1. Introduction

1.1. Background

Atmospheric nitrogen deposition is a key threat to the integrity of semi-natural ecosystems in the UK that are protected under both UK and European legislation. The principle cause of change is recognized to be through the eutrophication effect of additional nitrogen, which alters the competitive balance between different species. Further additional impacts include the contribution of nitrogen to the acidification of soils and freshwaters, as well as the direct effects of individual components contributing to nitrogen deposition (NEGTAP 2001).

While the effects of deposition on eutrophication and acidification have been assessed in the past using the critical loads approach, the direct effects on vegetation have been assessed using critical levels of concentrations. Critical levels have been established for oxides of nitrogen (NO_x), ammonia (NH_3) and cloud water acidity (Achermann and Bobbink 2003). In the UK, reduced nitrogen is now the dominant component of total nitrogen deposition. There is limited field evidence to suggest that given a particular dose of N (as kg N ha^{-1} y^{-1}), the severity of effect on vegetation is NH_3 (gas) > NH_4 (wet) (Leith *et al.* 2001). This may be in contrast with the fact that current critical level (8 µg m^{-3}) for NH_3, which is rarely exceeded at typical concentration in the UK (except close to point sources) and therefore would imply only limited direct effect of NH_3. However, recent evidence suggests that this value is too high (e.g. Pitcairn *et al.* 2003, Sutton *et al.* 2004b). Hence a range of different N containing pollutants appear to be affecting semi-natural ecosystems in the UK, while there remains a key question regarding the relative contribution to these impacts from different N forms.

The Habitats Regulations introduces new site safeguard commitments for the conservation agencies and environmental agencies (i.e. Environment Agency (EA), Scottish Environmental Protection Agency (SEPA), Environment and Heritage Service (EHS)). There are also legislative and policy commitments for the protection of designated sites in the UK. Nitrogen deposition and concentrations are a threat to the condition and long-term maintenance of sensitive habitats and species.

The current policy assessment for atmospheric N deposition impacts at a UK scale utilises the critical loads and levels approach, in particular the use of N deposition maps across the UK in conjunction with critical loads exceedance, associated with mapped distribution of different habitat types. This national mapping approach can be used to give an initial estimate of critical loads and levels exceedance at specific designated sites (e.g. SACs, SSSIs). The approach is currently used by the conservation and environmental agencies as a risk assessment of air pollution impacts on the integrity of designated sites. The critical loads approach is, however, limited to indicating an increased risk of environmental impact as exceedance of critical loads does not equate exactly to an impact on ‘site condition’. Furthermore, there are limitations with the resolution of the deposition modelling and habitat and soils mapping which means there are many uncertainties in applying such an approach at an individual site level.

As part of the statutory role of the conservation agencies the condition of designated nature conservation sites is monitored under the Common Standards Monitoring (CSM) programme. The CSM provides an assessment of the condition of interest features on SSSIs as a single snapshot in time, which presently does not include a predictive assessment of the long-term risk to sites. The use of nitrogen biomonitors, either as a single measurement or repeated measurements over time could complement the condition monitoring assessments and provide an indication of the impact of N deposition on a specific site and the potential impacts on the long term integrity of the site/interest features. Although in most cases any applied biomonitor method may not target specifically the interest features of the designated site, it could provide appropriate information which would indicate the potential for N pollution impacts on the site as a whole.

Bioindication and biomonitoring represent a complementary approach to the assessment of air pollution impacts of nitrogen. Bioindication for atmospheric nitrogen represents the use of biological measurements on a specific site of interest to indicate either a level of **exposure** (N deposition or concentration) and/or **ecosystem impact**. Where bioindicators are well characterized, they should be able to provide quantitative information from measurements conducted at a site at any given time regarding the level of exposure or impact associated with the site condition. Biomonitoring represents the extension of bioindication to consider the status of the site through time, and therefore represents the repeated application of given bioindicator methods.

To identify generalised N biomonitoring techniques which could be used as complementary tools in the condition monitoring of the statutory sites, Sutton *et al*. (2004a) provided a comprehensive review of existing N bioindicator methods. The objective was to identify those methods, which would provide an early indication of N effect, N exposure or ecological impact on statutory nature conservation sites. The applicability of the identified methods for general biomonitoring was assessed by empirically scoring the different methods for general application, according to criteria reflecting i) robustness, ii) ease of use and iii) extent of method development/establishment. In addition, a decision approach reflecting the different specific potential purposes of the bio-monitoring methods was also used.

