

# Assessing the effects of small increments of atmospheric nitrogen deposition (above the critical load) on semi-natural habitats of conservation importance

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# Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

## Background

The work was commissioned as part of a review of the thresholds used in air quality impact assessments that was required through the Inter-agency Air Quality Technical Advisory Group. The report aimed to analyse existing scientific data to demonstrate and quantify the effect of incremental additions of atmospheric nitrogen deposition (above the critical load) on different semi-natural habitat types.

The report will be used to inform specialist advice on air quality effects on habitat that is used in planning advice, agri-environment schemes and to protect and enhance designated sites. The Environment Agency

have planned (subject to approval) to use this science report to review the thresholds they use for controlling ammonia emissions from intensive farming.

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**Assessing the effects of small increments  
of atmospheric nitrogen deposition (above  
the critical load) on semi-natural habitats  
of conservation importance**

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## Summary

1. Around two thirds of all Sites of Special Scientific Interest in the UK exceed their critical loads as a result of current atmospheric nitrogen (N) deposition. In regions where the critical load is already exceeded there is a need to understand how further increases in N deposition may affect ecological communities.
2. The objective of this report was to examine recent vegetation survey data to understand the relationships that exist between species (composition and richness) and nitrogen deposition, and to determine the effect of incremental increases in N. Vegetation data were analysed from 226 sites, collected over 8 surveys of 5 UK priority habitats for conservation (sand dune, bog, lowland heath, upland heath, acid grassland). Further evidence was gained from published survey data and the network of UK nitrogen addition experiments.
3. The relationships examined in this report use modelled annual N deposition as the pollutant variables; however, the relationships studied between N deposition and species richness and presence have developed over many years of pollution. The current rate of N deposition is used as a proxy for long-term cumulative N deposition. In some/many cases, sites will have experienced high N deposition for many years and, because of this legacy, it is unlikely that an increase or decrease in nitrogen deposition will immediately cause changes in species richness or composition.
4. Across the habitats and datasets, increasing N deposition (total, reduced or oxidised) was correlated with quantifiable declines in species richness and changes in species composition. Species richness was also correlated to climate, with increasing species richness being a function of increasing precipitation and decreasing temperature. Evidence from the literature review (N addition experiments where climatic drivers are controlled for and other field surveys) supports the findings from the data that N is driving considerable change within the habitats studied.
5. When all the habitats are considered separately, the response of species richness to long-term N deposition is curved, with sharper losses in diversity from well below the habitat-specific critical load range. At levels of N deposition at and above the upper end of each habitat-specific critical load, additional increments of long-term N are associated with further declines in species richness.
6. Not all species responded negatively, nitrogen loving plants such as the graminoids (grasses and sedges) *increased* their cover in response to increasing N deposition in bog, heath and sand dune habitats. This may result in the loss of key habitat species due to increased competition from faster-growing species, and further threaten site integrity. In addition, some species groups responded in some habitats but not in others, for example bryophyte species richness.
7. Gaps in the data mean that there remain many habitat types in the UK for which the responses to N deposition are not fully understood. Ecosystems which share similarities in species and soil type are likely to show similar responses to those found within this report. In these cases it is recommended that the findings in this report, subject to local conditions, be used to predict responses to an incremental increase in N deposition sustained over the long term. Further work should be undertaken to fill the data gaps in these habitats and those that are dissimilar to the ones studied.
8. The atmospheric concentration of NO<sub>x</sub> and NH<sub>3</sub> can also influence responses. Over the long-term, changes in pollutant concentration are reflected by changes in deposition, therefore changes in annual mean concentrations could be converted to N deposition and responses predicted using the relationships developed in this report. However, it is important to recognise that the differing effects between concentration and deposition are unclear and high pollutant concentrations, even in the short-term, may be very damaging, especially for lower plants. Dose-response relationships to changes in N concentration are not fully understood and should be further researched experimentally.

9. The findings of this work, in conjunction with other recent studies, have important implications for the way that pollution regulators and the conservation agencies assess new or existing pollution sources and the assessment thresholds applied.

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# 1. Introduction

## The nitrogen problem

Emissions to the atmosphere of ammonia (NH<sub>3</sub>) and nitrogen oxides (NO<sub>x</sub>) dramatically increased in the 20<sup>th</sup> century due to increased combustion of fossil fuels and intensification of agriculture. Ammonia is volatilised from intensive agricultural systems such as dairy farming and intensive animal husbandry, while nitrogen oxides come mainly from burning of fossil fuel by traffic and industry (Asman *et al.*, 1988; Galloway, 1995; Bobbink and Hettelingh, 2011). These combined activities result in a more than doubling of the deposition of reactive nitrogen compounds to the earth's surface (Galloway *et al.*, 2004). The problems that result from increased aerial deposition of reactive nitrogen compounds have been recognised only in recent decades but are now believed to be widespread in ecological communities on regional and global scales (Emmett, 2007; Phoenix *et al.*, 2006; Bobbink *et al.*, 2010). There is particular concern over the impacts on natural and semi-natural ecological communities, where the normal low rates of nitrogen supply often provide important limits to ecological processes. For this reason the most obvious potential influence of pollutant nitrogen deposition is as a fertilizer, i.e. eutrophication, threatening the natural composition of those ecological communities that are well adapted to nutrient-poor soils. Another ecological impact of nitrogen deposition results from soil and water acidification which affects some species directly but also causes impacts through release of toxic metals such as aluminium (Stevens *et al.*, 2010). A wider range of biogeochemical changes are also likely to occur in impacted sites such as nitrogen leaching and nutrient imbalances in soils and vegetation (see RoTAP, 2012).

## The evidence base

The scientific evidence demonstrating that nitrogen pollution can affect ecosystems in the UK and elsewhere has grown substantially in the past decade and has recently been re-evaluated in RoTAP (2012). Much of the early knowledge about nitrogen impacts on ecological communities came from laboratory and field experiments which have demonstrated the potential for change in structure and function of ecosystems and communities. An alternative approach using field-based monitoring and targeted vegetation surveys provides complementary and compelling evidence that the changes seen in nitrogen addition experiments have actually occurred in the field as a result of atmospheric deposition. Various vegetation monitoring schemes such as the UK Countryside Survey (Maskell *et al.*, 2010) and specific habitat surveys across deposition gradients (e.g. Stevens *et al.*, 2006), supported by experiments (see Emmett *et al.*, 2007), indicate that long range nitrogen pollution is or has been responsible for community changes and significant losses of plant diversity across large areas of the UK.

## Critical loads

The growing knowledge base from the combined experimental studies and field surveys enable us to generate, for several plant communities, nitrogen dose – ecological response relationships and these can be used to evaluate and position critical load guidelines. The Critical Loads approach is a tool used to judge the risk of harm to the environment from several forms of air pollutants. Critical Loads are defined as: “a quantitative estimate of exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge”. The empirical Nutrient nitrogen critical loads were revised in June 2010 (Bobbink and Hettelingh, 2011) and are shown for all EUNIS habitats in Appendix 6 and summarised in Table 1 where they relate to specific habitats studied here. Large areas of the country now exceed the critical loads for nutrient N and are predicted to continue to do so in 2020 despite reductions

in emissions of reactive N gases (Hall *et al.*, 2006). For an overview of Critical Loads see the UK Air Pollution Information System ([http://www.apis.ac.uk/overview/issues/overview\\_Cloadslevels.htm](http://www.apis.ac.uk/overview/issues/overview_Cloadslevels.htm)).

Table 1: Summary of current critical loads relevant to the habitats studied in this report

Ecosystem type	EUNIS code	2011 critical load (kg ha <sup>-1</sup> yr <sup>-1</sup> )	Indication of exceedance
<b>Upland and lowland heath</b>	F4.2	10-20	Transition from heather to grass dominance, decline in lichens, changes in plant biochemistry, increased sensitivity to abiotic stress
<b>Sand dune grassland</b>	B1.4	8-15	Increase in tall graminoids, decrease in prostrate plants, increased N leaching, soil acidification, loss of typical lichen species
<b>Bog (raised and blanket)</b>	D1	5-10	Increase in vascular plants, altered growth and species composition of bryophytes, increased N in peat and peat water
<b>Acid grassland</b>	E1.7	10-15	Increase in graminoids, decline of typical species, decrease in total species richness

## Environmental protection

An application for an Environment Permit (under the Environmental Permitting Regulations 2010) from an industrial installation wishing to commence or expand activities triggers an assessment under the Conservation of Habitats and Species Regulations 2010 (known as the 'Habitats and Species Regulations'), in relation to European protected sites, and the Wildlife & Countryside Act 1981, as amended by the Countryside & Rights of Way (CROW) Act 2000 in relation to SSSIs. In accordance with the legislation, permits should only<sup>1</sup> be given where it is possible to conclude that the installation will have no adverse effect on the integrity of a European site (SAC, SPA or, by Government Policy, Ramsar site) and is not likely to damage a SSSI. This assessment is considered in context with the thresholds within the Environment Agency (EA) H1 guidance, together with the understanding that around two thirds of all protected sites are already predicted to exceed their nitrogen critical loads as a result of existing levels of air pollution (RoTAP, 2012).

This raises a key question for the Government nature conservation advisors and environmental regulator: if there is already an identified risk of harmful effects from existing air pollution (i.e. predicted critical load or critical level exceedance), what, if any, additional air pollution arising from a new installation is acceptable. Furthermore, what, if any, benefits are likely to be evident due to a reduction of pollutant exposure while the critical load or level remains exceeded?

In order to address these questions of the consequences of changes in nitrogen deposition above and below the critical load, this report will consider in detail the form and the quantitative nature of the relationships between atmospheric nitrogen deposition and ecological response in a number of different important UK ecological communities using

<sup>1</sup> With the exception where Overriding Public Interest is determined by the Secretary of State or Welsh Assembly Government, under the Habitats Regulations.

recent evidence from surveys and experiments. The aim is to quantify the effect of incremental changes in long-term nitrogen deposition both above and below the critical load on important measures of plant community diversity and species composition. The approach is to use recently available scientific data, including the UKREATE (Terrestrial Umbrella) survey dataset, and apply new statistical analysis such as canonical correspondence analysis, stepwise and LOESS (Locally weighted scatter plot smoothing) regression, and constrained cluster analysis to define and visualise the response variables within a range of habitats. Other published studies and experimental information are also assessed. The nature of the relationships between these response variables and nitrogen deposition are then examined. The results of our analysis are discussed in context with incremental increases in N deposition above and below the critical loads.

## **Report structure**

First the report introduces the data sets and statistical methods used. The report is then structured by Tasks:

### **Tasks 1 and 2: To collate the relevant scientific information and categorise by habitat type**

These tasks collated the available scientific information and categorised these data by habitat. Vegetation data sets from a number of surveys were used for the project, together with responses found at a number of nitrogen-addition experimental sites: details of these are provided here. In addition, key literature reporting survey responses from each habitat is also summarised in this section. In some habitats data were not available; these are identified and discussed separately in Task 6.

### **Task 3: To identify the relevant response variables for each habitat type**

This studied each habitat for which vegetation survey data were available and how species richness or species composition varied across each dataset. The responses of these variables are analysed alongside ecological driver data such as nitrogen and sulphur pollution, temperature and precipitation and response variables strongly associated with nitrogen pollution are identified.

### **Task 4: To determine the relationship between nitrogen deposition and the key response variables**

The nature of the relationship between the response variables identified in Task 3 and nitrogen pollution is considered further in Task 4.

### **Task 5: To assess the effects of different increments of nitrogen deposition above the critical load**

Here the relationships identified in task 4 between N deposition and the response variables within each habitat were further considered and the effect of an incremental increase in long-term N pollution upon each was derived. This is reported as percent change in species richness or cover of selected indicator species for a  $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$  rise in long-term N pollution, and the amount of long-term N that would lead to a reduction in species richness of 1 species at different background levels of N pollution. Results for other increments of nitrogen are provided in Appendix 5. Comparable responses from dose-response experiments and the literature are also presented for Tasks 4 and 5.

### **Task 6:**

This task reviews the information presented in the preceding tasks and assesses whether the relationships between the response variables and N can be applied to habitats where survey datasets were not available for analysis. Similarities between habitats in soil type and vegetation are used to complete this task. This task also considers whether the

relationships can be used when considering pollutants concentration i.e. critical levels rather than loads. A final discussion draws together the information presented within the report.

## 2. Methods

### 2.1 Vegetation survey data

This project analysed vegetation survey data collected during 8 surveys of 5 key UK semi-natural habitats between 2002 and 2009 encompassing the 2009 Terrestrial Umbrella (TU) multi-habitat survey, the 2006 TU Moorland Regional Survey (MRS), a 2002 Sand dune survey (Jones *et al.*, 2004), and the BEGIN UK Acid Grassland dataset (Stevens *et al.*, 2010). Mean vegetation data were collected over 5 separate quadrats per site (in most cases these quadrats were 2 x 2 m<sup>2</sup>) and vegetation cover of all the species present within the quadrat was estimated. In the case of the Moorland Regional Survey, 0.5 x 0.5 m<sup>2</sup> quadrats were used. Full details of each survey are included under Tasks 1 and 2 and in Tables 2 and 3.

For the quadrat-survey technique to be directly comparable, quadrat size should be identical. It is important to note that this measure of species richness is a probability of finding a species at each site; it does not necessarily mean that fewer species are present at each location, although this may be the case. However, it does imply that the evenness of species is reduced and there is a tendency for the vegetation community to be dominated by fewer species and individual species to be present at lower frequencies. Figure 1 overleaf illustrates this concept.

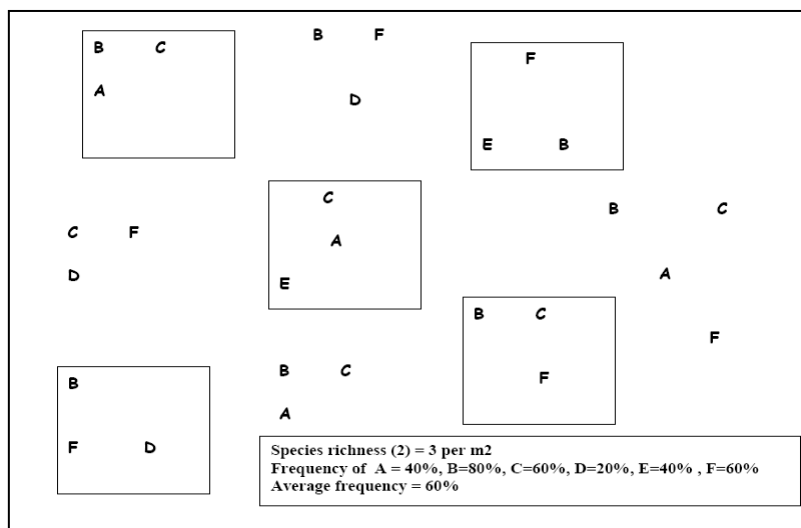
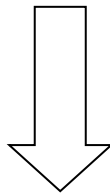
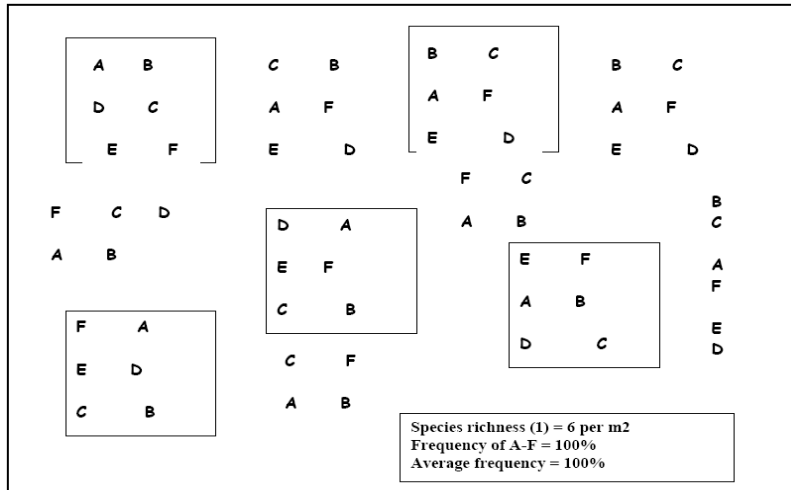


Figure 1: Species richness reduction vs. species loss. In both Figure 1a) and 1b), six species are present in the field. Both fields are surveyed using five 1 m<sup>2</sup> quadrats. In Figure 1 a), each quadrat surveyed contains 6 species and measured species richness is therefore 6. In Figure 1 b), each quadrat only contains 3 species, giving a species richness of 3. The same species are present in both fields, but at a lower frequency in the second: this generates a lower measure of species richness. Figure by N. Dise.



## 2.2 Environmental and pollutant driver variables

Species richness and composition may be affected by a number of physical and chemical variables. These drivers include variables such as temperature, precipitation, pH etc. Driver data shown in Table 2 were assembled from national datasets for use in the multivariate analyses. The climate data used were based upon UK 5 km<sup>2</sup> gridded data sets provided by the Met Office. Variables representing total annual precipitation and temperature were used, the latter represented by growing degree data (sum of degree days above 5°C) in the TU and BEGIN survey data and by extreme temperature range in the MRS data. Both precipitation and growing degree data were averaged over the period 1997-2006.

The pollutant deposition data used were the 5 km<sup>2</sup> Concentration Based Estimated Deposition (CBED) values for 2004-2006, provided by the Centre for Ecology and Hydrology (CEH). Variables for total nitrogen deposition (further divided into wet and dry and reduced and oxidised forms), total sulphur deposition (split further into wet and dry) and non-marine calcium + magnesium deposition were included in the data analysis.

Co-correlation of deposition data between pollutant types and the response variable is acknowledged due to the intrinsic link between some pollutants, for example, nitrogen and sulphur are both by-products of fossil fuel combustion and therefore fluctuations in deposition of each follow broadly the same spatial pattern. Therefore in some cases a judgement was made of the ecological significance of a particular type of pollutant deposition. Such judgements were based where possible on the observed effects: for instance if nitrogen and sulphur were closely correlated but the effects were typical of eutrophication rather than acidification it was possible to exclude sulphur as a possible cause. It is also recognised that different forms of a pollutant have different effects on an ecosystem (e.g. oxidised and reduced nitrogen) and that more than one form of a pollutant can be correlated to ecological change. Separating closely correlated environmental variables is difficult in exploratory analyses such as the gradient studies presented here. We consider that it is better to include all possible environmental variables rather than to make a *priori* judgements about which variables are important. In cases where many variables are highly correlated the selection of one highly-correlated variable over another must be interpreted with caution. Although in many of our analyses we present results for the variable with strongest correlation statistics, in general these are best viewed as representing a broader gradient. So, for instance, although we might find strongest correlation statistics with dry deposition of oxidised nitrogen typically this variable is very strongly correlated with other forms of nitrogen deposition and the result is best seen as simply representing 'nitrogen pollution'.

A further environmental variable included within the bog habitat was a hydrological index based upon field observations on a scale 1-5, with 1 relatively dry (similar to an upland heath) and 5 very wet: a quaking or floating bog. The environmental variables used in the statistical analysis and their acronyms when included on ordination plots are summarised in Table 2. Variables such as radiation index, latitude and longitude were not included as these are correlated with both climate and pollution; variability in these is accounted for by precipitation and growing degree days.

It is recognised that in these semi-natural habitats site management is an important determinant of vegetation structure. The term 'management' encompasses a range of human interventions (burning, grazing, drainage) that are difficult to quantify and for which national-scale data is rarely available. We attempt to account for these variables using field-observed indices. For the bog data, the hydrological index largely reflects the history of drainage and peat cutting. For the Moorland Regional Survey we included a 'habitat' term which captured the development phase of the *Calluna vulgaris* growth cycle i.e. 'pioneer',

'building', 'mature', 'degenerate' (Gimingham, 1972). For the TU heathland and sand dune data no management variables were included. In these studies (indeed in all of the studies) site and quadrat selection was carefully considered to maximise consistency between sites.

Table 2: Summary of driver variables used in the statistical analysis

Driver variables	Acronym	Comment
Growing degree days	growdeg	sum of degree days above 5°C
Precipitation	precip	
Extreme temperature range		Moorland regional survey only
Habitat		Moorland regional survey only
Grazing		Acid grasslands only
Altitude	altimetr	not sand dunes
Hydrology	bog_hydr	Bog habitat only
pH	pH	
Loss on ignition	LOI	
Total acid deposition	aciddepo	
Sulphur deposition	sulpdepo	
Nitrogen deposition	Nitrdepo	
Oxidised nitrogen deposition	oxiNdepo	
Reduced nitrogen deposition	redNdepo	
Calcium + magnesium deposition	Cmgdepo	
Wet sulphur deposition	wet_sulp	
Dry sulphur deposition	dry_sulp	
Wet oxidised nitrogen deposition	wet_oxiN	
Dry oxidised nitrogen deposition	dry_oxiN	
Wet reduced nitrogen deposition	wet_redN	
Dry reduced nitrogen deposition	dry_redN	

## 2.2.1 Cumulative N versus recent N deposition

The relationships examined in this report use modelled recent annual N deposition as the pollutant variable(s), however, the relationships studied between N deposition and species richness and presence have developed over many years (Dise *et al.*, 2011). The recent rate of N deposition is primarily a proxy for longer-term cumulative N deposition. Thus we would not expect that a change in N deposition, either increasing or decreasing, would immediately change species richness or composition, but instead these would be gradually influenced by longer-term changes in N deposition. However, different plant groups respond in different ways: bryophytes and lichens are likely to respond quicker than vascular plants and the responses of both may be affected by management interaction which could alter inter-species composition.

