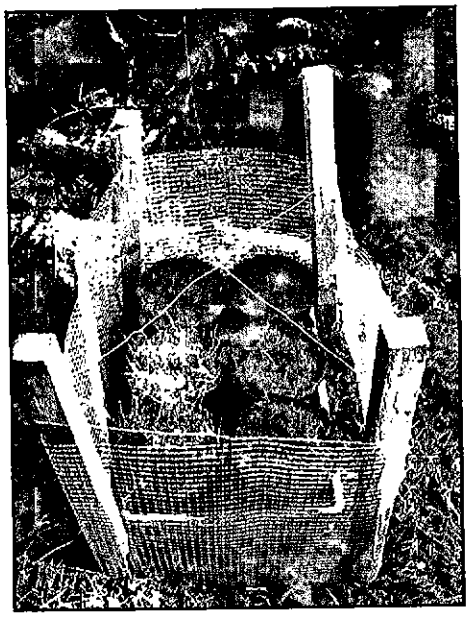
The pots were then placed in a heated Glasshouse (25 ^{0}C) for 7 days to encourage germination. Once the seeds had germinated and the young tillers had reached a height of 3-4 cm, the pots were moved to a sheltered location outside. There was a 100% germination rate i.e. all 45 pots successfully germinated. During this period (29 August –1 October 2002) the pots received rainfall but were also watered on 3 occasions.

Pre-treatment harvests

On 17 September 2002, two weeks prior to exposure, all the tillers were cut to a height 2-3 mm above the rim of the pot. This was done to prevent the grasses becoming too elongated and also encourage growth during the 38-day exposure period. On 1 October 2002 pre-treatment samples were taken for %N from 5 pots.

Experimental procedure (Poultry Farm NH_3 Transect))

A 276 m, six site transect was set up at Heathery Poultry Farm near Earlston in the Scottish Borders on 1 October 2002. The six sites were in a managed mixed coniferous woodland at 16, 30, 46, 76, 126 and 276 m from the Poultry farm (for individual site details see Figure A.1 and Pitcairn *et al* 1998).



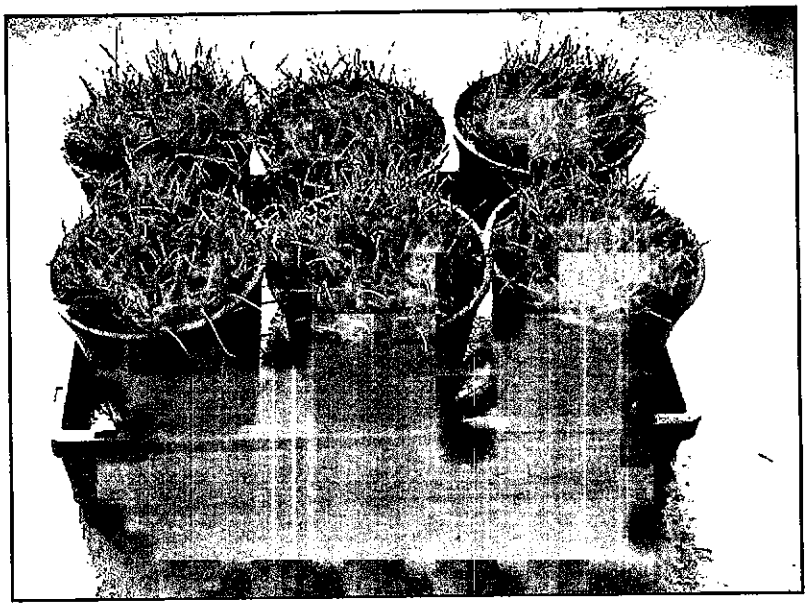


Figure C1. Site 4 with *L. perenne* pots and Alpha sampler post. **Figure C2**. *L. perenne* and protective wire cage. **Figure C3**. 6 pots of *L. perenne* in water reserve tray. **Figure C4** Individual pot of *L. perenne* with wicking system.

At each site, six pots of *L. perenne* were placed in rectangular grey water reserve trays (EuroBioNet; 46 cm x 36 cm x13 cm) with a 4 cm deep polystyrene insert fitted into the bottom of each tray (figure C1). The wicks were spread out on the polystyrene base and rainwater was added to the trays until it was at a level of 1.5 cm above the bottom of the pots (figure C2). The pots were protected from wild

life by 45 cm high birdcage wire mesh supported by 60 cm wooden posts (Figure C3). Wire was strung over the top of the *L. perenne* pots to damage from wildlife.