Seven methods were identified from the empirical selection process as being the most suitable for general application. These were:

Chemical methods

- Total foliar N concentration (primarily of bryophytes, but also of higher plants).
- Soluble foliar N concentration (evaluating foliar ammonium, nitrate and total soluble nitrogen).
- Measurement of the pH of tree trunks and twigs (as a supporting parameter to help interpret lichen diversity measurement; see below).

Diversity methods

- Ellenberg nitrogen index values of higher plants and bryophytes
- Measures of lichen diversity in relation to nitrogen preferences (using the van Herk and Wirth approaches), applied for twigs and trunks of trees.

Transplant methods

- Standardised model plants (e.g. *Lolium* spp., *Deschampsia* spp.) grown under standard conditions, and then exposed at monitoring, followed by measurement of growth and N inventory.
- Native reciprocal transplants (e.g. bryophytes), where natural plant material is exchanged between sites of varying N exposure, followed by measurement of growth and N inventory.

It was noted that each of these methods would benefit from further testing and application in the UK, and these form the basis of methods tested in the present study. It was recognized that the last of these (native reciprocal transplants, e.g. Mitchell *et al.* 2004) is more resource demanding than the others, and this approach was therefore not tested in the present project.

1.2. Objectives of the project

The main objective of the project was to refine, test and subsequently recommend bioindicator/biomonitoring methods for assessing the impacts of atmospheric nitrogen deposition or concentrations on statutory nature conservation sites. To do this, the work focused on contrasting situations with different habitat types and atmospheric pollutant combinations, and also included development work to refine the procedures for measurement of soluble foliar nitrogen, as a bioindicator of N pollution.

This study should help the conservation and environmental agencies to better understand the effects of nitrogen on site condition and integrity. These biomonitoring methods should also help validate the use of critical loads in providing a national overview of risk to the site series.

1.3. Report Field studies

The two main field components of the project were:

1) To test selected bioindicator methods in detail at four key contrasting sites.
The four sites referred to as the "intensive" sites were:

Piddles Wood, Dorset, a lowland woodland with dry deposition of agricultural NH_3 as the main source of nitrogen pollutant:

Happendon Wood, Lanarkshire, a wooded site adjacent to the M74 motorway with dry deposition of vehicular NO_x (plus associated NH_3) as the pollutant of interest.

Bowbeat Hill, a high altitude moorland dominated by wet deposition of N from NH_x and NO_y. This site is contrasted with a nearby low altitude moorland, **Auchencorth Moss**, with lower wet deposition inputs.

Whim Moss a nitrogen manipulation study on a *Calluna vulgaris*/*Eriophorum vaginatum* mire comparing the effect of different forms of N deposition (dry NH_3, vs. wet NO_3^- vs. NH_4^+).

2) To apply the simplified methods validated in (1) at the UK scale using sites with existing robust measurements of atmospheric N exposure.

Overall thirty-two sites (referred to as the "extensive" sites) were selected covering the devolved regions of the UK.

Two other objectives were:

3) To refine the soluble nitrogen methodology and apply it at the intensive and UK extensive sites.

4) To synthesise, using the results from the Intensive and UK Extensive sites, the applicability of N biomonitors and biomonitoring for the conservation agencies, SEPA and EHS in their condition monitoring and integrity assessment of designated sites (i.e. SSSIs, SACs etc).

1.4. Structure of the report

This report describes the results of the different bioindicator methods tested at both the intensive and UK extensive sites and gives an overview of their general applicability to conservation agencies, SEPA and EHS.

The study was divided into four main components a) development of the methodology for soluble NH_4-N concentration determination, focusing on the measurement of foliar ammonium concentrations, (Appendix I) b) the testing of simplified biomonitoring methods in parallel at four intensive sites (Sections 3-8) c) the application of selected simplified methods at the UK scale in the extensive study (Sections 9-13) and d) applying the results from the intensive and extensive to assess N impacts on condition and integrity of SSSI's and production of protocols and 'Bench marking'for specified habitat/pollutant scenarios (Sections 14, 15 and 16).