Since cumulative deposition data from the survey locations was not available for use in this study, the current N deposition was instead used as a proxy for cumulative deposition. If cumulative data are estimated from current deposition patterns (as in Dupre *et al.*, 2010) we would expect to see very similar results. However, if cumulative deposition data based on emission changes over time were available, this may show different results. An example of this could be in an area where agricultural N emissions have increased markedly in recent years: long term cumulative N deposition based upon current N deposition would over-estimate the total N deposited to the site. Conversely, the use of current N deposition

estimates for an area to which N deposition has reduced dramatically over recent years at a rate different to broad-scale trends would under-estimate the cumulative N deposited. Fowler *et al.* (2004) calculated cumulative N deposition for 1900-2000 based upon historic emissions data. A comparison of the cumulative N deposition map for 1900-2000 with modelled nitrogen deposition maps for the year 2000 revealed that broad-scale patterns of N deposition across the country were very similar in these datasets; however, one area of notable difference is apparent. Relatively recent modelled N deposition in East Anglia is much greater than that indicated long-term cumulative N, presumably owing to growing agricultural emissions.

Given the potential impacts of high N deposition both over the long term and currently, a sensible approach would be to consider both N deposition scenarios when judging the vulnerability of a site to raised N and assessing the impact of N deposition at a site. However, given the similarities between the broad-scale spatial patterns of cumulative and contemporary N deposition for much of the UK, the use of current modelled deposition as a proxy for long-term N to predicting responses to increases in N deposition seems reasonable.

## **2.3 Experimental data**

A number of long-term nitrogen addition experiments exist in the UK, many within the DEFRA 'UK Research on The Eutrophication and Acidification of Terrestrial Ecosystems' (UKREATE) project, and data from these were included where relevant to the findings of this project. These sites are summarised in Task 3 and Tables 5 and 6.

## **2.4 Data analysis**

Several analytical techniques have been used to determine the effect of air pollution and environmental variables on key response variables in the survey data. Responses studied included species richness, species composition, changes in species richness of different functional groups and the response of certain individual species. The techniques used include ordination analysis, stepwise and conventional regression, LOESS (Locally weighted scatterplot smoothing) regression and cluster analysis.

### **2.4.1 Ordination analysis**

Ordination analysis is a suite of techniques for the analysis of multivariate data in which the aim is to arrange samples along axes on the basis of their species compositions. At their simplest ordination techniques function as a dimension-reduction technique allowing the representation of difference or similarity in species composition of samples in a simple two-dimensional plot. Axes can be determined simply by the species composition of those samples (unconstrained ordinations, also termed indirect gradient analysis) or can be constrained to be composed of linear combinations of measurable environmental variables (constrained ordinations, also termed direct gradient analysis). In our analyses of gradient studies ordination techniques allowed us to understand and visualise the relationships between overall community composition and the environmental gradients which drive changes in that composition (pollution, climate etc). Furthermore, ordination plots allow us to identify individual species which are particularly responsive to individual environmental variables and which may function as indicator species. These analyses are therefore separate from, but complimentary to, analyses of univariate variables which integrate some aspect of community composition such as species richness or functional group ratios. Environmental controls on overall species composition are separate from, but often overlap with those on species richness.

A Detrended Correspondence Analysis (DCA) was first performed to analyse the length of environmental gradients underlying each dataset. Then either Redundancy Analysis (RDA) or Canonical Correspondence Analysis (CCA) were used as appropriate, assuming linear and unimodal species responses along the environmental gradients respectively (Leps and Smilauer, 2003). We present two key outputs from RDA or CCA, the numerical results namely the % variance explained (analogous to the  $R^2$  of a regression) and P-value (determined by Monte Carlo permutation test) and the ordination plot. Large-scale vegetation datasets will typically contain considerable noise due to non-measured variables and random variability so the proportion of variance explained by environmental variables is often low. However the relationships identified can still be highly significant with small P-values.

Initially, all environmental variables were entered into each analysis and a forward selection procedure using Monte Carlo permutation tests carried out to establish a minimal suite of variables that independently explained significant variance in the data. The variable that explained greatest variance in the data (greatest marginal effect) was first selected in the analysis, and then included as a co-variable in subsequent analysis. The variance explained by all other variables was then tested to identify the next variable that then explained the greatest *additional* variance. This variable could then be tested for significance, and if  $P < 0.05$  included in the analysis. The selection process continued until no further variables explained significant additional variance. This approach enabled the statistical effect of climate and pollutant variables to be separated, however, it can mean that variables that are correlated with each other (such as different types of air pollution) may be excluded as only the variable with the strongest association is included, and when this variable is included as a covariable, the other variables that are correlated with it may be removed from the analysis as they explain no additional variation.

Subsequently variance partitioning was carried out to test the % variance and significance of each forward selected variable with other selected variables as co-variables. CANOCO software for Windows version 4.53 (ter Braak and Smilauer, 2004) was used for both CCA and RDA analysis.

An example ordination plot from an RDA analysis is provided below (Figure 2). In all ordination plots the values of the axes and the position of species relative to those axes is of less interest in general terms than the position of species relative to other species and environmental variables. The red arrows represent environmental variables and their direction of influence relative to the species shown in the diagram. The strength of each driver variable on species composition is represented by the relative length of arrow, in this example 'redNdepo' (reduced nitrogen deposition) has the longest red arrow and as such exerts the most influence on species composition. The correlation between environmental variables can be judged by their similarity of direction, arrows pointing in the same direction represent correlated variables. In the plot below it will be noted that the three red arrows point in different directions, there is little correlation between variables. Similarly, individual species are shown on the plot and positioned relative to the driver variables and the influence of the driver variables shown by relative length of grey arrow presented on the plot. The direction of an arrow relative to the axes does not imply a positive or negative direction of influence. In the example below, most species are ordinated away from redNdepo implying that the cover of these species is negatively affected by deposition of reduced N. These species are also correlated with each other, the vectors for *Cladonia portentosa* (cladport) and *Cladonia gracilis* (cladgrac) are adjacent to each other, most likely the species are found in the same sites. *Cladonia fimbriata* (cladfima) is the only species that appears positively associated to 'redNdepo', however, it also appears associated with 'growdegr' (growing degree days) suggesting that its relative cover is influenced both by reduced N deposition and temperature.

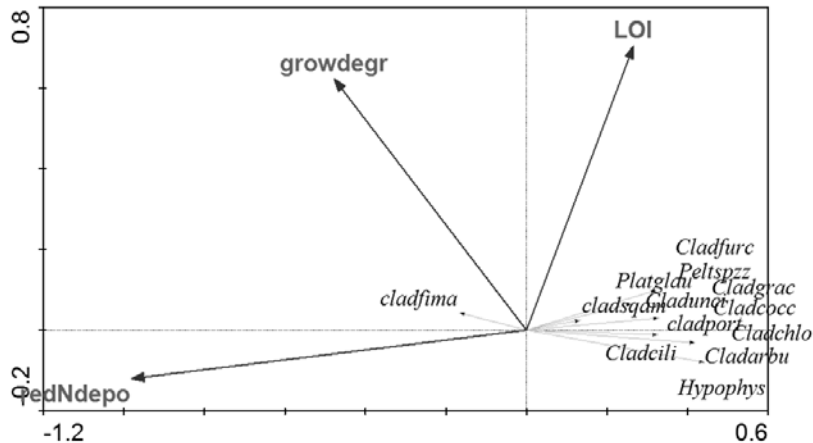


Figure 2: Example ordination plot

### 2.4.2 Stepwise and conventional regression

Regression techniques were used to model change in species richness with regard to an individual variable (simple regression) or a combination of variables (stepwise regression) that explain the most variation in the independent variable, in this case species richness. Stepwise regression is a form of multiple regression using a combination of forward and backward selection of variables. Variables are included if they explain significant variation in addition to those already in the model, and excluded if their removal does not increase the residual sum of squares. In both forms of regression a test for normality was performed and corrections made as necessary.

### 2.4.3 LOESS regression

LOESS (locally weighted scatterplot smoothing) regression is a form of non-parametric regression which acts as a 'smoothing' tool to aid visualisation of the response of taxa (individual species or functional groups) to a variable such as nitrogen pollution.

Linear regression techniques (including simple, multiple and stepwise) assume that the underlying relationship between dependent and independent variables is linear. LOESS regression enables a curve to be fitted to the data without making any assumptions about the form of the underlying relationship. In doing so it allows more flexibility than classical regression by fitting a smoothing function that varies with the data. However, simple mathematical equations describing the relationships cannot be generated with LOESS as with linear techniques.

PAST software version 2.06 (Hammer *et al.*, 2001) was used for this analysis.

### 2.4.4 Cluster analysis, sample grouping and ecological thresholds

An important question that is rarely explicitly addressed is whether the response of plant communities to nitrogen deposition is linear, or if there are ecological thresholds. If threshold responses do exist, this may have important implications for regulation of pollution loading, suggesting that there are points above which, or below which, further nitrogen deposition may have a disproportionate impact on the ecosystem. As detailed elsewhere in this report, gradient studies are now available for a large number of semi-natural habitats within the United Kingdom. Results from these studies show a reduction in species richness along the nitrogen pollution gradient and characteristic changes in plant communities.

Here we apply constrained cluster analyses to these datasets. This analysis attempted to identify non-linearities in the community response using a variety of statistical techniques originally developed for time-series data from bio-stratigraphy but theoretically equally applicable to changes along any gradient. These techniques are similar to conventional cluster analysis techniques but with the constraint that clusters be composed of samples with similar levels of nitrogen deposition.

In the context of this report this analysis has two important functions. Firstly it enables us to validate the results of the ordination analyses (discussed below) which show nitrogen pollution to be an important environmental control on the species composition of many habitats. If significantly different groupings can be identified solely on the basis of their nitrogen-loading this provides convincing evidence that nitrogen is an important control on community composition. Secondly, the location of 'break-points' between sample groupings is of interest because these may relate to ecological threshold responses. It is important to note that our approach is subtly different from direct identification of a threshold. If a threshold is abrupt the groups of samples on either side of a break-point will be distinctly different and are likely to be easily separated by clustering, if however the threshold is more gradual there may be variability in the group to which marginal samples are assigned.

We trial three methods derived from two contrasting approaches. We first test an agglomerative approach: with groups built by successively combining samples, as for many conventional cluster analysis techniques. Our approach is based on Ward's method (Ward, 1963) where clusters are built so as to minimize the increase in total within-cluster sum of squares. Conventional cluster analysis produces groups that are difficult to interpret in terms of a single environmental variable. To avoid this problem we introduce a constraint that clusters must be composed of samples with adjacent levels of nitrogen deposition. Essentially, we force the cluster analysis to produce groups which represent differing levels of nitrogen deposition. This method – constrained incremental sum of squares (CONISS) – is widely used for temporally-structured data (Grimm, 1987). CONISS produces a dendrogram, but only the first few splits are likely to be ecologically meaningful. Table 1 presents the first two. A limitation of this technique is that Ward's method has an inherent tendency to produce clusters of similar size (e.g. Morse, 1980).

The other two methods take a contrasting approach: instead of building up groups by successively adding samples in an agglomerative approach, they consider the whole dataset and the reduction in overall variance which may be achieved by the insertion of zone boundaries. As such the methodology is more focused on the sequence as a whole, unlike the agglomerative methodology which is more focused on the individual samples. We treat our samples as a transect along the gradient of total nitrogen deposition (as for CONISS), and test the validity of inserting splits in all alternative positions. Two variants of this divisive methodology are examined, with variance assessed by information content (SPLITINF) or least squares (SPLITLSQ; Gordon and Birks 1972, Birks and Gordon 1985). The SPLITINF and SPLITLSQ techniques are binary approaches that first split the overall dataset in two and then successively split these zones into smaller sub-divisions. As for CONISS, the first two divisions are presented in Table 17. We apply all three of these techniques using ZONE vers.1.2 (Juggins, 1992) with a squared Euclidean distance matrix.

Results of constrained agglomerative techniques can be presented as a dendrogram showing the relationships of samples along the gradient. An example of such a dendrogram is shown in Figure 3 below. The relationships of individual samples are shown by the proximity of their branches. Although such dendrograms present a large amount of information, typically only the first 'branches' are significant and useful. In the results of this analysis we only present the locations of the first two sample divisions.

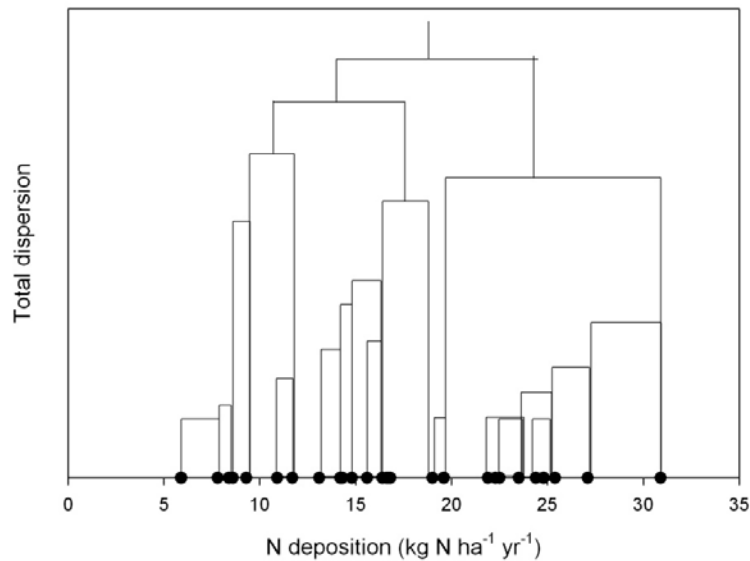


Figure 3: Example of a dendrogram showing the relationships of samples along a gradient

None of these three methods include a test of the validity of the clusters produced. To determine whether the community composition of the different clusters is significantly different, we apply a simple test of community similarity using ANOSIM. ANOSIM is a non-parametric test of similarity between pre-defined groups (Clarke, 1993). The test statistic ( $R_{\text{ANOSIM}}$ ) has a value between -1 and +1 (although negative values are unusual); a value of 0 indicates the null hypothesis, that there is no difference between groups, while a value of 1 indicates that all samples within groups are more similar to one another than to any samples from different groups. Significance testing is achieved using permutation tests.

We applied ANOSIM with a Bray-Curtis distance measure and 10,000 permutations in PAST ver. 1.71 (Hammer *et al.*, 2001). The ANOSIM results tell us whether the groups of samples are different, but give no assessment of whether those boundaries are in the optimum position (i.e. the probability of any random division producing a significant result in ANOSIM is relatively high). To give some assessment of the distinctness and validity of the cluster boundaries, we can compare the results of the three methods used. As these techniques rely on different underlying principles, if they identify similar cluster boundaries, this can give us confidence that the groupings are valid and useful. Additionally, where we have replication within an ecosystem type (acid grasslands and upland heaths) we can compare the results between the datasets.

Two general problems occur with all of these three approaches. Firstly there are issues with confounding environmental variables. In all of the datasets, many variables other than total nitrogen deposition affect plant communities, particularly climate. The response of the plant communities to these other variables is likely to complicate the identification of meaningful groups according to nitrogen deposition values. A second general problem is the size of the datasets and inconsistent sampling along the nitrogen gradient. With the exception of the larger Stevens *et al.* (2004, 68 sites) dataset the number of sites in each dataset is small (22-29 sites) and at the lower limit of the sample size for which many multivariate techniques are appropriate. Partly as a result of this limited sample size, the distribution of samples along the N deposition gradient is non-uniform, with gaps apparent in some datasets (for instance, no sites between 30.3 and 40.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the TU-acid grasslands data, and no sites between 5.9 and 10.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the TU-lowland heath data). Inconsistent sampling along the gradients both reduces the precision with which cluster boundaries can be located and increases the probability of false identification of a group boundary.

### 3. Tasks 1 and 2: Collation of scientific information and categorisation by habitat type

The bulk of the data used in this report is from a compilation of vegetation survey data from 8 field surveys encompassing vegetation quadrat data from a total of 226 UK sites surveyed between 2002 and 2009 (see Figure 4). The habitats are: dwarf shrub heathland (upland and lowland), acid grassland, bog and sand dune (fixed-dune grassland). Each represents a UK Biodiversity Action Plan (UK BAP) priority habitat for conservation (Natural England, 2011).

The surveys comprise of a 2002-2003 acid grassland survey, a 2002 sand dune survey, a 2006 upland heath survey, a 2007 acid grassland survey and 2009 surveys of sand dune, bog, upland and lowland heaths. Where data were comparable between surveys, results were combined. Details of the surveys are provided in Table 3.

Within each survey, locations were carefully chosen to enable site comparisons to be made, ensuring vegetation structure between sites was consistent. Locations were identified along a UK nitrogen pollution gradient which was typically cleaner in the north and more polluted in the south. This UK pollution gradient also closely follows a climate gradient, with northern sites being cooler and wetter than their southern counterparts. For this reason an east-west gradient was also maximised within each habitat to provide survey locations that were 'clean and warm', 'polluted and cooler', 'wetter and polluted' and 'drier and less-polluted'. Such an approach aided statistical partitioning of the effects of pollutant and climatic drivers of vegetation change.

Further data were obtained from a network of UK nitrogen addition experiments funded as part of the Terrestrial Umbrella (TU) UKREATE project (<http://ukcreate.defra.gov.uk/>) which include habitats similar to the surveys. Data from these experiments that support or challenge findings from the gradient surveys are presented and details of these experiments are shown, in Tables 5 and 6.

Published data from similar habitats to the survey data have also been drawn upon and reviewed as part of this report: these are summarised in Task 4 and Table 4.



Figure 4: The survey locations of the 226 sites from which data has been used in the analysis for this project



Table 3: Survey data sets by habitat type under new analysis as part of this report

Habitats	New analysis here or Reviewed	NVC	Survey date	Location	Method of data collection/ number of sites	Author /affiliation	Reference
<b>Acid grassland</b>	New	U4	2002/3	GB	Quadrat survey/68 sites	C. Stevens, Open University/CEH	Stevens <i>et al.</i> (2004)
	New		2007		Quadrat survey/22 sites		BEGIN grassland survey
<b>Bogs</b>	New	M19, M18	2009	GB	Quadrat survey/ 29 sites	TU consortium led by MMU	UKREATE, 2010 (TU report for 2007-2010)
<b>Lowland heath</b>	New	H2-H13	2009	GB	Quadrat survey/27 sites	TU consortium led by MMU	UKREATE, 2010 (TU report for 2007-2010)
<b>Upland heath</b>	New	H12	2009	GB	Quadrat survey/25 sites	TU consortium led by MMU	UKREATE, 2010 (TU report for 2007-2010)
<b>Sand dunes</b>	New	SD12, SD8 transitional	2009	GB	Quadrat survey/24 sites	TU consortium led by MMU	UKREATE, 2010 (TU report for 2007-2010)
<b>Sand dunes</b>	New	SD8, SD11, SD12 transitional	2002	GB	Quadrat survey/11 sites	L. Jones, CEH Bangor	Jones <i>et al.</i> (2004)
<b>Upland heath</b>	New	H12	2006	GB	Quadrat survey/20 sites	J. Carroll & S. Caporn, MMU	TU report, Caporn <i>et al.</i> , (2007) JNCC report, Stevens <i>et al.</i> , (2009)

Table 4: Published data by habitat type reviewed in this report

Habitats	New analysis here or Reviewed	Survey date	Location	Method of data collection	Author /affiliation	Reference
<b>Acid grassland</b>	Reviewed	2002-3	GB	Field survey	Stevens <i>et al.</i>	Stevens <i>et al.</i> (2004, 2006)
<b>Acid grassland</b>	Reviewed	2007	Europe inc. GB	Field survey	BEGIN consortium	Stevens <i>et al.</i> (2010)
<b>Calcareous grassland</b>	Reviewed		GB	Field survey	L. Van den Berg	Van den Berg <i>et al.</i> (2010)
<b>Acid grassland Calcareous grassland Mesotrophic grassland Heathland</b>	Reviewed	1998	GB	Field survey	CEH Countryside Survey	Maskell <i>et al.</i> (2010); JNCC report Stevens <i>et al.</i> (2009)
<b>Acid grassland Calcareous grassland Heathland Bogs</b>	Reviewed	Range	-	Collation of archived field survey	Stevens & CEH	Stevens <i>et al.</i> (2011)
<b>Upland dry heath</b>	Reviewed	2005	England, Wales	Field survey	J. Edmondson, MMU	Edmondson, <i>et al.</i> (2010)

Table 5: Name, location, vegetation type, soil type, and background atmospheric N deposition rates for the 9 TU sites

	Site name	Location in UK	Vegetation type: NVC classification	Soil type	Approx N dep. at site (kg N ha <sup>-1</sup> yr <sup>-1</sup> )
Heath	Ruabon	North East Wales	Upland heath: H12 <i>Calluna –Vaccinium</i>	Peaty podzol	20
	Budworth	North west England	Lowland heath: H9 <i>Calluna –Deschampsia</i>	Humo ferric podzol	20
	Thursley	Southeast England	Lowland heath: H8 <i>Calluna</i>	Podsol, over lower greensand	10-15
	Culardoch	Northeast Scotland	Low Alpine Heath: H13 <i>Calluna-Cladonia</i>	Sub-alpine podsol	11
Bog	Whim	Southern Scotland	Ombrotrophic bog: M19, <i>Calluna-Eriophorum</i>	<i>Sphagnum</i> peat	8-10
Grassland	Pwllperian	Central Wales	Upland acid grassland U4	Shallow ferric stagnopodzol	25
	Wardlow	Central England	Acid grassland: U4e <i>Festuca-Agrostis-Galium</i>	Paleo-argillic	20-25
		Central England	Calcareous grassland: CG2d <i>Festuca –Avenula</i>	Rendzina	20-25
Sand dune	Newborough	Northwest Wales	Fixed sand dune grassland: SD8 <i>Festuca – Galium</i>	Rendzina	11

Table 6: Experimental site name and simulated N deposition treatments for the 9 TU sites. <sup>1</sup>= first experiment (treatments ceased in 1996 to follow recovery); <sup>2</sup>= ongoing experiment; <sup>4</sup> = plots where treatments are no longer ongoing. <sup>5</sup>=includes plots split in half with recovery since August 2005; <sup>6</sup>=includes plots split in half with recovery since spring 2003; <sup>9</sup>= a number of experiments have some plots where treatments have ceased in order to assess recovery.