Biomass measurements

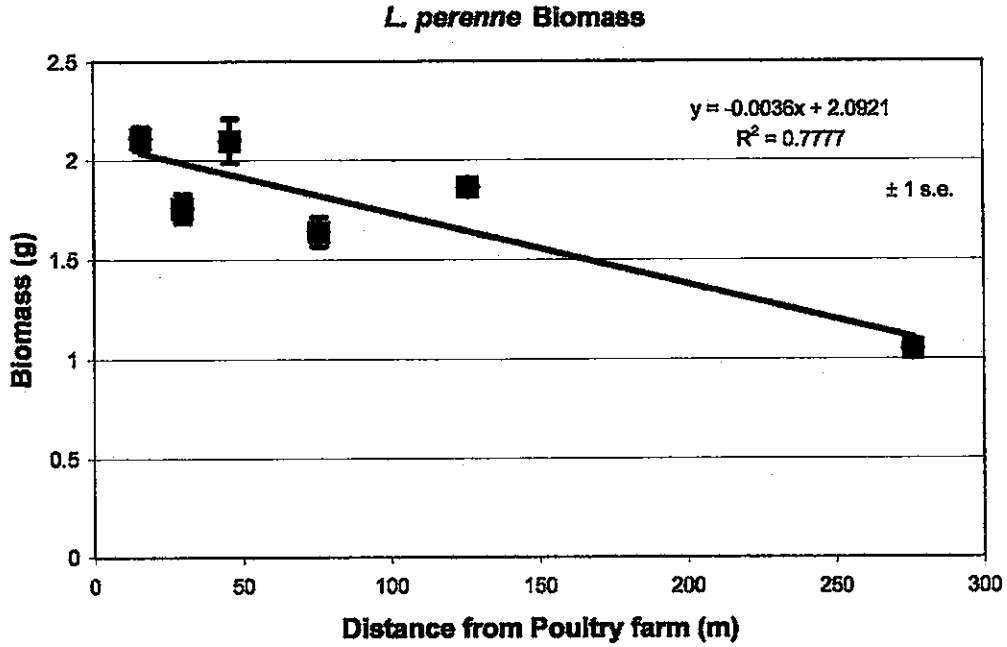


Figure B6a: Relationship between tiller biomass and distance from the NH_3 point source.

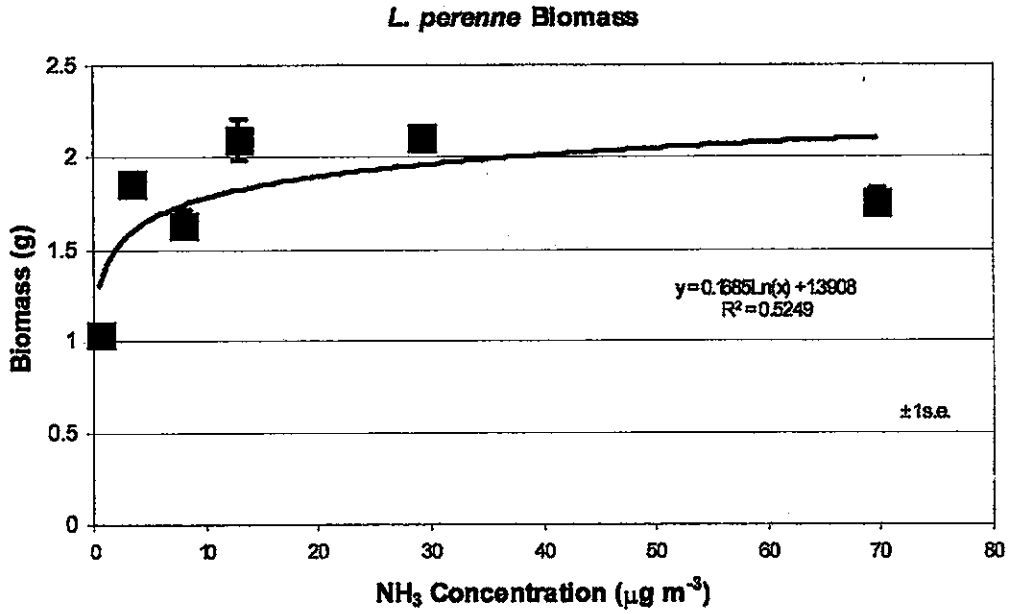


Figure B6b: Logarithmic relationship in tiller biomass with increasing ambient NH_3 concentrations.