	Site name	N treatment rates (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	N form (as solution unless stated)	Year started	Duration of N treatment years to date or until ceased <sup>9</sup>	Key references to experiment
Heath	Ruabon	0,40,80,120	NH <sub>4</sub> NO <sub>3</sub> solution	1989	22	Pilkington <i>et al.</i> (2005); Edmondson <i>et al.</i> (2010)
		0,10,20,40, 120		1998	13 <sup>6</sup>	
	Budworth	0,20,60,120	NH <sub>4</sub> NO <sub>3</sub> solution	1996	115	Wilson (2003), Field (2010) Lageard <i>et al.</i> (2005)
	Thursley	0, 7.7, 15.4 <sup>1</sup>  0, 30 <sup>2</sup>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1989- 1996 <sup>1</sup>  1998 <sup>2</sup>	7 <sup>1</sup>  12 <sup>2</sup>	Power <i>et al.</i> , 1998; Barker <i>et al.</i> , 2004; Power <i>et al.</i> , 2006 Barker, 2001, Green, 2005
Culardoch	0, 10, 20, 50	NH <sub>4</sub> NO <sub>3</sub>	2000	11	Britton and Fisher, 2007	
Bog	Whim	8,24,56 for wet dep.	NH <sub>4</sub> Cl NaNO <sub>3</sub>	2002	8	Sheppard <i>et al.</i> (2004). Sheppard <i>et al.</i> (2008)
		NH <sub>3</sub> transect 70- 4	NH <sub>3(g)</sub>	2002	8	
Grassland	Pwllperian	10, 20	NaNO <sub>3</sub> NH <sub>4</sub> SO <sub>4</sub>	1996	12	Emmett <i>et al.</i> (2007)
	Wardlow	35, 70, 140	NH <sub>4</sub> NO <sub>3</sub>	1990	12 <sup>4</sup>	Morecroft <i>et al.</i> (1994) Horswill <i>et al.</i> (2008)
		35, 140		1995	15 <sup>5</sup>	
	Wardlow	35, 70, 140	NH <sub>4</sub> NO <sub>3</sub>	1990 <sup>4</sup>	12 <sup>4</sup>	Morecroft <i>et al.</i> (1994) Horswill <i>et al.</i> (2008)
35, 140		1995 <sup>5</sup>		15 <sup>5</sup>		
Sand Dune	Newborough	7.5, 15	NH <sub>4</sub> NO <sub>3</sub>	2003	7	Plassmann <i>et al.</i> (2009)

## 4. Task 3: Identify the relevant response variables for each habitat

### 4.1 Introduction

This section of the report uses stepwise regression and ordination analysis to identify the key potential response variables in the survey datasets for each of the habitats studied. Stepwise regression considers overall changes in species richness and, where the data exist, by functional group. Ordination analysis considers changes in the composition of the vegetation community. Both analyses measure these changes relative to climatic and pollutant driver data.

Key potential response variables are also identified from published literature and from the Terrestrial Umbrella experiments.

### 4.2 Vegetation species richness responses to nitrogen pollution

Across all the datasets studied, increasing nitrogen deposition (total, reduced or oxidised) was correlated with reductions in species richness. This pattern was similar across all habitats. In many cases, climate was also correlated with species richness, with increasing species richness being a function of increasing precipitation and decreasing temperature (expressed as growing degree days or extreme temperature range). An exception to the latter was sand dunes, with  $\text{pH} \geq 6.5$  where increasing temperature was correlated with an increase in overall species richness. The output from the stepwise regressions are summarised in Table 7 and the relationship between nitrogen deposition and species richness presented in more detail in Task 4.

Consistency in survey methods and data collection in habitats visited as part of the TU 2009 survey enabled a direct comparison across all habitats (upland and lowland heath, sand dune, bog and acid grasslands – the latter representing a subset of 23 of the sites visited by Stevens *et al.* (2004)). For this cross habitat comparison, species richness was converted and expressed as a percentage of the maximum number of species recorded in that habitat. From this stepwise regression, nitrogen deposition explained most of the reduction in species richness (expressed as either total nitrogen deposition or dry-oxidised nitrogen deposition), followed by mean annual temperature.

Many plant groups were negatively associated with N pollution: within bogs the relationship was strongest in forbs including *Drosera rotundifolia* and *Narthcium ossifragum* and lichens; in upland heaths mosses and lichens reduced in diversity, although the change in the former was more significantly correlated with sulphur deposition; and in acid grasslands forbs showed a reduction in both richness and diversity (as previously reported in Stevens *et al.*, 2006). For lowland heaths, wet-oxidised N deposition was significantly correlated with reductions in overall species richness however, climate explained more of the variation in species richness across the plant groups. This probably reflects the shift in lowland heath soil types as their geographical location changed from acid, base-poor, sandy soils of the Cornish heaths to the moister, more organic soils of the northern lowland heaths. Interestingly, across the ericaceous habitats which are often defined by a competitive balance between shrub, graminoid and moss species groups, graminoid species richness also fell as a function of rising N deposition. However, graminoid cover increased, suggesting a shift toward dominance by fewer species.

In sand dunes, moss species richness showed a strong reduction with increasing N deposition. Forb species richness was more weakly correlated with N pollution. However,

when sand dune type was split by pH these responses were only seen in more calcareous sand dunes with pH  $\geq$  6.5, although limited data were available from sites with pH less than 6.5. In general, sand dune species richness was more strongly correlated to pH and the extent of decalcification. Some responses were, however, seen with soil N indicators in sand dunes (not shown in this report) such as N% and mineralisation at sites with pH lower than 6.5; this could indicate a longer-term response to N deposition.

The different forms of N pollution were also related to responses in species richness. However, it is difficult using modelled data to attribute change to a specific form of N pollution, and specific locations may be more vulnerable to either reduced or oxidised N dependent upon their proximity to a point source. In some cases, for example upland heath moss species richness and bog species richness, sulphur deposition was more strongly correlated with the species richness. It is difficult to be certain if these relationships are ecologically significant as sulphur levels are low across the range of the dataset, or indicative of a legacy effect from earlier years of high sulphur deposition. In general, significant correlations between pollutants exist and in both of these specific cases a form of N was also strongly correlated with the response variable and at levels more likely to elicit an ecological response.

Table 7: Summary of changes in overall species richness and the response of different functional groups (richness and cover where appropriate) using stepwise regression

Habitat /Survey	Response variable	Best fit model parameters from stepwise regression and influence of an increase in the parameter on the response variable ( $\uparrow\downarrow$ )	Variance explained by model and statistical significance
<b>All habitats from TU 2009 survey</b>	Overall species richness (% of maximum species recorded in each habitat)	Dry-oxidised nitrogen deposition ( $\downarrow$ ) Growing degree days ( $\downarrow$ )	$R^2=0.37$ , $P<0.001$
<b>Upland heathland (TU 2009)</b>	Overall species richness (total number of species recorded)	Reduced nitrogen deposition ( $\downarrow$ )	$R^2=0.39$ , $P=0.002$
	Moss species richness	Sulphur deposition ( $\downarrow$ ) (Wet-oxidised Nitrogen deposition also significant : $\downarrow$ )	$R^2=0.25$ , $P=0.011$ ( $R^2=0.21$ , $P=0.021$ )
	Lichen species richness	Reduced nitrogen deposition ( $\downarrow$ )	$R^2=0.26$ , $P<0.01$
	Graminoid species richness	Dry-reduced nitrogen deposition ( $\downarrow$ ) Altitude ( $\downarrow$ )	$R^2=0.46$ , $P<0.001$
	Graminoid cover (%)	Dry-reduced nitrogen deposition ( $\uparrow$ )	$R^2=0.24$ , $P=0.014$
<b>Upland heathland (MRS)</b>	Overall species richness (total number of species recorded)	Dry-reduced nitrogen deposition ( $\downarrow$ ) Altitude ( $\uparrow$ ) Temperature extreme range ( $\uparrow$ )	$R^2=0.87$ , $P=0.001$
<b>Lowland heathland (TU 2009)</b>	Overall species richness (total number of species recorded)	Growing degree days ( $\downarrow$ ) Altitude ( $\downarrow$ ) Wet-oxidised nitrogen deposition ( $\downarrow$ )	$R^2=0.64$ , $P<0.001$
	Moss species richness	Growing degree days ( $\downarrow$ ) pH ( $\uparrow$ )	$R^2=0.42$ , $P=0.005$
	Lichen species richness	no combination of variables explain significant variation in the data	-
	Graminoid species richness	Growing degree days ( $\downarrow$ )	$R^2=0.46$ , $P<0.001$
	Graminoid cover (%)	Dry-reduced nitrogen deposition ( $\uparrow$ )	$R^2=0.35$ , $P=0.001$

Habitat /Survey	Response variable	Best fit model parameters from stepwise regression and influence of an increase in the parameter on the response variable (↑↓)	Variance explained by model and statistical significance
<b>Bog (TU 2009)</b>	Overall species richness (total number of species recorded)	Dry-sulphur deposition (↓) (Dry-oxidised Nitrogen deposition also significant : ↓)	R <sup>2</sup> =0.56, P=0.01 (R <sup>2</sup> =0.50, P=0.01)
	Moss species richness	no combination of variables explain significant variation in the data	-
	Lichen species richness	Dry-oxidised nitrogen deposition (↓)	R <sup>2</sup> =0.37, P<0.01
	Forb species richness	Total acid deposition (↓) (Nitrogen deposition also significant : ↓)	R <sup>2</sup> =0.39, P=0.002 (R <sup>2</sup> =0.38, P=0.002)
	Graminoid cover (%)	Wet-reduced nitrogen deposition (↑) Growing degree days (↑)	R <sup>2</sup> =0.68, P<0.001
<b>Sand dunes TU 2009 (all sites)</b>	Overall species richness (total number of species recorded)	pH (↑) Wet-oxidised nitrogen deposition (↓)	R <sup>2</sup> =0.57, P<0.005
	Moss species richness	oxidised nitrogen deposition (↓) LOI (↑)	R <sup>2</sup> =0.67, P<0.001
	Forb species richness	pH (↑) Wet-oxidised nitrogen deposition (↓) Wet-sulphur deposition (↓)	R <sup>2</sup> =0.53, P<0.001
<b>Sand dunes pH &lt;6.5 (TU 2009)</b>	Overall species richness (total number of species recorded)	no significant relationship with N	-
	Moss species richness	wet-sulphur deposition (↓)	
<b>Sand dunes pH ≥6.5 (TU 2009)</b>	Overall species richness (total number of species recorded)	Oxidised nitrogen deposition (↓) Ca + Mg deposition (↑) Growing degree days (↑)	R <sup>2</sup> =0.76, P<0.001
	Moss species richness	Oxidised nitrogen deposition (↓)	R <sup>2</sup> =0.62, P<0.001
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>	Overall species richness (total number of species recorded)	pH (↑) Nitrogen deposition (↓)	R <sup>2</sup> =0.55, P<0.001
	Moss species richness	Total acid deposition (↓)*	R <sup>2</sup> =0.31, P=0.001
<b>Acid grasslands (BEGIN UK)</b>	Overall species richness (total number of species recorded)	Nitrogen deposition (↓) Precipitation (↑)	R <sup>2</sup> =0.38, P=0.001
	Forb species richness	Nitrogen deposition (↓)	R <sup>2</sup> =0.48, P<0.001

\*Total acid deposition incorporates both nitrogen and sulphur deposition as a total acid equivalent

Bryophyte species richness reduced in lowland heaths, sand dunes and the upland heath MRS survey – the latter included liverworts whereas the TU upland heath survey did not. Lichen species richness reduced in bogs and upland heaths and forb species richness reduced across acid grasslands, bogs and sand dunes. Graminoid species richness reduced in both heathland types whilst graminoid cover increased in all ecosystems except acid grasslands. However, within sand dunes, whilst this increase was significant, graminoid cover was more strongly associated with soil pH which could reflect either an interaction with precipitation and decalcification or acidification caused by pollutant deposition.

### 4.3 Vegetation community composition responses to nitrogen pollution

Table 8 presents the results from the ordination analysis. Vegetation community composition was also related to nitrogen (N), with a form N of nitrogen the first variable through the forward selection process in all habitats except sand dunes. In sand dunes, change was more strongly associated with climate (either growing degree days or precipitation) and the effect of rainfall on leaching of base cations, decalcification and acidification. Climate explained significant additional variation in the all the habitats after N pollution. In bogs, hydrology (typically influenced by management and drainage rather than rainfall) also was significantly related to change in species composition. Similarly, in upland heaths the amount of soil organic matter (LOI – loss on ignition) was related to community composition as was pH in lowland heaths: both these responses are indicative of changes in the soil from organic and peaty to calcareous and, at a broad-scale, driven by rainfall and climate.

The percentage of variance explained by these models is often low. Many factors drive variation within different habitats other than the ones chosen for this analysis and there is considerable heterogeneity between sites and also within a site. The fact that the relatively small number of environmental drivers explains as much variance as they do is testament to their strength as drivers of change in species composition.

Table 8: Summary of the analysis of species community composition using ordination (RDA) in CANOCO. ns = not significant

Habitat /Survey	Statistically significant drivers of change in species composition	Variance explained by model and statistical significance	Variance partitioning by driver*	Specific species showing a strong response to Nitrogen with good distribution across dataset (direction ↑↓)
<b>Upland heathland (TU 2009)</b>	Reduced nitrogen deposition	36.3%	15.8% P=0.001	<i>Cladonia fimbriata</i> (↑) <i>Cladonia portentosa</i> (↓) <i>Deschampsia flexuosa</i> (↑)
	Growing degree days		7.9% P=0.006	<i>Brachythecium rutabulum</i> (↑) <i>Hylocomium splendens</i> (↓)
	Loss on ignition		7.4% P=0.01	
<b>Upland heathland (MRS 2006)</b>	Dry-oxidised nitrogen deposition	87.8%	37.7% P=0.001	<i>Campylopus introflexus</i> (↑) <i>Hylocomium splendens</i> (↓)
	Reduced nitrogen deposition		22.1% P=0.001	
	Consecutive dry days		15.1% P=0.02	
	Habitat		12.9% P=0.033	
<b>Lowland heathland (TU 2009)</b>	Dry-oxidised nitrogen deposition	32%	8.2% P=0.005	<i>Cladonia fimbriata</i> (↑) <i>Cladonia portentosa</i> (↓) <i>Brachythecium rutabulum</i> (↑)
	Growing degree days		13.3% P=0.001	<i>Campylopus introflexus</i> (↑) <i>Hylocomium splendens</i> (↓)
	Soil pH		6.5% P=0.019	



Habitat /Survey	Statistically significant drivers of change in species composition	Variance explained by model and statistical significance	Variance partitioning by driver*	Specific species showing a strong response to Nitrogen with good distribution across dataset (direction ↑↓)
<b>Bog (TU 2009)</b>	Dry-reduced nitrogen deposition	22.6%	7.0% P=0.004	<i>Eriophorum vaginatum</i> (↑) <i>Sphagnum fimbriatum</i> (↑) <i>Cladonia portentosa</i> (↓)
	Hydrological index		8.3% P=0.001	
	Dry-oxidised nitrogen deposition		5.9% P=0.011	
<b>Sand dunes TU 2009 (all sites)</b>	Growing degree days	36.6%	5.6% P=0.03	<i>Hylocomium splendens</i> (↓) <i>Ammophila arenaria</i> (↓)
	Precipitation		10.6% P=0.001	
	Dry-reduced nitrogen deposition		5.1% P=0.035	
	pH		14.6% P=0.001	
<b>Sand dunes pH &lt;6.5 (TU 2009)</b>	Growing degree days	38.6%	18.8% P=0.035	<i>Hylocomium splendens</i> (↓)
	Dry-oxidised nitrogen deposition		22.0% P=0.008	
<b>Sand dunes pH ≥6.5 (TU 2009)</b>	Precipitation	29.3%	17.1% P=0.001	<i>Hylocomium splendens</i> (↓)
	Dry-reduced nitrogen deposition		9.5% P=0.025	
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>	Precipitation	28.1%	4.9% P=0.06	<i>Hylocomium splendens</i> (↓)
	Ca+Mg deposition		3.2% ns	<i>Carex arenaria</i> (↑ but ns). The relationship is significant when the 2002 data is analysed independently.
	Dry-sulphur deposition		3% ns	
	Dry-oxidised nitrogen deposition		3% ns	
	pH		8.2% P=0.006	
<b>Acid grasslands (BEGIN UK)</b>	Nitrogen deposition	15.5%	3.1% P=0.002	<i>Deschampsia flexuosa</i> (↑) <i>Hypnum cupressiforme</i> (agg.) (↑)
	Growing degree days		3.8% P=0.001	<i>Nardus stricta</i> (↑) <i>Carex panicea</i> (↑)
	Precipitation		2.8% P=0.001	<i>Euphrasia officianlis</i> (↓) <i>Hylocomium splendens</i> (↓)
	Ca+Mg deposition		2.1% P=0.02	<i>Lotus corniculatus</i> (↓)

\*the sum of the variance explained by individual drivers will not always equal the total variance explained due to the use of covariables in the variance partitioning process

The species richness relationships detailed in the previous section were also largely reflected in community composition, for example, see Figure 5 which illustrates the response of sensitive lichen species in the upland heath habitat to N deposition: in this case reduced N deposition was the most strongly correlated variable (N.B. all species were included in the ordination but only lichen species are shown on the plot, for other ordination plots refer to appendix 1). The ordination process also suggested individual species that appeared strongly associated, either positively or negatively, with N and specific species for each habitat are suggested in Table 8. For example, in Figure 5, *Cladonia fimbriata* (cladfima) is the only species positively associated with N deposition while other lichen species are associated with low N conditions.