L. perenne exposure period.
All plants were exposed from 1 October 2002 until 7 November 2002, when the female poultry were removed from the houses. Ambient NH_3 concentrations were also continuously monitored throughout this period as shown earlier.

L. perenne destructive harvest
After 38 days of field exposure all pots were collected on 7 November 2002 and returned to CEH Edinburgh for destructive harvest. To provide sufficient sample for both foliar and soluble N analysis three replicate samples were taken by bulking two replicate pots (2 pots x 3 replicates).
Using disposable gloves the tillers were cut at 2 mm above the level of the pot (figure C4).

L. perenne tiller, biomass, foliar %N and soluble N
The tillers were immediately weighed and a fresh weight determined. A 1.5 g fresh weight sample (approximately 50 tillers) was washed in de-ionised water to remove any surface dry N deposition then immediately placed in a plastic bag and stored in a –18 ^{0}C freezer for soluble N determination. The remainder of the sample was washed, oven dried at 70 ^{0}C for 3 days then dry weighed. To calculate the total biomass per replicate, the fresh to dry weight ratio was determined and the 1.5 g fresh weight sample incorporated.
The dried samples were then ground using a hammer mill (sieve size 0.8mm) and digested and analysed for NH_4-N by the Indophenol-blue method (Grimshaw et al 1989). The above samples have been sent to our chemistry section at CEH Merlewood. Results should be received by early January 2003. Soluble N will be determined at CEH Edinburgh.

These results suggest that the *L. perenne* is responding positively to the increased N availability but until we have the %N data this cannot be confirmed. The results indicate that even though the experiment was conducted at the end of the growing season for *L. perenne* with a reduced growth rate it is still able to observe an effect of increased ambient NH_3 on biomass. It should be noted that site 1 is east of the site and not in the northern woodland transect. Conditions of light and exposure differ from those at sites 2-6.

Biochemical analysis

Two moss species, *Rhytidiadelphus triquetrus* and *Hypnum cuppressiforme,* were sampled from 5 sites, and only *Eurynchium praelongum* (the sole species present) was sampled from site 1. Disposable gloves were used for all parts of the protocol, and samples were stored in polythene bags at 4°C prior to sorting. In the laboratory, samples were 'cleaned ' to obtain a pure sample of the desired species by removing unwanted material (plant, soil and litter), then washed quickly with deionised water to remove any surface contamination without leaching ions from the cells. The sample was then split, half was frozen for soluble N determinations and the other half was oven dried at 70°C for total tissue N content. In selected samples, part of the frozen portion was freeze dried for amino acid analysis.

Soluble substrate nitrogen
Collected samples are stored in the freezer at -18°C. Small portions of leaf or moss shoot are blotted dry with thin clean laboratory tissues, cut into small segments and placed into the cup of a grinder (10 cm diameter ceramic morter). The segments are frozen with liquid nitrogen (approx 10 ml) while quickly grinding the leaves into a thin powder with a ceramic pestle. Two samples of the ground (powdered) plant material are taken (each approximately of 0.1 g) and put into small plastic tubes (1.5 ml Eppendorf). Having previously established the weight of an empty tube, the filled tubes are

then weighed precisely, to establish the mass of leaf material. Then 1 ml of de-ionised water is added in each tube, which is closed, shaken and quickly frozen. Samples are stored in the freezer (-18°C) until analysis.

Analysis of NH_4^+ concentration

Defrosted samples are centrifuged (10 minutes, 2000 gravities) to settle the solid parts of ground leaf material. Alternatively, if no centrifuge is available, the solid parts of ground leaf may be settled by letting the samples in a tray for few minutes. The supernatent (clear solution on top) is then taken off by pipette and filtered to remove any remaining plant material (useing laborabory cotton wool filters in the tip of a plastic microsyringe). The filtered solution is then analysed for total ammonium, according to the method available in the laboratory using e.g. AMFIA, o-phalaldehyde

Results

Results of soluble N determinations should be available by early January 2003.

Foliar N Content

Dried samples were hammer-milled to < 0.8 mm and sent to CEH Merlewood for analysis. The ground powder will be analysed for total N at CEH Merlewood using the CNS Analyser (Elementar Model: Vario EL).

Results

Results of all foliar N determinations should be available by early January 2003.

Natural History Museum Interim report to JNCC
Pat Wolseley and Peter James

PRELIMINARY FIELD TRIAL OF LICHENS AS BIO-INDICATORS OF ATMOSPHERIC NITROGEN

Background

Lichens have been widely used as bio-monitors of atmospheric pollution due to acidification and heavy metal accumulation across Europe, and for this purpose corticolous substrata have been widely used. The use of lichens of corticolous, saxicolous and terricolous substrata has recently been used to assess environmental conditions across land use units in 7 European countries in a project called BIOASSESS. Their use in biomonitoring of nitrogen compounds in the atmosphere has been investigated in a number of countries and the trial of these methods in a single site in Britain has provided data with which to evaluate the methods.

Objective

- To trial appropriate methods for assessing lichens as bioindicators of atmospheric nitrogen
- To prepare a data set that can be used to compare and contrast methods in current use.

Selection of Earlston poultry farm as site for field trial

Following discussion between CEH Bush and NHM a site was selected where previous emissions of ammonia had been recorded in the vicinity of poultry rearing units at Earlston in southern Scotland. The site is surrounded by *Picea* trees in close proximity to the sheds and a c.40 year old *Pinus sylvestris* plantation to the north and west. There were large *Fagus sylvatica* trees along the tracks

but the only native tree frequent on the site was *Betula pubescens.* Ammonia has been measured within a 300m transect from the source, mainly in a north to northwest direction correlating with prevailing winds.

OUTLINE METHODS

Field work was undertaken between 16 - 19th September 2002 by Wolseley and James.

At Earlston the ground layer was very disturbed and the only walls on this site were deeply shaded by beech trees along the track. In this situation only corticolous substrata were sampled. The use of lichens on other substrata will be considered in the review.

Sample stations coincided with the location of ammonia monitoring points established by CEH Edinburgh. However those points in the middle of the plantation were well shaded and did not conform to sampling conditions as defined by Asta et al (2002) (LDV) or van Herk (1999) (NIW and AIW) where a condition of sampling procedure requires well-lit trees.

Due to limitation of available tree species on site, conifer trees were used for all lichen sampling on trunks and *Betula pendula* twigs were sampled at all sites. In order to trial several methods in use for bio-monitoring sampling was undertaken on 5 trees of conifer and 5 trees of *Betula pendula* at each site. Girth of all tree species was recorded. Bark was collected from all trees sampled for lichens and twig lengths were also cut from sampled trees. All specimens were dried and stored in paper bags prior to pH determination. Bark pH of trees sampled will be recorded using a flat-top surface electrode and on twigs by waxing twig ends of a 5 cms sample and soaking in distilled water as outlined by Kermit & Gauslaa (2001).

The whole site at Earlston is surrounded by agricultural land and there is no adjacent site of natural vegetation. In order to assess deviation from naturality of the site for lichens as defined by Loppi, a nature reserve at Gordon Moss 4.7 km east of the site dominated by *Betula pubescens* was included in the field survey of lichens on twigs. No ammonia data exists for this site, but concentrations are estimated to be <0.5 $\mu g\ m^{-3}$.

In order to conform to data collection by a range of methods, lichens were recorded as follows:
All lichens recorded on a trunk and assigned frequency values as outlined by van Herk (1999), nitrophyte and acidophyte species were distinguished.

LDV and BIOASSESS

On each tree, 4 quadrats (50 x 10 cm with 10 cm grids) were placed at compass points and lichen presence recorded in each 10 cm square for each compass point.

Lichens on twigs

Birch trees (*Betula pubescens*) were widely distributed throughout the site and were used to assess lichens on twigs adapted from the method outlined by Wolseley et al. (2002) 5 birch trees were selected at each site, if possible with a girth of > 20 cm and lichen presence recorded on accessible twigs and branches up to 2cms diameter on each tree.

RESULTS

Data were entered in EXCEL files according to prescribed methods and indices of NIW, AIW and LDV calculated. Indicator scales used by Lallemant (1997) in western France were also assessed. Mean species diversity was calculated per site and per aspect (for LDV) and results are shown in Table C2. Further analysis using multivariate techniques will be carried out to establish the correlation of lichen species and distribution with environmental factors.

Results demonstrate a strong shift from nitrophyte species to acidophyte species between site 2 and site 6 as shown by results using NIW and AIW species. Indices of diversity as calculated for LDV do not correlate with ammonia concentrations due to the replacement of acidophyte diversity by nitrophyte diversity in sites close to the source of ammonia.