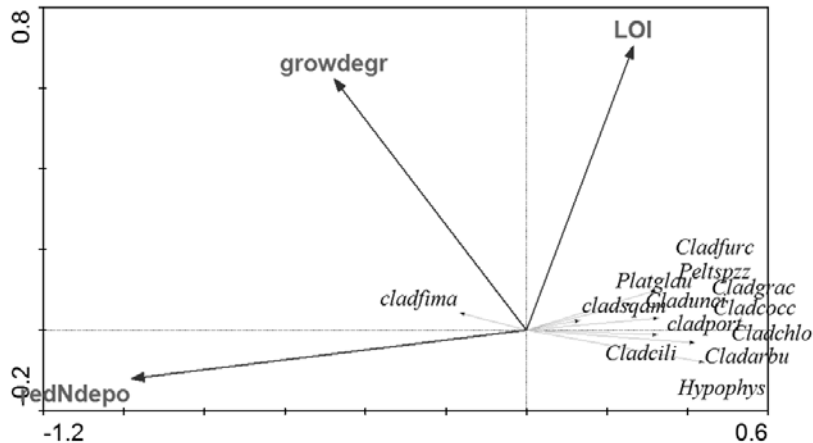


Figure 5: Ordination plot produced from RDA of upland heath data (TU 2009), lichen species only shown

In the example ordination plot above, many lichen species are ordinated away from N. This reflects reductions in lichen species richness as a function of N deposition, however, few lichen species are represented consistently across the dataset to enable a relationship with N to be examined with any statistical confidence. *Cladonia fimbriata* and *Cladonia portentosa* are two widely-represented lichen species, and these relationships alongside the other species shown in Table 8 are examined in greater detail in Task 4.

Typically, the strongest species related to N within the survey were mosses and lichens, showing both positive and negative relationships to N. Across the heathland, acid grassland, and sand dune datasets, the moss *Hylocomium splendens* showed a consistent negative response to N. The MRS survey of upland heaths also recorded presence of liverworts and these too were strongly sensitive to pollution. Within upland heath and bog habitats the graminoid species *Deschampsia flexuosa* and *Eriophorum vaginatum* showed positive responses to N deposition. In the 2009 sand dune survey, the grass *Ammophila arenaria* (marram grass) decreased in cover with increasing N deposition, however, this is at odds with findings from the 2002 survey where an increase in *A. arenaria* cover was found (Jones *et al.*, 2004). *Carex arenaria* showed an increase with N, although this was non-significant. This difference in response may be related to the differing types of sand dune surveyed between the surveys, since the 2009 survey focused on older de-calcified habitats where *Ammophila* persists only at low cover with low vigour, and is termed 'relict *Ammophila*'. Within the acid grassland habitat, forb species were strongly negatively correlated to N deposition, most notably *Lotus corniculatus* and *Euphrasia officianlis*.

Moss, lichen and forb species are an important component of the biodiversity within all the habitats studied, and key to maintaining high species richness and favourable habitat condition. Within the nature conservation agencies Common Standards Monitoring is a tool used to assess the condition of the habitat or feature. In heathlands, bryophytes and lichens play an important role in the overall habitat and are indicators of favourable condition in Common Standards Monitoring (CSM) (JNCC, 2006). The balance between graminoid and shrub cover is important in maintaining the intrinsic diversity in ericaceous habitats. In some cases, high graminoid cover may be detrimental to site integrity, for example, *Deschampsia flexuosa* cover above 25% in lowland heaths (JNCC, 2004). In two of the lowland heaths surveyed graminoid cover exceeded 30% and in several upland heaths graminoid cover was above 25 % suggesting that N deposition posed a long-term threat to site integrity.

## 4.4 Key response variables in the reviewed literature

Numerous vegetation categories have been described in the literature, such as individual species, botanical groups (grasses, bryophytes etc), functional groups (e.g. Ellenberg score) and plant characteristics (e.g. canopy height). The main ones relevant to N pollution response are listed in Table 9, while their relationships with nitrogen deposition are discussed under Task 4.

Table 9: Response variables frequently reported in the literature on vegetation and N deposition

Habitats	Reported response variable	Reference
<b>Upland dry heath</b>	Bryophytes richness	Edmondson <i>et al.</i> (2010)
<b>Upland dry heath</b>	Bryophytes richness, total spp. richness, individual species	Stevens <i>et al.</i> (2009) - pooled data of Edmondson <i>et al.</i> (2010) and Payne <i>et al.</i> 2014)
<b>Acid grassland</b>	Forbs, grass, bryophyte richness and cover, grass/forb ratio  Individual species	Stevens <i>et al.</i> (2004, 2006, 2009)
<b>Acid grassland (includes European sites)</b>	Forbs, grass, bryophyte richness	Stevens <i>et al.</i> (2010)
<b>Calcareous grassland</b>	Species richness & diversity  Functional groups species richness, Ellenberg N & R, individual species including (rare & scarce) species	Van den Berg <i>et al.</i> (2010)
<b>Acid grassland Calcareous grassland Mesotrophic grassland Heathland</b>	Vascular, bryophyte species richness	Maskell <i>et al.</i> (2010)
<b>Acid grassland</b>	Acid preference index  Ellenberg N & R  Competitive, stress tolerant, ruderal strategy	Stevens <i>et al.</i> (2010)
<b>Acid grassland Calcareous grassland Heathland Bogs</b>	Ellenberg N & R, canopy height, specific leaf area, species richness, individual species occurrence	Stevens <i>et al.</i> (2011)

## 4.5 Key response variables in the experimental site data

In the Terrestrial Umbrella (TU) experiments, vegetation and soils have been subjected to detailed study over many years, and several variables, both ecological and biogeochemical, were found to respond to additions of nitrogen. In relation to this report, the key response variables of interest considered were: changes in presence and abundance of individual species, botanical groups (vascular plants, bryophytes and lichens); changes in visible plant injury due to stress (winter damage, heather beetle); and changes in plant and soil chemistry (with potential consequences for nutrient imbalance, soil leaching and pH).

## 4.6 Conclusion and summary of response variables taken forwards to task 4

The results from the survey datasets strongly support the findings from the literature review. Increasing nitrogen deposition is strongly associated with both detrimental changes in species composition and reductions in species richness. In some cases a specific form of N was more strongly associated with a response however, to enable comparison with critical loads, total N deposition is used in further analysis. This will not affect the overall relationship as modelled total nitrogen deposition was strongly correlated with both modelled reduced and oxidised N deposition over the data used ( $R^2=0.89$  and  $0.72$  respectively, both  $P<0.01$ ). The nitrogen addition experiments provide data to support the hypothesis that nitrogen pollution, in the absence of change in other environmental variables, has a direct adverse effect on community composition in many different types of vegetation. Important changes in habitats were seen especially regarding the abundance of sensitive species and some of these are described under Task 4.

Table 10 below summarises the response variables that are strongly associated with N deposition for each habitat, these are further analysed in Task 4. The consistency shown between the new research discussed here and the published data is remarkable and reflects the strength of N as a driver of change within the UK's semi-natural ecosystems. The responses to N that are found are in addition to those explained by a climatic gradient and, in many instances, N deposition is statistically the strongest driver of change.

Table 10: Summary of the strongest response variables found during the statistical analysis that will be carried forward to Task 4. '#' indicates found within vegetation datasets analysed as part of this report, '\$' indicates found within the literature reviewed as part of this report.

Response variable	Acid grassland	Bog	Upland heath	Lowland heath	Sand dune
Species composition	#\$	#\$	#\$	#\$	#
Total species richness	#	#\$	#\$	#\$	#
Bryophyte species richness	\$		#\$	#	#
Lichen species richness		#	#		
Forb species richness	#\$	#			#
Graminoid species richness			#	#	
Graminoid cover	\$	#	#	#	#

In some cases, for example lowland heath mosses or lichens, N deposition did not emerge from the stepwise regression as a potentially significant driver of change in species richness. However, in these cases N deposition was associated with changes in species composition in the ordination analysis, with individual moss, lichen, forb and graminoid species strongly associated with changes in a form of N deposition.

Changes in certain individual species were related to changes in N deposition and these are summarised in Table 11 overleaf. Many more species appeared to show some relationship to N but, low frequency in the dataset meant that this was not significant. However, their response does contribute to changes in overall species richness and the species richness of functional groups.

Table 11: Summary of individual species that showed a strong response to N in the ordination analysis. The nature of these relationships with N deposition is examined further under task 4.

<b>Habitat</b>	<b>Species with strong response (direction of response)</b>
<b>Upland heath (TU &amp; MRS)</b>	<i>Cladonia fimbriata</i> (↑) <i>Cladonia portentosa</i> (↓) <i>Deschampsia flexuosa</i> (↑) <i>Brachythecium rutabulum</i> (↑) <i>Hylocomium splendens</i> (↓) <i>Campylopus introflexus</i> (↑)
<b>Lowland heath</b>	<i>Cladonia fimbriata</i> (↑) <i>Cladonia portentosa</i> (↓) <i>Brachythecium rutabulum</i> (↑) <i>Campylopus introflexus</i> (↑) <i>Hylocomium splendens</i> (↓)
<b>Acid grassland</b>	<i>Deschampsia flexuosa</i> (↑) <i>Hypnum cupressiforme</i> (agg.)(↑) <i>Nardus stricta</i> (↑) <i>Carex panicea</i> (↑) <i>Euphrasia officianlis</i> (↓) <i>Hylocomium splendens</i> (↓) <i>Lotus corniculatus</i> (↓)
<b>Bog</b>	<i>Eriophorum vaginatum</i> (↑) <i>Sphagnum fimbriatum</i> (↑) <i>Cladonia portentosa</i> (↓)
<b>Sand dune</b>	<i>Hylocomium splendens</i> (↓) <i>Ammophila arenaria</i> (↓)

## 5. Task 4: Determine the relationship between N deposition and the key response variables

### 5.1 Introduction

The relationships between N deposition and the key response variables determined in Task 3 are examined in more detail here. This task presents results over 6 main sections: 1) those from the gradient surveys analysed in this report; 2) supporting evidence of change from the dose response experiments; 3) evidence from the literature; 4) a review of the relationships between N and the response variables found in the JNCC collation report (Stevens *et al.*, 2011); 5) the use of cluster analysis to determine if any relationship exists between species composition and nitrogen deposition in the survey datasets and the possible presence of threshold responses and 6) Concluding comments.

The relationships examined in this chapter use modelled annual N deposition as the pollutant variables, however, the relationships studied between N deposition and species richness and presence have developed over many years. The current rate of N deposition is primarily a proxy for long-term cumulative N deposition. Thus we would not expect that a change in N deposition, either increasing or decreasing, would immediately change species richness or composition, however, long-term changes in N deposition are likely to affect species richness and composition. Furthermore, different species groups will respond in different ways: bryophytes and lichens with no root structure are likely to respond quicker than vascular plants and the responses of both may be affected by management interaction which could alter inter-species composition. Refer to the methods section for further information.

### 5.2 Relationships determined in the gradient surveys

The nature and strength of the relationships between N deposition and total species richness, functional group richness and plant cover, where significant, are summarised in Table 12 and the relationships between N and individual species cover or presence are presented in Table 13. Regression curves for each of these relationships are provided in Appendix 2 for total and functional group species richness and Appendix 3 for the individual species responses, however, the broad relationship between N and the percentage of the maximum number of species within habitats with comparable survey techniques (TU 2009 upland heath, lowland heath, bog, sand dune and subset of BEGIN/Stevens *et al.* (2004) grasslands) is shown in Figure 6.

Across the 135 survey sites represented in this plot, a highly significant pattern of species richness reduction as a function of increasing atmospheric nitrogen deposition is apparent with a wedge shaped response. The pattern indicates that at low N deposition the species number can be both high and low, but at high N deposition the species number is always low. The large scatter in the data is related to the variation between- as well as within- habitats. Within this overall dataset, a negative-linear relationship best describes the response, however, within each habitat and functional group a negative, curvi-linear relationship is more common, indicating a more rapid loss of species associated with increasing N deposition at

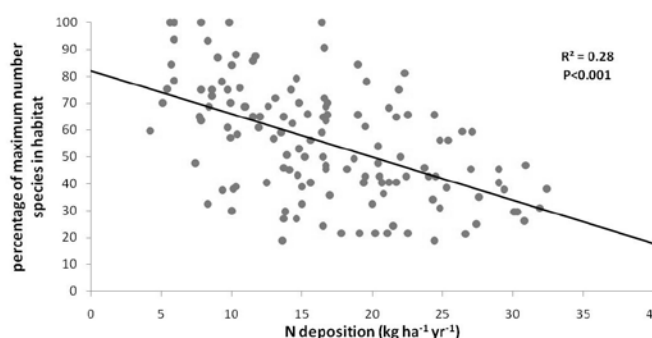


Figure 6: Change in % of maximum species richness as N deposition increases at 135 sites studied in the TU 2009 multi-habitat survey

lower levels of N pollution. Species richness curves from the TU sand dune (all pH) and TU upland heath surveys and are shown in Figure 7 below, and a complete set of response curves provided in Appendix 2. A curvi-linear response suggests that less-polluted sites are more sensitive to increases in N deposition and that at sites already receiving high levels of pollution, much species diversity has already been lost. In all habitats, the magnitude of the response is large, with 50-75% fewer species in the least diverse sites within each habitat, compared with the most diverse.

Loss of species richness related to increases in N deposition is consistent across all the habitats and functional groups, with the exception of sand dunes with a pH less than 6.5, where limited data and strong climatic effects occurred. Whilst graminoid species richness also declined, graminoid cover increased, and this relationship was also curvilinear, indicating more rapidly increasing cover of fewer graminoid species as N deposition increased. In this respect, the potential for adverse change in each habitat increased at locations with a higher background N deposition.

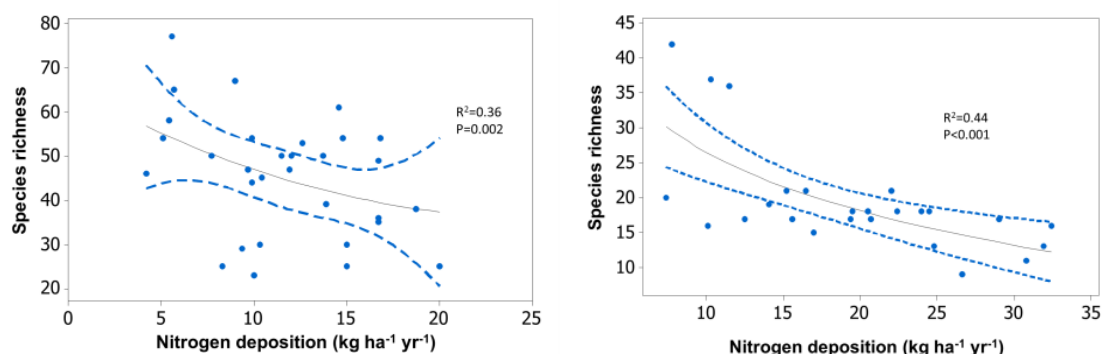


Figure 7: Measured species richness within a) TU 2009 survey sand dunes (all pH) and b) TU survey upland heaths as nitrogen deposition increases

To further examine the range over which the response variables from Task 3 show the most rapid change, each variable was analysed using LOESS regression. Summary data from these analyses are presented in Tables 12 and 13 and example regression curves for TU Sand dune (all pH) and TU Upland heath surveys are shown in Figure 8.

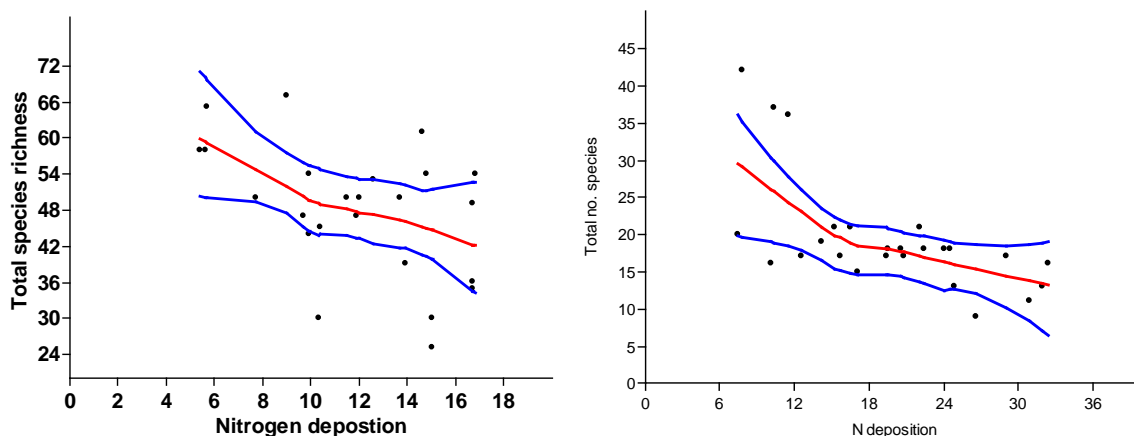


Figure 8: LOESS regression curves showing change in species richness as nitrogen deposition increases for a) TU 2009 survey sand dunes (all pH) and b) TU survey upland heaths. Best fit to data line in red, 95% confidence limits shown in blue and fitted by bootstrapping.

Although data in most habitats is limited to around 25 sites, clear response points were usually found, and these mostly supported the curvilinear relationships from the linear regressions. In many cases the inflection was at an N deposition level typically between 17 and 22 kg N ha<sup>-1</sup> yr<sup>-1</sup> for many negative and positive responses to N. The general exception to this was *Cladonia fimbriata* cover which showed a ‘humpback’ uni-modal response in upland (16-25 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and lowland heaths (17-28 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

Table 12: Summary of relationship type and direction between modelled nitrogen deposition ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) and species richness/cover for each habitat. Relationship equations shown:  $y$ =species richness;  $x$ =nitrogen deposition ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ). LOESS regression range highlights the range over which the species response is the most responsive, a dash indicates no tipping point was apparent.

Habitat/ Survey	Response variable	Direction of response to N	Relationship type	Statistical significance	Relationship equation	LOESS regression range of max. loss or gain
<b>All habitats (from TU 2009)</b>						
Total species richness	negative		linear	$R^2=0.28$ $P<0.001$	$y=1.56*x + 82.9$	n/a
<b>Upland heathland (TU 2009)</b>						
Total species richness	negative		mild curvilinear	$R^2=0.44$ $P<0.001$	$y=54.37 - 12.11*\ln(x)$	7-17 kg N
Moss species richness	negative		curvilinear	ns	-	-
Lichen species richness	negative		linear	$R^2=0.23$ $P=0.015$	$y=11.34 - 3.18*\ln(x)$	7-16 kg N
Graminoid species richness	negative		mild curvilinear	$R^2=0.27$ $P<0.01$	$y=9.62 - 2.28*\ln(x)$	7-22 kg N
Graminoid cover	positive		mild curvilinear	$R^2=0.31$ $P=0.017$	$y=0.042x^2 - 0.88x + 9.19$	>22 kg N
<b>Upland heathland (MRS)</b>						
Total species richness	negative		mild curvilinear	$R^2=0.61$ $P<0.001$	$y = 0.011*x^2 - 0.709*x + 19.8$	20 kg N
<b>Lowland heathland (TU 2009)</b>						
Total species richness	negative		mild curvilinear	$R^2=0.32$ $P=0.002$	$y=-11.25*\ln(x) + 47.27$	< 17 kg N
Moss species richness	negative		mild curvilinear	$R^2=0.15$ $P<0.05$	$y = -3.29*\ln(x) + 14.685$	-
Graminoid species richness	negative		mild curvilinear	$R^2=0.26$ $P<0.01$	$y=0.16+38.99/x$	-
Graminoid cover	positive		mild curvilinear	$R^2=0.25$ $P<0.05$	$y=8.45 - 1.15*x+0.05x^2$	> 23 kg N
<b>Bog (TU 2009)</b>						
Total species richness	negative		linear	$R^2=0.23$ $P=0.009$	$y=27.9 - 0.30*x$	> 19 kg N
Lichen species richness	negative		linear	$R^2=0.19$ $P=0.018$	$y= 4.79 - 0.13*x$	-
Forb species richness	negative		mild curvilinear	$R^2=0.46$ $P<0.001$	$y = 8.07 - 2.33*\ln(x)$	-
Graminoid cover	linear		linear	$R^2=0.27$ $P=0.004$	$y=1.35*x + 17.4$	-
<b>Sand dunes TU 2009 (all sites)</b>						
Total species richness	negative		mild curvilinear	$R^2=0.36$ $P=0.002$	$y=30.4 + 194.6/x$	< 10 kg N
Moss species richness	negative		strong curvilinear	$R^2=0.81$ $P<0.001$	$y= -1.3 + 84.4/x$	< 12 kg N
Graminoid cover	positive		mild curvilinear	$R^2=0.17$ $P<0.05$	$y=75.3 - 214.8/x$	< 10 kg N
Forb species richness	negative		mild curvilinear	$R^2=0.17$ $P<0.05$	$y=12.8 + 84.1/x$	-
<b>Sand dunes TU 2009 (pH &lt;6.5)</b>						
Total species richness	too few data points		-	-	-	-



Habitat/ Survey	Response variable	Direction of response to N	Relationship type	Statistical significance	Relationship equation	LOESS regression range of max. loss or gain
<b>Sand dunes TU 2009 (pH ≥6.5)</b>						
Total species richness	negative		mild curvilinear	R <sup>2</sup> =0.42 P=0.009	y=94.1- 16.8*ln(x)	-
Moss species richness	negative		mild curvilinear	R <sup>2</sup> =0.85 P<0.001	y= -1. + 86.1/x	
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>						
Total species richness	negative		mild curvilinear	R <sup>2</sup> =0.27 P<0.001	y=104.3- 22.6*ln(x)	-
Moss species richness	negative		mild curvilinear	R <sup>2</sup> =0.26 P=0.002	y=22.9- 6.98*ln(x)	
<b>Acid grasslands (BEGIN)</b>						
Total species richness	negative		mild curvilinear	R <sup>2</sup> =0.29 P<0.001	y = 0.0052x <sup>2</sup> - 0.68*x + 34.6	-
Forb species richness	negative		linear	R <sup>2</sup> =0.48 P<0.001	Y= 11.8 - 0.35*x	-

Some of the individual species also revealed an apparent threshold level above or below which the response was strong. Most notable were the rapid reduction in the probability of presence (number of quadrats in which a species was found) of *Hylocomium splendens* in both upland heath surveys and the sand dunes at N deposition above 20 kg N ha<sup>-1</sup> y<sup>-1</sup> and the rapid increase in *Brachythecium rutabulum* at a similar point. *H. splendens* is thought to be sensitive to N however, its absence should not lead to the assumption that a site is negatively affected by N as the moss generally only occurs at less-polluted sites that are also moist. Similarly, *B. rutabulum* exhibits a preference for moister sites. It is therefore important that other factors be considered when making judgement of a site's N-status by the presence or absence of a single species.

Table 13: Summary of key relationships between nitrogen deposition and the individual species in each habitat identified in Task 3. Type, direction, statistical significance and equation of curve shown.

Habitat/ Survey	Response variable	Direction of response to N	Relationship type	Statistical significance	Relationship equation
<b>Upland heathland (TU 2009)</b>					
	<i>Hylocomium splendens</i> cover (%)	negative	mild curvilinear	R <sup>2</sup> =0.16 P<0.05	y = 0.038x <sup>2</sup> - 2.37*x + 36.44
	<i>Hylocomium splendens</i> probability of presence	negative	threshold	ns	-
	<i>Cladonia portentosa</i> cover	negative	curvilinear	ns	-
	<i>Deschampsia flexuosa</i> cover	positive	mild curvilinear	R <sup>2</sup> =0.30 P=0.018	y = 0.04x <sup>2</sup> - 0.75*x + 6.52
	<i>Cladonia fimbriata</i> cover	positive	curvilinear	ns	-
	<i>Brachythecium</i> <i>rutabulum</i> cover	positive	curvilinear	ns	-
	<i>Brachythecium</i> <i>rutabulum</i> probability of presence	positive	threshold	ns	-
<b>Upland heathland (MRS)</b>					
	<i>Hylocomium splendens</i> presence (%)	negative	threshold	R <sup>2</sup> =0.65 P<0.001	y= -1.33 +39.74/x
	<i>Campylopus introflexus</i> presence (%)	positive	threshold	R <sup>2</sup> =0.39 P=0.01	y = 0.003x <sup>2</sup> - 0.05*x + 0.23

Habitat/ Survey	Response variable	Direction of response to N	Relationship type	Statistical significance	Relationship equation
<b>Lowland heathland (TU 2009)</b>					
	<i>Hylocomium splendens</i> cover (%)	negative	curvilinear	R <sup>2</sup> =0.44, P<0.001	y=6.21+ 119.51/x
	<i>Hylocomium splendens</i> probability of presence	negative	curvilinear	R <sup>2</sup> =0.35, P<0.001	y=6.69-2.14 *ln(x)
	<i>Cladonia portentosa</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.35, P<0.001	y=11.08 -3.60 * ln(x)
	<i>Cladonia portentosa</i> presence	negative	strong curvilinear	R <sup>2</sup> =0.37, P<0.001	y=8.72 -2.69 *ln(x)
	<i>Cladonia fimbriata</i> cover	positive	linear	ns	-
	<i>Cladonia fimbriata</i> presence	positive	linear	R <sup>2</sup> =0.14, <b>P=0.06</b>	-
	<i>Campylopus introflexus</i> cover	positive	curvilinear	ns	-
	<i>Brachythecium rutabulum</i> cover	positive	curvilinear	ns	-
	<i>Brachythecium rutabulum</i> presence	positive	strong curvilinear	R <sup>2</sup> =0.25, P<0.05	y=0.06 -0.014*x + 0.005*x <sup>2</sup>
<b>Bogs (TU 2009)</b>					
	<i>Cladonia portentosa</i> cover	negative	linear	R <sup>2</sup> =0.13, <b>P=0.055</b>	-
	<i>Cladonia uncialis</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.25, P=0.006	y=0.89 – 0.29*ln(x)
	<i>Cladonia uncialis</i> presence	negative	mild curvilinear	R <sup>2</sup> =0.53, P<0.001	y= -1.17 +24.52/x
	<i>Eriophorum vaginatum</i> cover	positive	linear	R <sup>2</sup> =0.41, P<0.001	y=1.48*x + 5.27
	<i>Sphagnum fimbriatum</i> cover	positive	mild curvilinear	R <sup>2</sup> =0.20, P=0.015	y= -0.58 + 0.24*ln(x)
	<i>Sphagnum fimbriatum</i> presence	positive	mild curvilinear	R <sup>2</sup> =0.21, P=0.013	y= -2.0 + 0.83*ln(x)
<b>Sand dunes (TU 2009 all sites)</b>					
	<i>Hylocomium splendens</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.21, P<0.01	y= -5.84 +106.88/x
<b>Acid Grasslands (BEGIN)</b>					
	<i>Hylocomium splendens</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.16, P=0.001	y= -1.01 +42.06/x
	<i>Hypnum cupressiforme</i> cover	positive	linear	R <sup>2</sup> =0.16, P<0.001	y = 0.19*x - 2.07
	<i>Nardus stricta</i> cover	positive	linear	R <sup>2</sup> =0.11, P=0.003	y = 0.30*x - 2.38
	<i>Carex panacea</i> cover	positive	linear	R <sup>2</sup> =0.08, P=0.014	y = 0.072*x – 0.88
	<i>Euphrasia officianlis</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.11, P=0.005	y= 1.83 - 0.54*ln(x)
	<i>Lotus corniculatus</i> cover	negative	mild curvilinear	R <sup>2</sup> =0.09, P=0.009	y= 3.58 – 1.03*ln(x)

## 5.3 Evidence from Dose–response relationships in the TU experiments

The UKREATE Terrestrial Umbrella (TU) project is funded by the Department for Environment Food and Rural Affairs (Defra) and the Natural Environment Research Council (NERC). The nine UKREATE sites (see Tables 5 and 6) are long term experiments in locations representing a broad range of priority UK habitats. Although established at different times over the past 22 years and involving different levels of nitrogen additions, the common features of their research design and monitoring enable consistent comparisons to be made among the sites. A recent overview is presented in RoTAP (2012), while the published sources of results from individual sites are given in Table 6 as well as in the report section on the UKREATE web site (<http://ukreate.defra.gov.uk/publications/reports/index.htm>).

### 5.3.1 Complementary nature of the Field surveys and the UKREATE experiments

The national-scale field surveys and the UKREATE experiments provide different, but complementary information. The changes observed in the spatial field surveys take place in the ‘real-world’ under normal timescales but may also be driven by climate, management, air pollution, soil chemistry and a host of other factors. Where air pollution, and in particular nitrogen deposition, is separated out as a main driver of change, the timescales of the influence of pollutants are very lengthy, possibly at least over the two centuries since the Industrial Revolution and periods of agricultural expansion but certainly over recent decades (Fowler *et al.*, 2004).

The UKREATE experiments are short by comparison and in most cases involve nitrogen additions that are beyond the normal range of current deposition starting from ambient loadings which are already around the critical load. However, the controlled experiments provide evidence for ecosystem responses that result directly from nitrogen addition since other factors (soils, climate management etc) are a constant. They can also reveal the potential for changes that may occur at higher levels of nitrogen deposition but are not yet detectable in the natural landscape. Furthermore, some of the UKREATE experiments include the cessation of treatments along with maintained monitoring in order to investigate the consequences of reduction of nitrogen inputs.

Within this report the results of the UKREATE experiments are used to provide evidence regarding the nature of changes in plant community composition and individual species in response to added nitrogen.

### 5.3.2 Responses of vegetation to nitrogen addition and recovery

Selected examples of responses of vegetation to nitrogen additions in the UKREATE experiments are presented below. A general outcome from the experiments to date is that bryophytes and lichens are strongly and negatively affected by the nitrogen additions, but that changes in the vascular flora are modest and have appeared more slowly (RoTAP, 2012; Phoenix *et al.*, 2012). One important exception is that both vascular species as well as lower plants in the Whim bog experiment have been greatly affected by gaseous ammonia treatments (Figure 9). The data from Whim bog show that responses in the most sensitive plants, in this case the bryophytes, are evident even at the lowest level of N addition i.e. 8 kg N ha<sup>-1</sup> y<sup>-1</sup> above a background of the same. At the higher end of the nitrogen addition range there is evidence of increasing damage to bryophytes at additions beyond the critical load range (for bogs 5-10 kg N ha<sup>-1</sup> y<sup>-1</sup>). In the gaseous NH<sub>3</sub> experiment, increased nitrogen also led to substantial community change with large increases in *Eriophorum* and loss of *Calluna* (Figure 9, top).

The sensitivity of bryophytes and lichens to wet deposited nitrogen treatments was also demonstrated at the original Ruabon upland heath (heather moorland) experiment (Carroll *et al.*, 1999). In the newer Ruabon experiment, which used a wider range of treatments, a gradual pattern of change in lichen cover (mainly *Cladonia portentosa*) was observed (Figure 10) which after 5 years showed sensitivity to just 10 kg N ha<sup>-1</sup>y<sup>-1</sup> above the ambient input of around 20 kg N ha<sup>-1</sup>y<sup>-1</sup>. This being the upper end of the critical load range for heathland (Pilkington *et al.*, 2007). At Ruabon the same experiment also found that certain liverworts were particularly sensitive to increasing nitrogen additions (Figure 11), and again their abundance continued to decline as loadings were raised above the heathland critical load (Edmondson, 2007).

The sensitivity of lichens to nitrogen pollution, a clear outcome of the JNCC collation report (Stevens *et al.*, 2011) was further confirmed in the experiment at Culardoch in the Cairngorms where lichens are an important part of the *Calluna-Cladonia* montane heath (Figure 12). Lichen cover was also very sensitive to nitrogen additions of just 7.7 kg N ha<sup>-1</sup> y<sup>-1</sup> (above a background deposition of around 8 kg N ha<sup>-1</sup> y<sup>-1</sup>) at Thursley Common (Figure 13) and here the effect still persisted at least 8 years after the nitrogen treatments had ceased (Power *et al.*, 2006).

The above examples from the UKREATE experiments are consistent with the overall results from the field surveys since they show that small, realistic nitrogen additions can have adverse effects on the abundance of sensitive vegetation, even where the site is already close to the critical load. Increasing nitrogen inputs beyond the critical load can also change the character of the community by increasing the cover of dominant graminoid species.

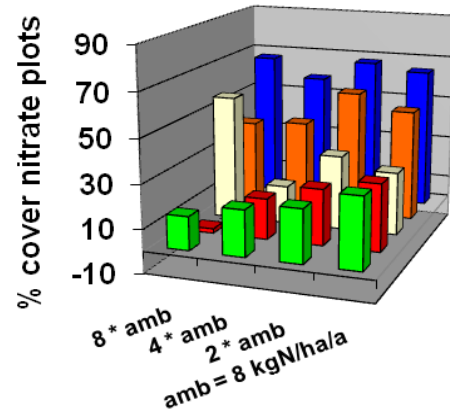
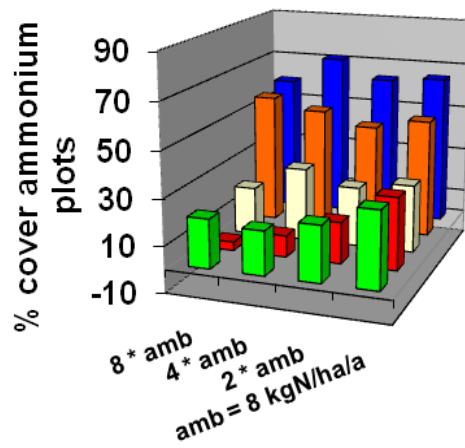
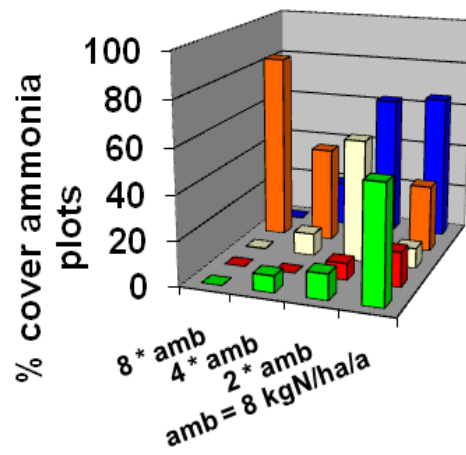


Figure 9: Response to different forms of nitrogen addition on Whim bog in 2009 after seven years treatment: ammonia gas (top), wet ammonium (middle), wet nitrate (lower) in different plants from front to back – *Sphagnum capillifolium*, *Pleurozium schreberi*, *Hypnum jutlandicum*, *Eriophorum vaginatum*, *Calluna vulgaris* (Sheppard, unpub). Ambient (amb) nitrogen deposition (approx. 8 kg N ha<sup>-1</sup> yr<sup>-1</sup>) at the right hand side of each graph, increasing N deposition towards the left.

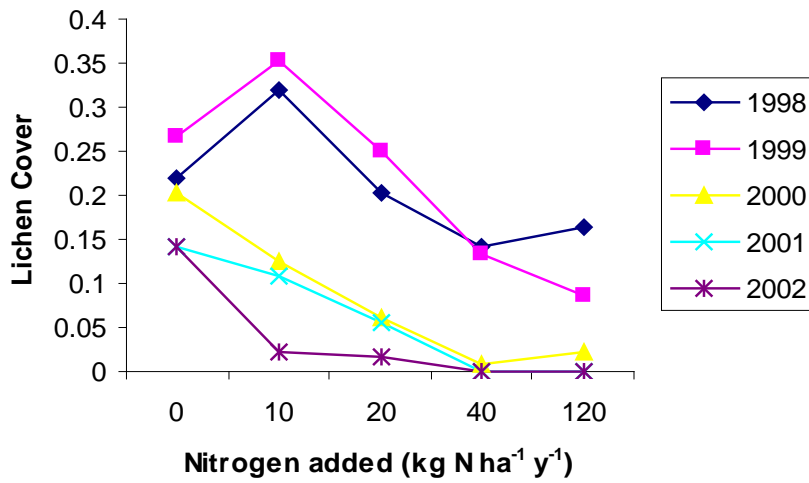


Figure 10: Nitrogen dose-response of lichen cover (mean touches/pin) at the Ruabon upland heath experiment new plots in the first 5 years of treatment. Ambient deposition circa 20 kg N ha<sup>-1</sup> yr<sup>-1</sup>. (Pilkington *et al.*, 2007).

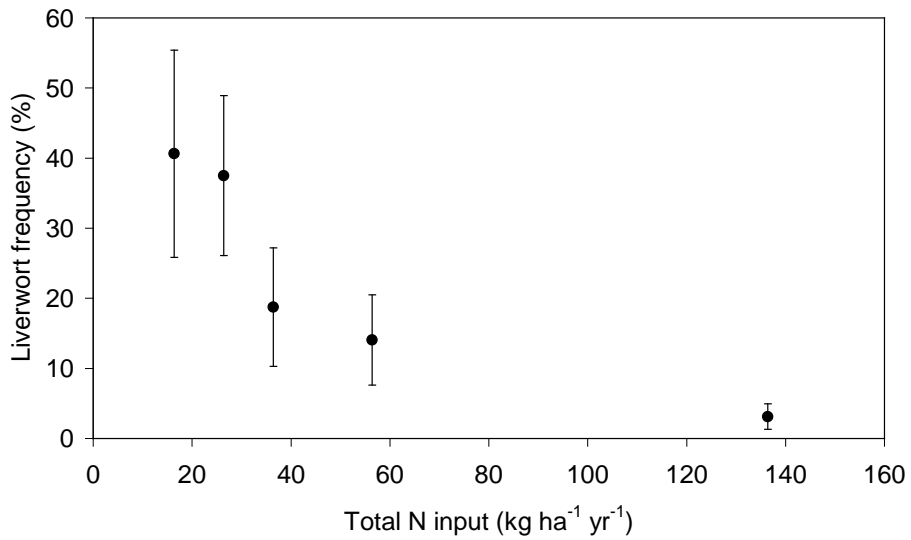


Figure 11: Relationship between total N input (N treatment + ambient deposition) and total liverwort frequency at the Ruabon upland heath experiment new plots. Ambient deposition circa 20 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Edmondson, 2007).

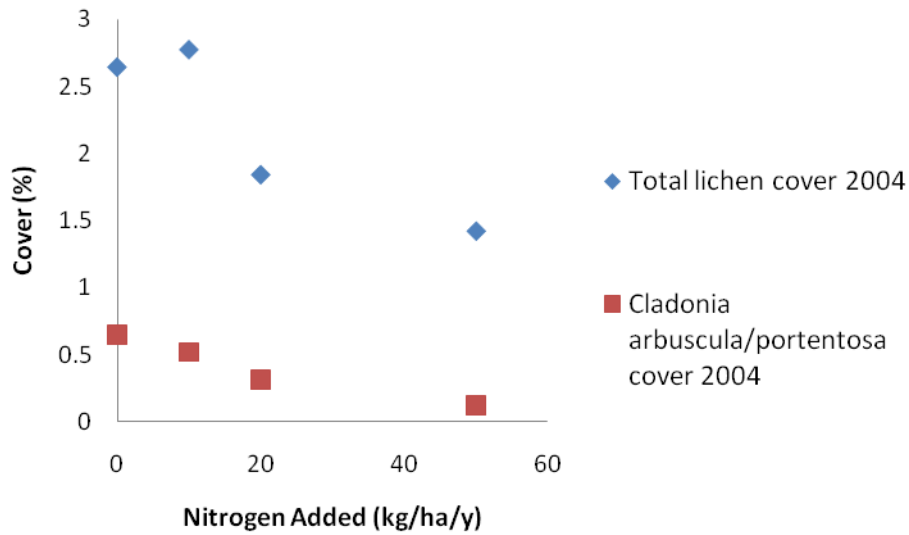


Figure 12: Response of lichens to nitrogen addition in montane heath at Culardocho in the Cairngorms (Britton & Fisher, 2007). Background N deposition circa  $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ .

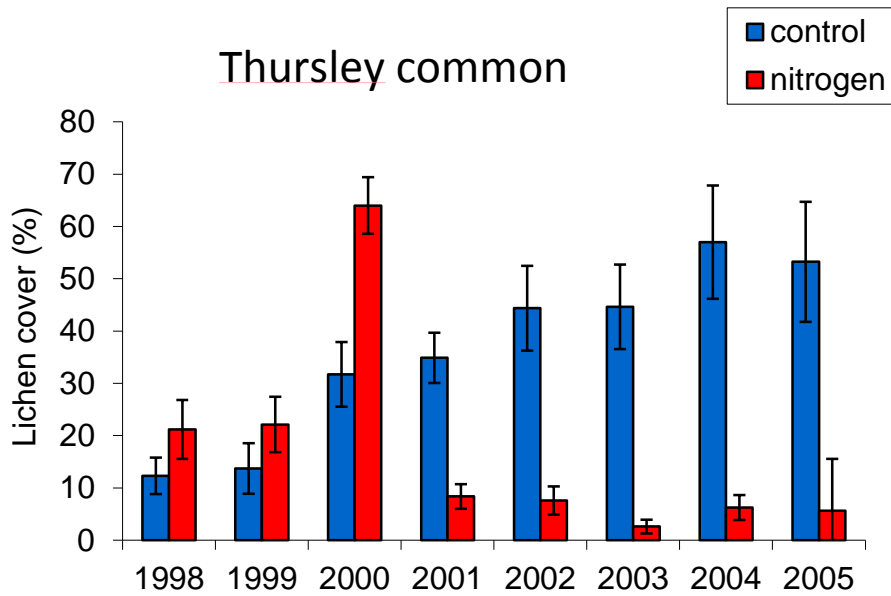


Figure 13: Effect of nitrogen additions ( $30 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) on lichen cover at Thursley common, lowland heathland in Surrey (Sally Power, pers. comm.). The large decrease between 2000 and 2001 is thought to be related to a closing higher plant canopy following management interaction in 1998.