Tree trunks were not equally exposed to light sources across the site so that sites 3, 4, 5 and 5a in even-aged *Pinus sylvestris* plantation were exposed to very low light levels. Mean diversity for NIW and AIW species was low in these sites. The trunks were covered with green algae and remnants of infertile *Scoliciosporum chlorococcum,* where lichen establishment was limited. It was observed that significant pollen deposition probably influenced trunk flora. In this heavily shaded situation lichen sampling did not fulfil guidelines outlined by LDV or NIW/AIW that only exposed trees should be sampled. However, in these sites twig diversity on *Betula pubescens* was less affected by these environmental conditions.

Site 1 is on exposed *Picea* trees planted as a shelter-belt adjacent to the source. Sites 2 and 6, on the edge of the *Pinus sylvestris* plantation where trunks were exposed to higher light levels had similar species diversity on trunks, but in site 2 this was composed of nitrophyte species with an absence of acidophytes whereas in site 6 it was composed of acidophyte species with an absence of nitrophytes. The use of nitrophyte and acidophyte species as defined by van Herk, allows a comparison between the sites which corresponds to recorded ammonia concentrations. The exception is site 1 where *Xanthoria parietina* covers all exposed *Picea* twigs and trunks but contributes to a lower NIW than at site 2 where other nitrophytes are present. From these results it would appear that there is a range of sensitivities to critical levels among nitrophytic species.

Using indicator scales developed in the Netherlands (van Herk) and in western France (Lallemant) regional maps have been produced of areas affected by ammonia deposition. The selection of indicators is based on regional data and there is little overlap between van Herk and Lallemant's indicator species. There is a considerable overlap between species found at Earlston with van Herk's definition of NIW and AIW species and little overlap with Lallement's indicators in western France. Further analysis of these data will detect regional elements in the Earlston data and compare this with other sites.

Although nitrophytes are absent from the trunk from sites more than 125 m from the source (5, 5a and 6), they are present on twigs at all sites except 6. This may reflect the influence of nitrogen compounds other than ammonia at a background level in surrounding agricultural land even at Gordon Moss.

Lichen communities colonising newly available bark substrata on twigs appear to provide the most reliable estimate of present environmental conditions. Where the bark chemistry of trees has been altered during previous environmental conditions the trunk may carry remnants of previous cryptogamic communities. Analysis of pH data from all bark surfaces will allow us to assess the affect of bark pH on lichen communities at Earlston.

Table C2: Lichen survey data from Earlston poultry farm, September 2002

						mean frequency by aspect (LDV)				van Herk method			Lallemant method NH3		Twig method		
Site	distance from source (m)	direction from source	exposure levels 1-5	NH3 conc ug m-3	LDV	N	E	S	W	NIW	AIW	Trunk div.	no of ind. sp.	zones	Twig-NIW	Twig-AIW	Twig div.
1(2)	30	90°	5	70	17.4	5	3.8	4	4.6	2.2	0	1.2	1	zone 1	2.6	0	3.4
2(5)	16	0°	5	29	32.8	1.6	9.4	10	12	5	0	4.2	2	zone 1	4	0	4.8
3(6)	46	45°	1	9.5	2.2	0.2	0	1.6	0.4	0.8	0.2	1.2	1	zone 2	3.4	0	4
4(7)	76	0°	1	6.5	8.6	3	2	0.8	2.8	0.2	1.8	1.2	0		1	0	1.6
5(8)	126	350°	1	3.9	16	4	3.8	3.8	4.4	0	3.2	1.2	0		1	0	3
5a	c. 200	340°	2		35	9.2	8.8	7.6	9.4	0	4.8	2.4	0		1.2	1	6.2
6(9)	276	330°	4	1.6	47.6	9	11	15	13	0	7.4	3.8	1	zone 2-5	0	4.4	8
10 = Gordon Moss	4700	30°	4												0.6	7	11
Total div. by aspect																	

SUMMARY

- The use of an index of lichen diversity based on frequency alone (LDV) to assess ammonia levels around point sources does not appear to be appropriate, due to the replacement of acidophytic lichen diversity by nitrophytic diversity in sites with high concentrations of ammonia.
- There is a need to assess nitrophyte and acidophyte indicator species and to test these against corresponding ammonia values in order to accommodate a range of regional climatic and atmospheric conditions.
- Standardised recording methods (e.g. proposed European protocol) may not always be appropriate for use in a range of ecological conditions especially in humanly altered habitats.
- Lichens colonising newly available surfaces on twigs appear to provide the most realistic estimate of present environmental conditions whereas trunks may carry remnants of cryptogamic communities from previous conditions.