## **5.4 Reviewed literature – relationship between N deposition and the key response variables in the Countryside Survey and targeted habitat spatial surveys**

The form of the relationship between nitrogen pollution and plant community composition is reviewed here for two types of survey: firstly the Countryside Survey of Great Britain, using a stratified randomised approach, which in this case used data collected in 1998 (Maskell *et al.*, 2010). Secondly, several smaller scale surveys that have deliberately targeted particular habitats and enabled separation of air pollution signals from other important influences such as precipitation, temperature and management (Table 14).

The main focus of the Countryside Survey and targeted habitats spatial surveys was change in plant species richness, with further interest in individual sensitive species and functional groups. A range of important UK habitats was addressed, but some significant habitat gaps, notably woodland, remain. Data were gathered using small quadrats (2 x 2 m or 0.5 m<sup>2</sup> for bryophytes only) and analysed by multivariate statistical methods that examined a range of potential drivers including climate, air pollution and in some cases management (in other studies management level was kept a constant).

### **5.4.1 Habitat differences**

The results of these surveys (Table 14) show a large degree of agreement, but also some important differences, between habitats. In upland heath, acid grassland and mesotrophic grassland, the different studies show strong agreement i.e. species richness was negatively correlated with nitrogen deposition. However, the two independent studies examining calcareous grassland found no correlation between nitrogen deposition and species richness. The contrast between base-rich calcareous habitats and the others suggests at least one of the likely mechanisms of change in less buffered soils is a long-term shift in soil pH (Stevens *et al.*, 2010 Functional ecology paper) that could result from increased nitrogen deposition. While the calcareous grassland spatial survey in 1990-93 reported by Van den Berg *et al.* (2010) found no significant correlation with nitrogen deposition, the smaller repeat survey of 2006-2009 found an reduction in plant species diversity over the two decades that was greater in high-N deposition regions of the UK, particularly in the 25-35 kg N ha<sup>-1</sup> y<sup>-1</sup> areas. These changes comprised a decline in the frequency of characteristic calcareous grassland species and a lower number of rare and scarce species.

### **5.4.2 Taxonomic groups respond differently**

In surveys where a significant drop in overall species richness in response to nitrogen deposition was recorded, different plant groups did not always respond in the same way. In acid grasslands, Stevens *et al.* (2006) found a strong decline in richness of forbs, a much weaker (but significant) reduction in grass species richness and no change in bryophyte species richness although the moss *Hylocomium splendens* was consistently reduced. In the Countryside Survey, both bryophyte and total species richness significantly declined in acid grasslands but not in heathlands and mesotrophic grasslands, where total species richness reduced but, bryophytes showed no change (Maskell *et al.*, 2010). Bryophytes declined significantly with increasing nitrogen in the upland heath surveys.

### **5.4.3 The shape of the relationships between species richness and nitrogen deposition, and the critical load.**

The decline in total species richness with increasing nitrogen demonstrated in all surveys apart from calcareous grasslands are described by either linear or curvilinear mathematical relationships. Over the nitrogen range investigated in the UK there is no evidence of a limit at the low end of the range below which negative change does not occur. The linear



responses to increasing nitrogen found in a number of the studies (or plant groups within the studies) indicate that the rate of change in richness is constant across the nitrogen deposition range while the curvilinear fit that better describes other relationships indicate that reduction in species richness is greater at low than at high N deposition.

The slope of species richness vs nitrogen deposition is remarkably similar for the studies 1-5 (Table 11) (all except calcareous grassland, study 6) falling in a range of between minus 2.5 – 4.3 fewer species per quadrat per  $10 \text{ kg ha}^{-1}\text{y}^{-1}$ . Where curvilinear gradients were described (study 4) the rate of change was greater (minus 4.3) below  $10 \text{ kg ha}^{-1}\text{y}^{-1}$  and minus 2.9 above  $20 \text{ kg ha}^{-1}\text{y}^{-1}$ . These figures compare broadly with the survey data analysed in this report: see task 5, Table 19.

Note however, that fewer species in a quadrat, or a reduction in species richness, means neither species 'loss' from a site, nor local species extinction – it means that the frequency of at least one species has been reduced. Also note that it is very likely that any inferred reduction in species richness due to N is the product of many years of N deposition, so that the current rate of N deposition is primarily a proxy for this long-term cumulative N.

#### **5.4.4 Species richness declines in relation to the critical load**

For acid grasslands and heathlands (Table 14, studies 1-4 and part of 5) the critical load range is  $10\text{-}15 \text{ kg N ha}^{-1}\text{y}^{-1}$  and  $10\text{-}20 \text{ kg N ha}^{-1}\text{y}^{-1}$  respectively. These fall towards the lower end (left) of the nitrogen deposition range surveyed. This has the following important implications:

- (a) as would be expected, *above* the critical load range there is a substantial reduction in species richness (for both linear and curvilinear responses);
- (b) what was more unexpected is that where curvilinear responses in species richness are described, the greatest decline in richness is *below* the critical load.

Table 14: Details of other surveys that have targeted specific habitat types along pollution and climatic gradients

Habitats	Significant Trend in response to increasing N deposition	Critical Load N kg ha <sup>-1</sup> y <sup>-1</sup>	Nitrogen Range kg ha <sup>-1</sup> y <sup>-1</sup>	Slope	Type of Relationship of response variable with N deposition	Comment	Reference
<b>Upland dry heath</b>	Bryophytes richness declines	10-20	19.5-30.5	3.1 bryophyte species / 10 kg N	Linear best fit		Edmondson <i>et al.</i> , 2010
<b>Upland dry heath</b>	Bryophytes richness declines	10-20	8-31	2.4 bryophyte species/ 10 kg N	Linear best fit		Combined data of Edmondson & Carroll & Caporn (Stevens <i>et al.</i> , 2009)
<b>Acid grassland</b>	Forbs & grass richness declines, Bryophytes no change  Plant acid preference index score increases	10-15	6-36	4 species (all) / 10 kg N	Linear best fit to NH <sub>y</sub> and total N deposition;  Exponential curvilinear best fit with NO <sub>x</sub> deposition  Linear best fit of plant acid preference with total N deposition	Greater decline at low N deposition	Stevens <i>et al.</i> , 2004, 2006, 2010
<b>Acid grassland</b>	Forbs Grasses Bryophytes  All groups richness decline	10-15	2-44	4.3 species (all) / 10 kg N below 20 kg /ha/y  2.9 species (all) / 10 kg above 20 kg N/ha/y	Exponential curvilinear best fit with total N	Greater decline at low N deposition  BEGIN project Analysis included GB and European sites	Stevens <i>et al.</i> , 2010

Habitats	Significant Trend in response to increasing N deposition	Critical Load N kg ha <sup>-1</sup> y <sup>-1</sup>	Nitrogen Range kg ha <sup>-1</sup> y <sup>-1</sup>	Slope	Type of Relationship of response variable with N deposition	Comment	Reference
<b>Acid grassland</b>	(Vascular + bryophyte) richness decline  Bryophyte (alone) Decline	10-15	c. 5-40	2.5 (vasc+bryo species) / 10 kg N	Linear best fit	Countryside Survey 1998	Maskell <i>et al.</i> , 2010
<b>Calcareous grassland</b>	No response in richness	15-25	c. 5-40	No change	No change	Countryside Survey 1998	
<b>Mesotrophic grassland</b>	(Vascular + bryophyte) richness decline	15-25	c. 5-40	Not given	Linear fit	Countryside Survey 1998	
<b>Heathland</b>	(Vascular + bryophyte) richness decline	10-20	c. 5-40	3.2 (vasc+bryo) species / 10 kg N	Linear fit	Countryside Survey 1998	
<b>Calcareous grassland</b>	No response in richness (1990-1993 survey)	15-25	7-41	No change	Above 25 kg N/ha/y there was a lower number of rare and scarce species	1990-3 survey part repeated in 2006-9; Increasing decline in species diversity and evenness over +20 years	Van den Berg <i>et al.</i> , 2010

## 5.5 Review of relationships between N deposition and the key response variables from the JNCC Collation report

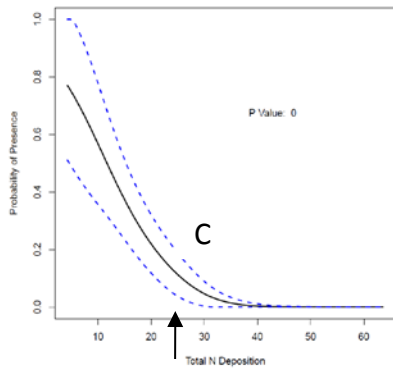
Two recent studies for JNCC have collated and analysed several different vegetation surveillance data sets (within UK or GB) in order to investigate relationships between community composition and nitrogen deposition (Stevens *et al.*, 2011) and the impact of this on critical loads and policy (Emmett *et al.*, 2011). A summary of relationships in selected data sets from the JNCC report is given here (Tables 16-17). This report will focus on the analysis of the vegetation datasets held within Stevens *et al.* (2011) although for a broader overview of policy implications the reader is directed to Emmett *et al.* (2012).

This JNCC collation study examines in detail the responses to nitrogen in a selection of habitats: heathlands, acid grasslands, calcareous grasslands and bogs. Large scale geographical distributions of plant species in relation to nitrogen deposition were examined using eight different surveillance data sets. Stevens *et al.* (2011) analysed spatial and in addition temporal changes over recent decades where data was available.

Discussion in this report is limited to examining the nature or shape of the spatial dose response relationships described in the collation report and covers the main databases providing detailed information on individual species over a wide geographical range: Vascular plant database (VPD), Botanical Society of the British Isles (BSBI), British Lichen society (BLS). Comment here is made regarding the British Bryological Society (BBS) data set.

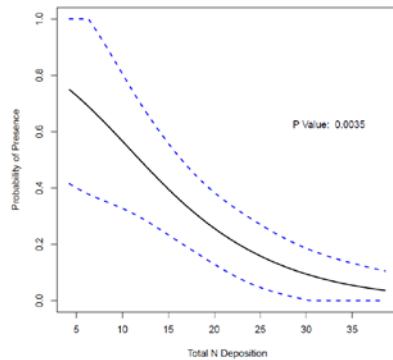
A range of dose-response relationships was mathematically described and plotted in the JNCC Collation report (Stevens *et al.*, 2011). Species presence - N relationships were not analysed by Stevens *et al.* (2011) where there were insufficient samples across the nitrogen deposition range. In the current report four types of relationships are proposed, the first three are negative responses of species to nitrogen, while the fourth is a positive response. In the cases of negative responses, an approximate deposition to result in 50% probability of presence is given based on visual assessment of the relationships; this is termed ND<sub>50</sub>. Examples of these responses taken from Stevens *et al.* (2011) are shown below overleaf. The four response types are categorised in the following way and illustrated in Figure 14. The mathematical relationships are represented by the solid line. Dotted lines illustrate the confidence intervals, where narrow our understanding of the response is strong, where the distance between these lines widens this reflects a less understanding of responses usually at extremes of the N deposition range:

- |         |  |
|---------|--|
| Type 1: | Strong negative curvilinear fall with a turning point ('heel') at point C  |
| Type 2: | Mild negative curvilinear fall, the shape approaching linear within the normal deposition range (up to 30-35 kg N) |
| Type 3: | Reverse sigmoid fall, indicating a shoulder at point A and a heel at point B                                       |
| Type 4: | Increase within normal deposition range  |



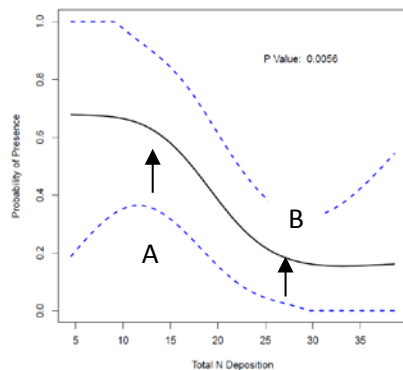
**Type 1: strong curvilinear fall**

Spatial change in the probability of presence of *Cladonia subulata* in heathland with increasing total current inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>). Data from BLS.



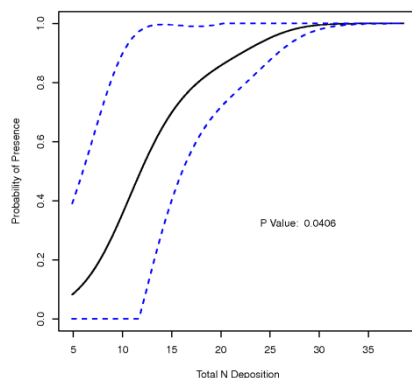
**Type 2: mild curvilinear fall**

Spatial change in the probability of presence of *Peltigera didactyla* in acid grassland with increasing total current inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>). Data from BLS.



**Type 3: reverse sigmoid fall**

Spatial change in the probability of presence of *Cladonia foliacea* in calcareous grassland with increasing total current inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>). Data from BLS.



**Type 4: Increase**

Spatial change in the probability of presence of *Alchemilla xanthochlora* in upland calcareous grassland with increasing total current inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>). Data from Vascular Plant Database.

Figure 14: Examples of relationships between individual species probability of presence and nitrogen deposition from JNCC report 447 (Stevens *et al.*, 2011) with proposed inflexion points A,B,C which are discussed here in the text

### 5.5.1 Other types of responses within the VPD, BSBI, BLS and BBS databases

In addition to the cases listed in the Tables 15-17 and shown in the graphs (see Stevens *et al.* 2011), there were also other cases of: no significant relationship; hump-backed responses, U-shaped responses; 'small magnitude changes.' These are not reported in the Tables 15-17 in the current report because they are difficult to interpret and suggest the strong influence of other factors. The tables below show cases where there were clear-cut, significant relationships with nitrogen deposition, both positive and negative.

Where there were sufficient data available for analysis, numerous observations of 'no significant response' were reported. Where significant changes were found the majority of these were negative i.e. decline in presence with increasing nitrogen deposition. There were also a significant number of species showing increasing presence, particularly amongst bryophytes, but the majority of responses within any plant group were negative.

The strongest responses were in the lichen data set (BLS) and these were consistently negative changes. All three decline curve types were seen, but the majority were Type 1 or 2, indicating that there was no minimum threshold and the decline in presence started from a very low dose (around  $5 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ). The fewer cases found of the reverse sigmoid curve type (Type 3) suggested that a decline commenced above a nitrogen deposition of approximately  $15 \text{ kg N ha}^{-1} \text{ y}^{-1}$ . The approximate value for the  $\text{ND}_{50}$  was, for the lichens, within or around the low end of the critical load range for the habitats, with an average of  $14 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for those lichens shown in Table 14.

There were fewer cases of clear relationships with nitrogen in the BSBI higher plant data sets (Table 16), but these were all negative with either Type 1 or Type 3 curves over the normal range of deposition. Sigmoid type curves (Type 3) for three species showed that decline commenced above a nitrogen deposition of approximately 5, 8,  $18 \text{ kg N ha}^{-1} \text{ y}^{-1}$  respectively. The mean  $\text{ND}_{50}$  for these vascular plants was approximately  $16 \text{ kg N ha}^{-1} \text{ y}^{-1}$ .

The Vascular Plant Database (VPD) analysis showed a larger number of significant positive and negative relationships between vascular species presence and Nitrogen deposition (Table 17). The most common decline was described by a strong curvilinear response (Type 1) with a 50% drop by around  $8\text{-}15 \text{ kg N ha}^{-1} \text{ y}^{-1}$ . A few species declined in a sigmoid manner (Type 3) with a shoulder at around  $10\text{-}15 \text{ kg N ha}^{-1} \text{ y}^{-1}$ .

Bryophyte response curves in the JNCC collation report were not analysed to the same level of detail in the current study. The individual species data for bryophytes are difficult to interpret with some species increasing and other decreasing and the authors could not find any clear and general trends in the data (Stevens *et al.* 2011).

### 5.5.2 Response patterns above the critical load

Examination of the individual responses from the BLS, BSBI and VPD datasets suggests that at the higher levels of deposition there usually is a turning point or 'heel' to a slower rate of decline (position C in a type 1 curve, and position B in a type 3 curve - see Figure 14). In the significant negative responses this turning point was observed around an average approximate nitrogen deposition of  $25 \text{ kg N ha}^{-1} \text{ y}^{-1}$  in lichens (BLS datasets) and  $23 \text{ kg N ha}^{-1} \text{ y}^{-1}$  in vascular plants (VPD and BSBI datasets).

## **5.6 Summary from the review of published dose response relationships between species richness or individual species presence and nitrogen deposition**

Evidence from the BLS, BSBI and VPD national datasets (summarised over Tables 15 to 17) which are based on national botanical surveillance data sets or targeted habitat surveys show a remarkable degree of consistency demonstrating evidence for nitrogen enrichment across several UK habitats. All habitats examined here show evidence of either broad scale reductions in species richness, decline in individual species or plant groups or increases in some nitrophilous species such as graminoids. However, it is also clear that species and habitats do not all respond in the same way to nitrogen deposition. For example, individual bryophytes show a range of different responses to nitrogen, some declining and others increasing that probably reflect the importance of other aspects of habitat/micro-habitat over nitrogen deposition in many species of this group. Calcareous habitats are less affected by nitrogen deposition than less well pH buffered systems suggesting a role for acidification in changes in plant communities.

Negative relationships with nitrogen in species richness or individual species presence tend to show a similar response form – namely either linear indicating an equal rate of decline across the nitrogen deposition range or curvilinear suggesting a greater rate of change at low nitrogen than at high deposition rates. The JNCC Collation study revealed a number of plant species that started to decline with increasing nitrogen inputs only after a level of deposition was reached, but in the majority of affected species no level was apparent implying that within the normal UK deposition range the vulnerable species start to decline with any increasing level of nitrogen and that this is typically at or below the critical load range for the habitats.

At the higher end of the nitrogen deposition scale, all the data sets investigated demonstrated clear evidence that species declines continue to occur above the habitat critical loads, so that small increments of nitrogen pollution within the range of 20-35 kg N ha<sup>-1</sup> y<sup>-1</sup> still have the potential to cause adverse and continuing change. Even in the less sensitive calcareous habitat, the temporal change study in calcareous grasslands found the greatest decline in individual species was in the areas receiving 25-35 kg N ha<sup>-1</sup> y<sup>-1</sup>. The importance of changes in nitrogen deposition at the higher end of the UK range should not be underestimated.

Table 15: Relationship between probability of presence and nitrogen deposition for lichen species from the BLS database spatial analysis for which significant responses were found. The turning point and ND50 (these are defined in section 5.5) values refer to nitrogen deposition ( $\text{kg N ha}^{-1} \text{y}^{-1}$ ) and were estimated by eye from published figures of Stevens *et al.* (2011) and Emmett *et al.* (2012). Examples of response types and turning points are shown in Figure 14.

British lichen society database Habitat / Species (Critical load)	Direction of change	Response Type	Turning Points ( $\text{kg N ha}^{-1} \text{y}^{-1}$ )	ND <sub>50</sub>
<b>Acid grassland (10-15 <math>\text{kg N ha}^{-1} \text{y}^{-1}</math>)</b>				
<i>Cetraria aculeata</i>	Negative	1	C=22	15
<i>Peltigera didactyla</i>	Negative	2		12
<b>Calcareous grassland (15-25 <math>\text{kg N ha}^{-1} \text{y}^{-1}</math>)</b>				
<i>Cladonia foliacea</i>	Negative	3	A=15, B=30	17
<b>Bog (5-10 <math>\text{kg N ha}^{-1} \text{y}^{-1}</math>)</b>				
<i>Cladonia portentosa</i>	Negative	3 (between 5-30 kg N)	A=15, B=30	22
<b>Heathland (10-20 <math>\text{kg N ha}^{-1} \text{y}^{-1}</math>)</b>				
<i>Cetraria aculeata</i>	Negative	1	C=20	10
<i>Cetraria muricata</i>	Negative	1	C=20	10
<i>Cladonia cervicornis cervicornis</i>	Negative	1	C=15	8
<i>Cladonia cervicornis verticillata</i>	Negative	2 (between 5-35 kg N)		10
<i>Cladonia portentosa</i>	Negative	2 (between 5-30 kg N)		15
<i>Cladonia subulata</i>	Negative	1	C=27	12
<i>Cladonia uncialis biuncialis</i>	Negative	1	C=18	9
<i>Peltigera hymenina</i>	Negative	3	A=15, B=40	25
				Mean = 14



Table 16: Relationship between probability of presence and nitrogen deposition for vascular plant species from the BSBI database local change spatial analysis for which significant responses were found. The turning point and ND<sub>50</sub> values refer to nitrogen deposition (kg N ha<sup>-1</sup> y<sup>-1</sup>) and were estimated by eye from published figures of Stevens *et al.* (2011) and Emmett *et al.* (2012). Examples of response types and turning points are shown in Figure 14.

BSBI database Habitat/Species (Critical load)	Direction of change	Curve Type	Turning Points (kg N ha <sup>-1</sup> y <sup>-1</sup> )	LD50
<b>Calcareous grassland</b>				
<b>Lowland (15-25 kg N ha<sup>-1</sup> y<sup>-1</sup>)</b>				
<i>Bromopsis erecta</i>	Negative	1 (over 5-25 kg N range )	C= 12	8
<i>Campanula glomerata</i>	Negative	3	A=18, B=30	27
<i>Carex spicata</i>	Negative	1	C=18	14
<i>Ononis repens</i>	Negative	3	A=8, B=18	15
<b>Upland Heathland (10-20 kg N ha<sup>-1</sup> y<sup>-1</sup>)</b>				
<i>Vaccinium vitis-idaea</i>	Negative	3	A=5 , B=27	18
				Mean = 16

Table 17: Relationship between probability of presence and nitrogen deposition for vascular plant species for which significant responses were found in the vascular plant database spatial analysis. The turning point and ND<sub>50</sub> values refer to nitrogen deposition (kg N ha<sup>-1</sup> y<sup>-1</sup>) and were estimated by eye from published figures of Stevens *et al.* (2011) and Emmett *et al.* (2012). Examples of response types and turning points are shown in Figure 14.

Vascular plant database Habitat/Species (critical load)	Direction of change	Curve Type	Turning Points	LD <sub>50</sub>
<b>Acid grassland: Lowland (10-15 kg N ha<sup>-1</sup> y<sup>-1</sup>)</b>				
<i>Cerastium arvense</i>	Negative	1	C=15	8
<i>Cerastium semidecandrum</i>	Negative	1	C=22	12
<i>Trifolium arvense</i>	Negative	1	C=19	12
<i>Vicia lathyroides</i>	Negative	1	C=19	10
<i>Viola canina</i>	Negative	1	C=24	15
<b>Calcareous grassland (15-25 kg N ha<sup>-1</sup> y<sup>-1</sup>)</b>				
Lowland				
<i>Allium vineale</i>	Negative	1	C=25	9
<i>Anacamptis pyramidalis</i>	Negative	1	C=30	8
<i>Carlina vulgaris</i>	Negative	2 (between 5-30)		16
<i>Cynoglossum officinale</i>	Negative	3	A=10, B=35	25
<i>Echium vulgare</i>	Negative	3	A=10, B= 30	Outside range
<i>Geranium columbinum</i>	Negative	1	C=25	12
<i>Lathyrus nissolia</i>	Positive	4 (between 15-30)		
<i>Ononis repens</i>	Negative	3	A=10, B= 22	20
<i>Spiranthes spiralis</i>	Negative	1	C=15	8
<i>Stachys officinalis</i>	Positive	4 (between 5-30)		
Upland				
<i>Alchemilla xanthochlora</i>	Positive	4 (between 5-30)		
<i>Melica nutans</i>	Negative	3	A=15, B=27	17
<b>Heathland (10-20 kg N ha<sup>-1</sup> y<sup>-1</sup>)</b>				
Lowland				
<i>Platanthera bifolia</i>	Positive	4 (between 5-35)		
<i>Viola canina</i>	Negative	3	A=12, B=25	18
Upland				
<i>Arctostaphylos uva-ursi</i>	Negative	1	C=15	9
				Mean = 13

## 5.7 Cluster analysis, sample grouping and ecological thresholds results

The analysis in this section of Task 4 sought to establish coherent ecological groupings along the N deposition gradient to support the suggestion that N is an important control on community composition and locate putative loads of N deposition where community composition is found to change significantly. Full details of the methodology used are provided in the methods section of this report.

The results are summarised in Table 18, split by survey dataset. In the acid grassland data (BEGIN) there are significant differences between the first two groups identified by both CONISS and SPLITLSQ. The break-points identified by these methods are different but rather similar, falling around 14.1 and 14.4 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In the TU sand dune dataset significantly different groups are identified with only one method and the groups identified are only marginally significant. In the TU bog dataset two methods identify first divisions in adjacent positions around 11-12 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In the TU upland heath data CONISS and SPLITINF identify adjacent break-points around 17 kg N ha<sup>-1</sup> yr<sup>-1</sup>, SPLITLSQ identifies a slightly lower first break at around 14.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> and then a second significant break around 25-26 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In the TU lowland heath data identical breaks are identified by all methods with only a first break significant at around 14.6 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

Table 18: Results of CONISS, SPLITLSQ and SPLITINF for seven nitrogen gradient studies. Results show only the first two break-points listing the samples (e.g. N14.4, N14.5 etc) between which a division falls and the total nitrogen deposition (N) values for those samples in kg N ha<sup>-1</sup> yr<sup>-1</sup>. Analyses were based on squared Euclidean distance using proportion data (mean percent cover in most datasets, proportion of total species occurrences in five quadrats in the moorland regional survey). Differences between groups were tested on the same datasets using ANOSIM with Bray-Curtis dissimilarity, significance testing with permutation tests (10,000 permutations). \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001, grey shading shows non-significant results. Second break-points are only counted as significant if all groups are significantly different (maximum P value between groups shown).

Dataset	CONISS		SPLITLSQ		SPLITINF	
	1 <sup>st</sup> division	2 <sup>nd</sup> division	1 <sup>st</sup> division	2 <sup>nd</sup> division	1 <sup>st</sup> division	2 <sup>nd</sup> division
<b>BEGIN- acid grassland</b>	(N14.4/14.5)**	(N22.8/23.8)	(N14.0/14.2)*	(N14.4/14.5)	(N24.8/25.0)	(11.0/13.4)
<b>Moorland regional survey</b>	N26.1/28.1)***	N20.4/20.6)**	(12.9/20.4)***	N26.1/28.1)***	(12.9/20.4)***	(N26.1/28.1)***
<b>TU-sand dune</b>	(N13.9/14.6)	(N15/16.7)	(N13.9/14.6)	(N15/16.7)	(N5.7/7.7)*	(N10.4/11.5)
<b>TU-bogs</b>	N10.9/11.7)***	(N19.6/21.9)	(N19.6/21.9)	(N14.2/14.3)	(N11.7/13.1)**	(16.6/16.6)
<b>TU- Upland heath</b>	(N16.5/17)**	(N24.5/28)	(N14.1/15.2)***	(N24.8/26.6)**	(N17/19.4)***	(N24.8/26.6)
<b>TU-lowland heath</b>	(N14.6/14.7)**	(N13/13.6)	(N14.6/14.7)**	(N13/13.6)	(N14.6/14.7)**	(N13/13.6)

Comparing the results between different surveys of the same or similar habitat types the case for consistent break-points is not overly strong. In the upland heath data from the TU and MRS there is overlap in some of the break-points however given the inconsistent sampling along the gradient it is difficult to place great confidence in the significance of this observation. The 16.5/17, 14.1/15.2 and N17/19.4 break-points in the TU data all lie within the wide gap between the MR15 and MR20 samples (12.9-20.4 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in the MRS data. As discussed above, comparison of the MRS results with other results is particularly difficult due to the differing methodologies. Comparing the TU upland and lowland heath data there is similarity in the position of a first break-point in the lowland heath dataset and the first break-point with SPLITLSQ in the upland heath data while the upland heath first splits with SPLITINF and CONISS are slightly higher.

The results show that although break-points are identified in all cases, not all of the identified groups are significantly different. In only two cases are groups identified by a second split in the data significantly different from each other. The most significant results are identified with the moorland regional survey with all methods producing significantly different groups; however these results should be treated with caution particularly in comparison to the other datasets. In contrast to other datasets instead of recording percent cover this survey recorded presence/absence in each of five quadrats; the data we analyse here is a proportion of total species count and therefore although incorporating some measure of abundance is more weighted towards presence/absence.

In general there is some similarity between the results of different methods, but in many cases also substantial differences. Perhaps surprisingly the results of the two divisive methods are not substantially more similar to each other than to the results of the agglomerative method.

In most datasets our analyses succeed in identifying groups of samples with significantly different plant communities according to levels of nitrogen deposition. In itself this is an interesting result, supporting the suggestion from the ordinations that nitrogen deposition modifies plant community structure. The identification of such groups may be a useful way to approach the output of gradient studies. For instance the identification of indicator species for these groups may allow new sites to be categorised according to their levels of nitrogen-loading, which may be a useful approach to bioindication.

In some datasets different statistical techniques suggest the same break-point locations whereas in others there are differences. Where there are similar results by different methods this provides evidence for the validity of these values. However, where results differ between methods it does not necessarily mean the results are invalid. As different methods work on different principles and responded to different elements in the data it is quite possible for different methods to identify different, but equally valid break-points.

This analysis has studied the relationship between species composition and nitrogen deposition. The analysis of the lowland and upland heath and acid grassland datasets suggest that significant changes in composition may occur at 15 – 17 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This value is in broad agreement with the conventional and LOESS regressions which looked for 'heels' in the species richness curves. Analysis of the bog vegetation data suggested a breakpoint where change occurs in species composition of 10-13 kg N ha<sup>-1</sup> yr<sup>-1</sup> which is lower than the 19-20 kg N ha<sup>-1</sup> yr<sup>-1</sup> suggested in the regression analysis of species richness data. Analysis of the sand dune data also suggested that changes in species composition may occur at much lower levels of N deposition than changes in overall species richness, 5-8 kg N ha<sup>-1</sup> yr<sup>-1</sup> compared to 10 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

These findings broadly support the species richness regressions and the levels of N deposition where the rate of change in species richness altered, however, they do highlight

the possibility that a change in species composition may occur before it is measured by a change in overall species richness. The implication of this is that ecosystems may be showing sensitivity to N deposition at much lower levels of N deposition than previously thought and certainly at the lower end of the critical load ranges.

## 5.8 Summary

The responses to nitrogen (N) across the habitats studied in the survey datasets are remarkably consistent with strong reductions in measured species richness associated with increases in N deposition. The fact that N deposition is so consistently related to changes in species richness (and composition) demonstrates the deleterious effects of N pollution on biodiversity. In many cases, bryophytes and lichens were shown to be most sensitive to N deposition although in grasslands, the intrinsic biodiversity of the habitat was affected by N through reductions in forb species richness. Within the heathland, bog and sand dune survey datasets graminoid cover increased exponentially with rising N deposition.

The results from the survey datasets are consistent with the findings from the dose-response experiments and the literature review. At a number of heathland experiments bryophyte and lichen diversity or cover reduced as N addition increased, notably so at the Culardoch montane heath where background N deposition was at the low end of the critical load range for heathlands ( $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). Reductions specifically in *Cladonia portentosa* cover at Ruabon and Culardoch supported the reductions seen in the heathland and bog surveys. Vascular plants were not strongly affected by N in the experiments although this perhaps reflects the dose/time compromise made. An exception to this was increase in *Eriophorum vaginatum* cover at Whim Bog along the gaseous ammonia release transect which reflected increases in cover this sedge as N deposition increased in the survey dataset. It should also be noted that the timescales of even the longest N-addition experiments are short by comparison to real-world N driven changes which have occurred over many decades. Furthermore, experiments tend to be limited by plot size which limits the possible response of vascular plants which may be reflected in change over a wider area. For these reasons, many of the experiments fail to show significant loss of vascular species although in some cases declines in cover are observed. Indeed, as has been observed, cover of certain graminoid species increased with N deposition although graminoid species richness tended to fall.

The similarity in responses seen at the experimental sites provides important supporting evidence that N deposition is driving the changes seen in the survey datasets and that change is not solely driven by a climatic gradient. Larger national survey datasets including the Countryside Survey and plant databases from the British Lichen Society (BLS) and the Botanical Society of the British Isles (BSBI) also provide evidence for widespread changes in response to N deposition. However, it is also accepted that climate plays an important role in influencing species richness and diversity with a tendency towards greater species richness in wetter and colder sites.

Much of the data presented and reviewed in this report suggested a curvilinear response to N with steeper responses at lower background levels of N ( $< 10 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ), decreasing at higher background N ( $>20 \text{ kg}$ ) with a turning point or heel in the response curves generally occurring between  $15 - 22 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . These N deposition loads were broadly supported by the cluster analysis that studied change using species composition as the variable, however, in sand dunes and bogs species composition appeared sensitive at much lower levels of N highlighting that ecosystem change occurs across the N deposition range. This is reflected by some of the changes in seen in individual species that are poorly distributed across the datasets but typically found at the less polluted sites. The change in the

frequency of presence or cover of some species may therefore be good indicators of N deposition even where a change in overall species richness is not observed.

Table 19 below summarises some of the key findings from the analysis of the vegetation datasets and supporting reviews of literature and experimental site work. The relationships found in the species richness, cover and individual species data will be carried forward to Task 5 where the effect of incremental increases in N deposition on the variables will be examined further. Other relationships exist with the review literature and the experiments as discussed in this chapter, however, where these were not found to be significant in the data analysed as part of this project they have not been carried forward.

Table 19: Summary of the key findings from the analysis of the vegetation datasets and supporting reviews of literature and experimental site work. <sup>1</sup>Details of the UKREATE experiments in this report is presented in Tasks 2, 3 and 4 summarised in Phoenix *et al* (2012). <sup>2</sup>For details of studies included in the literature review see Tasks 2 and 3.

Habitat	Response curve shape from data analysis	Supported by UKREATE experiments <sup>1</sup>	Literature <sup>2</sup>	Spp. richness LOESS max. sensitivity	Spp. composition Cluster analysis change range	Individual species
<b>All habitats</b>						
Species richness (SR)	linear	#	#	n/a	n/a	<i>Hylocomium splendens</i>
<b>Upland heath</b>					14-20 kg N	<i>Hylocomium splendens</i> <i>Deschampsia flexuosa</i>
Total SR	mild curvilinear		#	7-20 kg N		
Lichen SR	curvi-linear	#	#	7-16 kg N		
Graminoid SR	mild curvilinear			7-22 kg N		
Graminoid cover	mild curvilinear			>22 kg N		
<b>Lowland heath</b>					14-15 kg N	<i>Hylocomium splendens</i> <i>Cladonia portentosa</i> <i>Brachythecium rutabulum</i>
Total SR	mild curvilinear	#	#	< 17 kg N		
Moss SR	mild curvilinear	#	#	none		
Graminoid SR	mild curvilinear			none		
Graminoid cover	mild curvilinear			> 23 kg N		
<b>Bog</b>					10-13 kg N	<i>Cladonia uncialis</i> <i>Eriophorum vaginatum</i> <i>Sphagnum fimbriatum</i>
Total SR	linear			> 19 kg N		
Lichen SR	linear			none		
Forb SR	mild curvilinear			none		
Graminoid cover	linear	#		none		
<b>Sand dune</b>					5-8 kg N	<i>Hylocomium splendens</i>
Total SR	mild curvilinear					
Moss SR	strong curvilinear					
Forb SR	mild curvilinear					
Graminoid cover	mild curvilinear		#			

Habitat	Response curve shape from data analysis	Supported by UKREATE experiments <sup>1</sup>	Literature <sup>2</sup>	Spp. richness LOESS max. sensitivity	Spp. composition Cluster analysis change range	Individual species
<b>Acid grassland</b>					14-15 kg N	<i>Hylocomium splendens</i> <i>Hypnum cupressiforme</i> <i>Europhrasia officianalis</i> <i>Lotus corniculatus</i> <i>Carex panacea</i> <i>Nardus stricta</i>
Total SR	mild curvilinear		#			
Forb SR	linear		#			



## 6. Task 5: Determine the relative effect of incremental N

### 6.1 Introduction

The implications of the relationships between the response variables and nitrogen (N) deposition that were detailed within Task 4 are explored further within this task and the effect of incremental increases in long-term N deposition upon these response variables is considered.

### 6.2 Habitat specific difference in species richness along the survey gradients associated with different levels of N deposition

As described previously, the relationships between species richness and N deposition described by the spatial surveys have developed over many years, and the current rate of N deposition is primarily a proxy for this long-term cumulative N. Thus we would not expect that a change in N deposition, either increasing or decreasing, would immediately change species richness or composition. However, the spatial relationships described above can be examined to estimate how the species richness of different habitats has responded to different levels of long-term N deposition, as represented by differences in current N deposition. Recall from previously that a reduction in species richness does not necessarily mean that any species are 'lost' (see Figure 1), but that the frequency of some species is reduced.

This section 1) compares the difference in species richness for a  $1 \text{ kg N ha}^{-1} \text{ y}^{-1}$  increment of long-term N deposition along the gradient studies, expressed as a reduction of percentage of the maximum number of species recorded in each habitat (Table 20, other increments are shown in appendix 5), 2) expresses the response relationship as the difference in long-term N deposition associated with a species richness reduction of one species along the gradient (Table 21). For reference, the current (2011) critical load for each habitat is included in the results tables and full details of critical loads for all habitats are provided in appendix 6 with a summary in the introduction to this report).

When the linearly related TU survey data are combined, 1.6% of the maximum number of species within each habitat is reduced with every incremental increases of  $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$  of long-term N deposition. When all the habitats are considered separately, the typically curvilinear response of species richness to N deposition produces sharp losses in diversity from well below the habitat-specific critical load ranges. However, even at levels of N deposition at and above the upper end of each habitat-specific critical load, the effect of a 1 kg increase of N is considerable and at the mid-point of the critical load range the losses and subsequent threat to habitat integrity from loss of sensitive species and increases in graminoid cover are considerable. For example, within the upland and lowland heath habitats in the TU 2009 survey, species richness is reduced by nearly 1.0 (around 2 % of the maximum species richness) for each  $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$  increase in long-term N above the mid-point of the critical load range ( $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and by 1.0 for each 2 kg increase (above 1 % of maximum) increase in N deposition well above the critical load:  $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . The magnitude of change is less in the MRS Upland heath survey probably due to a different recording technique and the use of smaller survey quadrats due to the focus towards the lower plant species. These results from the heathland surveys reveal remarkably high losses in diversity in what are naturally low-diversity systems, typified by specialist low-nutrient plants and these losses highlight the vulnerability of this habitat to eutrophication by N enrichment.

The results from the other habitats follow a similar pattern of reductions in species richness as long-term N deposition increases. Sand dune ecosystems in particular appear to be

strongly sensitive to N deposition with a very rapid loss of species diversity as N increases from below the lower end of the critical load range. Even when sand dune type is split between decalcified and calcareous, species richness reduces by 1.2 % species for every 1 kg increase in long-term N deposition above the upper end of the habitat specific critical load ( $15 \text{ kg N ha}^{-1}\text{yr}^{-1}$ ), equivalent to a fall of 1 species for every  $1.1 \text{ kg N ha}^{-1}\text{yr}^{-1}$ . Moss diversity is particularly negatively related to N. Within the bog habitat, losses are less severe with species richness reducing by around 1 % for approximately every 3 kg increase in long-term N deposition across the range studied. This is likely due to the hydrology regime limiting species responses to N. The much larger acid grassland dataset (BEGIN) also showed reductions in richness to N and, due to a flatter response curve, these remain high with a 1 species drop in richness for approximately every 2 kg N increase above the upper end of the critical load range. Interestingly, when non-UK European grasslands are included (not included in this report) a curvilinear response is also apparent (Stevens *et al.*, 2010).

Table 20: Summary of relationships between nitrogen deposition and species richness/cover by habitat expressed as a percentage of the maximum in a habitat. Change in species richness associated with a 1 kg ha<sup>-1</sup> y<sup>-1</sup> difference in long-term N deposition along the survey sites is shown. Modelled relationship only applied over N deposition range in which survey sites fell, where no sites were surveyed at a given N deposition level '-' is shown.