Literature Review

The literature review is in progress and initial drafts have already been received on some topics. The workshops attended by staff at the Bradford University and Berne on the setting of critical loads for nitrogen provided useful discussion on several aspects of the literature review.

A preliminary lichen investigation was carried out to select appropriate methodology for use at selected field site.

References

Asta, J., Erhardt, W., Ferreti, M., Fornasier, F., Kirschbaum, U., Nimis, P.L., Printos, S., Purvis, O.W., Scheidegger, C., Van Haluwyn, C. & Wirth, V. (2002). Mapping lichen density as a indicator of environmental quality. In Monitroing with Lichens, P.L. Nimis, C. Scheidegger & P.A. Wolsey eds, , pp. 273-279, Kluwer, Dordecht.

EuroBioNet :Instruction Manual for Cultivation,exposure, injury assessment and sampling of the Bio-indicators species. Life99Env/d/000453. Universitat of Hohenheim. .

Fowler, D., Pitcairn, C. E. R., Sutton, M. A., Flechard, C., Loubet, B., Coyle, M. & Munro, R. C. (1998). The mass budget of atmospheric ammonia within 1 km of livestock buildings and effects of land use. *Environ. Pollut.*, **102**(S1), 343-348. .

Grimshaw H.M., Allen, S.E. & Parkinson. J.A. 1989. Nutrient elements. In: Chemical analysis of ecological materials (2nd ed.), edited by S.E. ALLEN, pp. 81-159. Blackwell Scientific Publications. Oxford.

Hill, M. O., Mountford, J. O., Roy, D. B. & Bunce, R. G. H. (1999). *Ellenberg's indicator values for British plants.* ECOFACT Vol. 2. Technical Annex. Centre for Ecology and Hydrology, Natural Environmental Research Council.

Lallemant, R. , Joslain, H., Houssay, I. & Cyprien, A-L. (2002) The use of lichens for estimating air pollution in Western France. Submitted for publication.

Lallemant, R. 1997. Etude de L'impact des emissions ammoniacales agricoles sur la qualite atmospherique. Report by University of Nantes.

Näsholm, T. , Edfast, A-B.,Ericsson, A. & Nordén, L-G. (1994). Accumulation of amino acids in some boreal forest plants in response to increased nitrogen availability. *New Phytol.*, **126**, 137-143.

Nordin, A., and Näsholm, T. (1997). Nitrogen storage forms in nine understorey plant species. *Oecologia*, **110**, 487-492.

Pitcairn,C. E. R., Leith, I. D., Sheppard, L. J., Sutton, M. A., Fowler, D., Munro, R. C., Tang, S. & Wilson , D. (1998). The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environ. Pollut.*, **102**(S1), 41-48.

Siebel, H. N. (1993). Indicatiegetallen van blad -en levermossen. IBN-rapport 047, Wageningen

Skiba, U., Sheppard, L.J., Pitcairn, C.E.R., Leith, I.D., Crossley, A., van Dijk, S., Kennedy V.J. & Fowler, D. 1998. Soil nitrous oxide and nitric oxide emissions as indicators of the exceedance of critical loads of atmospheric nitrogen deposition in semi-natural ecosystems. *Environ. Pollut.*, **102(S1)**, 457-461.

Skiba, U., Smuth, K.A., Fowler, D. 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. *Soil Biology and Biochemistry*, **25**, 1527-1536.

Tang Y.S., Cape J.N. & Sutton M.A. Development and types of passive samplers for monitoring atmospheric NO_2 and NH_3 concentrations. (2001). The Scientific World, **1**, 513-529.

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

Wolsey, P. and James, P. (2002) Assessing the role of biological monitoring using lichens to map excessive ammonia deposition in the UK. In: Effects of NO_x and NH_3 on lichen communites and urban ecosystems – a pilot study. Report to DEFRA by Imperial College and The Natural Histor Museum.

Wyers, G. P., Otjes, R. P. & Slanina, J. (1993). A continuous-flow denuder for the measurement of ambient concentrations and surface exchange fluxes of ammonia. *Atmos. Environ.* **A27**, 2085-2090.