Survey/ Habitat/	Max. species richness	Habitat/specie s critical load (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 1 kg increase in long-term N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total species richness	77 spp.	10-20	-1.6 % of maximum number of species/kg N increase					
<b>Upland heath (TU 2009 )</b>								
Total species richness	42 spp.	10-20	-5.7 %	-2.9 %	-2.0 %	-1.4 %	-1.2 %	-1.0 %
Lichen species richness	11 spp.	10-20	-5.4 %	-2.7 %	-1.8 %	-1.8 %	-1.0 %	-1.0 %
Graminoid species richness	7 spp.	10-20	-7.0 %	-2.9 %	-2.9 %	-1.4 %	-1.4 %	-1.0 %
Graminoid cover	n/a	10-20	-0.5 %	no change	+0.4 %	+0.8 %	+1.2 %	+1.6 %
<b>Upland heath (MRS)*</b>								
Total species richness	16 spp.	10-20	-3.4 %	-3.1 %	-2.5 %	-1.9 %	-1.3 %	-0.3 %
<b>Lowland heath (TU 2009)</b>								
Total species richness	37 spp.	10-20	-6.2 %	-3.5 %	-2.2 %	-1.6 %	-1.4 %	-1.0 %
Moss species richness	12 spp.	10-20	-5.8 %	-2.5 %	-1.7 %	-1.7 %	-1.7 %	-0.9 %
Graminoid species richness	9 spp.	10-20	-17.8%	-4.4 %	-2.2 %	-1.1 %	-1.1 %	-0.5 %
Graminoid cover	n/a	10-20	-0.6 %	no change	+0.5 %	+1.05 %	+1.6 %	+2.2 %
<b>Bog (TU 2009 )</b>								
Total species richness	32 spp.	5-10	-0.9 % of maximum number of species/kg N increase					
Lichen species richness	6 spp.	5-10	-1.7 %					
Forb species richness	6 spp.	5-10	-7.7 %	-3.9 %	-2.6 %	-1.9 %	-1.6 %	-1.3%
Graminoid cover	-	5-10	+1.5 % cover/kg N increase					
<b>Sand dunes (TU 2009, all sites)</b>								
Total species richness	77 spp.	8-15	-10.1%	-2.6 %	-1.2 %	-0.6 %	-	-
Moss species richness	16 spp.	8-15	-21.3%	-5.0 %	-2.5 %	-1.3 %	-	-
Graminoid cover	n/a	8-15	+8.6 %	+2.2 %	+1.0 %	+0.5 %	-	-
Forb species richness	33 spp.	8-15	-10.3%	-2.4 %	-1.2 %	-0.6 %	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total species richness	77 spp.	8-15	-4.4 %	-2.2 %	-1.4 %	-1.0 %	-	-
Moss species richness	16 spp.	8-15	-21.3%	-5.6 %	-2.5 %	-1.3 %	-	-
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>								
Total species richness	77 spp.	8-15	-4.4 %	-2.2 %	-1.4 %	-1.0 %	-	-
Moss species richness	16 spp.	8-15	-8.9 %	-4.4 %	-3.1 %	-2.5 %	-	-

Survey/ Habitat/	Max. species richness	Habitat/specie s critical load (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 1 kg increase in long-term N deposition at different background N deposition levels					
<b>Acid grasslands (BEGIN)</b>								
Total species richness	42 spp.	10-15	-1.5 %	-1.4 %	-1.2 %	-1.1 %	-1.0%	-0.9%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

The incremental effect of long-term N on species richness reduces as deposition levels increase above the upper end of the critical load for each habitat due to the curvilinear nature of the relationship between N and species richness. However, the positive, curvilinear relationship between graminoid cover in the heathlands means that graminoid cover increases dramatically above the critical load. This outcome is of key importance to site integrity, particularly within heathlands which have been shown to be vulnerable to conversion to grassland, most notably in the highly N-polluted areas of the Netherlands. Within the bog habitat, graminoid cover (principally the sedge, *Eriophorum vaginatum*) was found to increase by 1.5% per additional kg N across the deposition range studied suggesting that the balance between shrubs, graminoid and moss (mainly *Sphagnum* spp.) is at risk of moving towards dominance by sedge species. A similar result was obtained from the sand dune survey data although the relationship with N was weaker and more strongly associated with pH. Nevertheless, other studies have found sand dune integrity vulnerable to increases in graminoid cover (Remke *et al.*, 2009).

It is also important to highlight the differing results between surveys, particularly the TU 2009 Upland Heath Survey and the Moorland Regional Survey (MRS). The reason behind this is the different quadrat sizes used to measure species richness: the TU survey used 2 x 2 m quadrats whilst the MRS used 0.5 x 0.5 m quadrats. Both produce valid measures of species richness although the MRS was more focussed on lower plants. For this reason the TU 2009 survey provides the strongest data as the bigger quadrats capture more of a site's biodiversity and therefore are more representative of the changes that occur across the dataset.

Table 21: Summary of relationships between long-term nitrogen deposition and species richness by habitat expressed as the amount of incremental N deposition (in kg N ha<sup>-1</sup> yr<sup>-1</sup>) associated with a reduction in species richness of one species along the survey gradient sites. Modelled relationship only applied over N deposition range in which survey sites occurred; where no sites were surveyed at a given N deposition level '-' is shown.

Survey/ Habitat/	Max. species richness	Habitat/ species critical load kg N ha <sup>-1</sup> yr <sup>-1</sup>	Increase in N deposition (in kg N ha <sup>-1</sup> yr <sup>-1</sup> ) required to reduce measured species richness by 1 at different background long-term N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>Upland heath (TU 2009 )</b>								
Total species richness	42 spp.	10-20	0.4 kg	0.8 kg	1.3 kg	1.7 kg	2.0 kg	2.4 kg
<b>Upland heath (MRS)*</b>								
Total species richness	16 spp.	10-20	1.7 kg	2.0 kg	2.5 kg	3.3 kg	5.0 kg	20.0 kg
<b>Lowland heath (TU 2009)</b>								
Total species richness	37 spp.	10-20	0.4 kg	0.8 kg	1.3 kg	1.7 kg	2.0 kg	2.4 kg
<b>Bog (TU 2009 )</b>								
Total species richness	32 spp.	5-10				3.3 kg		
<b>Sand dunes (TU 2009, all sites)</b>								
Total species richness	77 spp.	8-15	0.1 kg	0.5 kg	1.1 kg	2.0 kg	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total species richness	77 spp.	8-15	0.3 kg	0.6 kg	0.9 kg	1.3 kg	-	-
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>								
Total species richness	77 spp.	8-15	0.3 kg	0.6 kg	0.9 kg	1.3 kg	-	-
<b>Acid grasslands (BEGIN)</b>								
Total species richness	42 spp.	10-15	1.7 kg	1.7 kg	2.0 kg	2.0 kg	2.5 kg	2.5 kg

\*in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

Table 22: Summary of relationships between long-term nitrogen deposition and species cover (C) or probability of presence (P) by habitat expressed as a percentage of the maximum in a habitat. Difference in species richness associated with a 1 kg ha<sup>-1</sup> y<sup>-1</sup> difference in long-term N deposition along the survey sites is shown. Modelled relationship only applied over N deposition range in which survey sites fell, where no sites were surveyed at a given N deposition level '-' is shown. When the relationship between N and species richness was not significant 'ns' is shown.

Habitat (Survey) /Species	Max cover/ presence (no. of quadrats)	Change in species cover expressed as a % of maximum species cover recorded in habitat with a 1 kg increase in long-term N deposition at different background N deposition levels					
		5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>Upland heath (TU 2009 )</b>							
<i>Hylocomium splendens</i> cover (C)	73	-2.0 %	-1.6 %	-1.2 %	-0.8 %	-0.4 %	-0.1
<i>Deschampsia flexuosa</i> (C)	37	-0.3 %	0.1 %	0.5 %	0.9 %	1.3 %	1.7
<b>Upland heath (MRS)*</b>							
<i>Hylocomium splendens</i> presence (P)	5	-1.3 %	-0.4 %	-0.2 %	-0.1 %	-0.1 %	-0.04
<i>Campylopus introflexus</i> (P)	2	0.0 %	0.02 %	0.04 %	0.07 %	0.10 %	0.13
<b>Lowland heath (TU 2009)</b>							
<i>Hylocomium splendens</i> (C)	31	-4.0 %	-1.1 %	-0.5 %	-0.3 %	-0.2 %	-0.1
<i>Hylocomium splendens</i> (P)	5	-0.4 %	-0.2 %	-0.1 %	-0.1 %	-0.1 %	-0.1
<i>Cladonia portentosa</i> (C)	11	-0.7 %	-0.3 %	-0.2 %	-0.2 %	-0.1 %	-0.1
<i>Cladonia portentosa</i> (P)	5	-0.5 %	-0.3 %	-0.2 %	-0.1 %	-0.1 %	-0.1
<i>Brachythecium rutabulum</i> (P)	5	0.0 %	0.1 %	0.1 %	0.2 %	0.2 %	0.3
<b>Bog (TU 2009 )</b>							
<i>Cladonia uncialis</i> (C)	1	-0.1 %	0.0 %	0.0 %	-0.01 %	-0.01 %	-0.01
<i>Cladonia uncialis</i> (P)	4	-0.8 %	-0.2 %	-0.1 %	-0.06 %	-0.04 %	-0.03
<i>Eriophorum vaginatum</i> (C)	65	1.5 %					
<i>Sphagnum fimbriatum</i> (C)	1	0.0 %	0.0 %	0.0 %	0.01 %	0.01 %	0.01
<i>Sphagnum fimbriatum</i> (P)	3	0.2 %	0.1 %	0.1 %	0.04 %	0.03 %	0.03
<b>Sand dunes (TU 2009 all sites)</b>							
<i>Hylocomium splendens</i> (C)	30	-3.6 %	-1.0 %	-0.4 %	-0.3 %	-	-
<b>Acid Grasslands (BEGIN)</b>							
<i>Hylocomium splendens</i> (C)	11	-1.4 %	-0.4 %	-0.2 %	-0.1 %	-0.1 %	0.0
<i>Hypnum cupressiforme</i> (C)	19	0.2 %					
<i>Nardus stricta</i> cover (C)	42	0.3 %					
<i>Carex panacea</i> cover (C)	13	0.1 %					
<i>Euphrasia officianlis</i> cover (C)	1	-0.1 %	-0.1 %	0.0 %	0.0 %	0.0 %	0.0
<i>Lotus corniculatus</i> cover (C)	11	-0.2 %	-0.1 %	-0.1 %	-0.1 %	0.0 %	0.0

### 6.3 Applicability of this work to pollutant (NO<sub>x</sub> and NH<sub>3</sub>) concentrations and critical levels

The work in this chapter has focused upon the relationship between nitrogen deposition, critical loads and species richness, however, it is recognised that the concentration of a pollutant and its critical level may also influence species responses. Critical levels were identified in order to protect particular species or groups of plants, for example the critical level for NH<sub>3</sub> is 3 µgm<sup>-3</sup> for vascular plants but 1 µgm<sup>-3</sup> for lower plants such as lichens. By contrast critical loads are habitat specific.

Nitrogen deposition data is based on measured concentration data (for wet deposition, ion concentrations in precipitation and the amount of rainfall, and for dry deposition, gas concentrations in air, measured at a specified height, usually 1.5 m from the ground surface, which represent an average over a defined period). Thus deposition reflects pollutant concentration and over the longer-term, vegetation responses to changes in deposition would be comparable to changes in pollutant concentrations of a similar magnitude. It would therefore be reasonable to use the data in this chapter to approximate the response of vegetation to a percentage increase in concentration and to compare responses between changes in long-term deposition and long-term mean concentrations. This could be done by converting an increment in concentration to an increment in N deposition. However, this would only be relevant over the longer-term, and it is important to understand that the differing effects between concentration and deposition over time are unclear. High pollutant concentrations in particular may be very damaging, especially for lower plants (Pearce and van der Waal, 2008).

At present, air concentrations of dry deposited gases are regulated through critical levels. Gaseous NH<sub>3</sub> and NO<sub>x</sub> concentrations can be measured relatively easily using passive samplers, normally exposed for one month, so that routine monthly measurements can be made to estimate an annual mean. However, the conversion of a gas concentration to an N load is not straightforward, as deposition velocity varies by species group, and meteorological conditions also affect deposition. In addition, the precise positioning of a new or expanding installation would also influence the frequency of high concentrations.

Much experimental evidence concerning the responses of a number of ecosystem types to changes in nitrogen deposition, mainly in form of wet NH<sub>4</sub>NO<sub>3</sub>, is available and we are confident that nitrogen deposition is in many cases the strongest driver of the changes in species richness and composition found in the survey data. However, the interactive effects of pollutant concentration and deposition are unclear and within the current report it was not the intention to attribute responses to specific forms of pollutant or changes in modelled concentration owing to co-correlation between these variables and the difficulty in separating out these across the survey sites.

Field experiments investigating the response of ecosystems to changing concentrations in wet deposition are rare and no contemporary experiments have studied the response of semi-natural ecosystems to changes in dry deposition of NO<sub>x</sub> at realistic concentrations, however, one notable UK experiment has studied the response of bog vegetation to gaseous NH<sub>3</sub>. The CEH Edinburgh Whim Bog experiment adds wet nitrogen deposition in oxidised and reduced N forms and dry-gaseous N deposition in the reduced form as NH<sub>3</sub> to a raised bog consisting mainly of ericoids, *Sphagnum* and cotton grass vegetation (Leith *et al.*, 2004; Sheppard *et al.*, 2004). Per unit N deposited, gaseous NH<sub>3</sub> has a much greater effect on soils and vegetation in comparison to similar deposition of the wet forms of N (Sheppard *et al.*, 2011). The stronger responses to gaseous NH<sub>3</sub> are thought to be linked to effects on the foliage which may in part reflect the intermittent high concentrations, although deposition is not linearly linked to concentration (Jones, 2006) and applying the critical level

of ammonia ( $1 \mu\text{g m}^{-3}$ ) to the bog provides greater protection than the critical load ( $5\text{-}10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ).

In conclusion, caution should be applied when using the approach developed in this report to legislate for small incremental increases in pollutant concentration as the incremental responses developed within this report are not directly comparable between deposition and concentration and the level of uncertainty from a direct conversion of load to level would be quite large. Projected increases in deposition are more appropriate in understanding the likely long-term change and mean levels of concentration could be converted to deposition to give an indication of the changes that would occur over many decades. However, particular care should be taken where installations are likely to produce high levels of a pollutant over short-timescales as these may be very damaging to vegetation within close proximity to the source.

With the exception of the Whim Bog facility, dose response relationships to changes in N concentration are not fully understood and should be further researched experimentally. Further work on the datasets used in this report could also be carried out to study responses to changes in  $\text{NH}_3$  concentration and deposition based on forthcoming 1 km gridded modelled pollutant datasets and the effect on bog vegetation based upon current experimental evidence. Given the commonalities between soil type and vegetation, this could also be used to experimentally support responses seen in some heathland systems and it may be possible to produce similar incremental relationships to those found with N deposition.

## 6.4 Summary

Even relatively small increases in long-term N deposition of  $1 - 2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  were found to have an impact on species richness across all habitats with the sand dune habitat appearing the most sensitive at low levels of N and the bog habitat the least. As N deposition increased upwards through the critical load range the rate of fall in species richness lessened but still existed and within the gradient of UK N deposition responses were still marked. These changes in rate of response reflected the curvi-linear relationships that were developed in Task 4.

Contrasting the falls in species richness, graminoid cover increased markedly with long-term N deposition and highlighted a potential threat to site integrity in the sand dunes, bogs and heaths. In the latter, cover increased exponentially and at some sites was above a level likely to cause concern and tip the balance between shrubs, lower plants and grasses.

The impacts illustrated in this chapter are calculated from the modelled relationships found in chapter 4. Only the relationships that were statistically significant were used to consider the impact of incremental N and in the most part these relationships were strong, however, with the exception of the acid grassland habitat, only 25-30 sites were visited within each habitat and this has produced some scatter within the data which is to be expected given the heterogeneous nature of site vegetation. The fact that the relationships found *were* so strong demonstrates the strength of the nitrogen signal in the data and if more sites were visited it is likely that the strength of the relationship would increase further.

The measures of species richness used in calculating the incremental effect of long-term N were obtained from a specific survey methodology that was consistently applied across all sites. The relationships produced are not rigid and they are not intended to be a precise prediction of the impact of incremental N although the direction of the impact of N (usually negative), the relative magnitude of the response and the shape of the response (often curvi-linear) would not be expected to change across an alternative selection of sites. In addition, many factors that impact biodiversity vary across the sites surveys including climate and other pollutants such as sulphur and these may also be correlated with changes in species



richness. It is important to remember that these, and the relationships used in this report are based upon correlations and which does not imply causation, however, the findings of this work are also supported by significant literature on the subject and work from experimental sites which control for N addition.

The data used in this chapter illustrates what the effect of long-term N deposition has been, and could be used in the assessment of the relative effects of incremental increases in N over the long-term from new sources at different background levels of N deposition. The impact of N on individual species should be more cautiously used as many factors may influence the response of a species at a particular site and the data on individual species within the surveys was limited.

## 7. Task 6: Applicability of results to other habitats with limited dose-response information

### 7.1 Introduction

This report has studied the responses of vegetation to long-term N deposition within data from five semi-natural UK habitats: acid grasslands, upland heath, lowland heath, bog and sand dunes, however, evidence suggests that other important UK habitats are also negatively affected by N. Task 6 will consider whether the results and relationships presented within this report can be reasonably applied to other habitats given similarities in soil type and vegetation. Focus will be given to Calcareous grasslands, vegetated shingle, fens and deciduous woodlands.

### 7.2 Comparisons of responses between habitats

Reductions in total species diversity were found in all the habitats studied in this report. Figure 15 below compares the response curves within each based upon the percentage of the maximum number of species at a given N deposition. Both heaths showed similar response gradients, and this reflects the changes in species richness illustrated in Task 5.

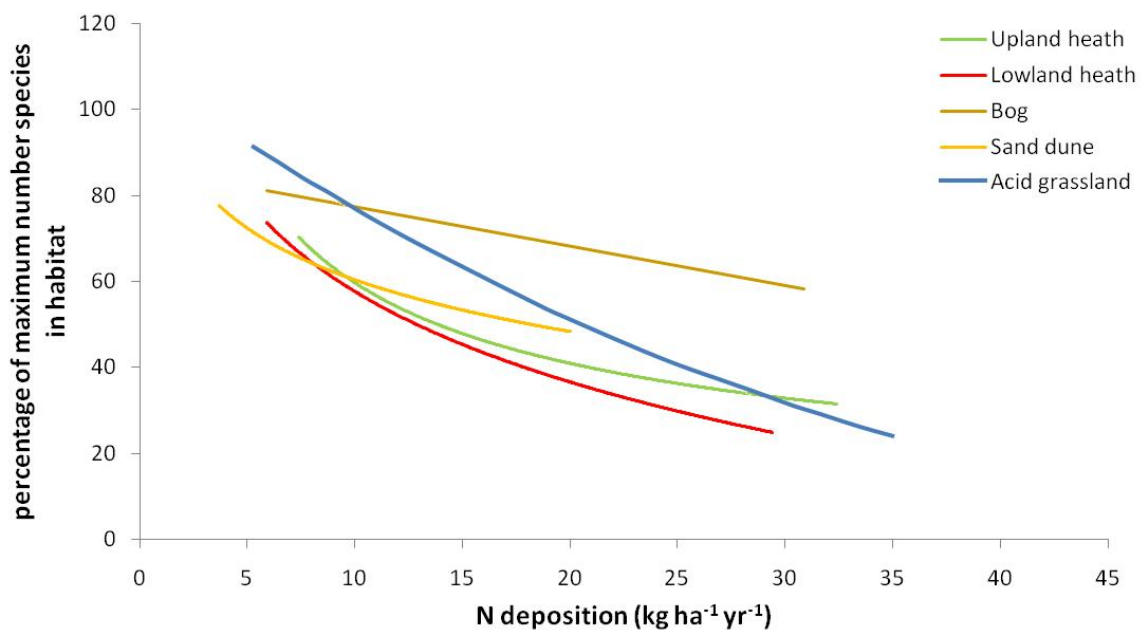


Figure 15: Modelled response curves showing the rate of change in species richness across the habitats studied as part of the TU 2009 multi-habitat survey.

Acid grasslands show a similar magnitude of response as the heaths, albeit less curvilinear, perhaps reflecting similarities in acidic, humus soil types. Changes in species richness in the bog habitat are shallower, indicative of the strong effects of hydrology limiting the response to N and a slightly less diverse habitat than the others. Sand dune response occurs more rapidly initially, then shallow over a much smaller range than the other habitats.

Whilst there is variability between the responses of the different habitats, the general magnitude of response is similar and when all the above data is considered together, as in Tasks 4 and 5, species richness reduces by 1.6% per 1 kg increase in long-term N. This broad approach could be used in similar habitats to those studied in this report, however, would be unsuitable for habitats which do not show change in species richness. An example

of the latter would be calcareous grasslands where survey work has found that species richness does not appear affected by N deposition but species composition does change and the frequency of rare or scarce plants reduces.

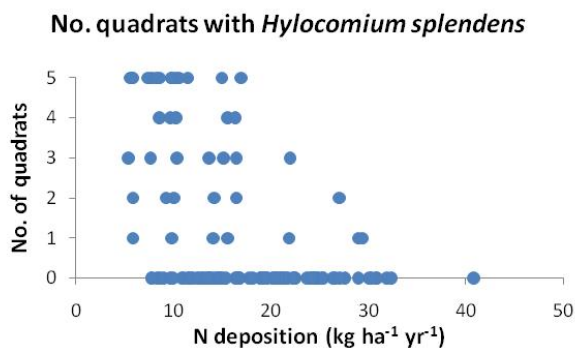


Figure 16. The presence of *Hylocomium splendens* across the habitats studied as part of the TU 2009 multi-habitat survey.

response to N across all the habitats except bogs, see Figure 16, with an abrupt reduction in probability of presence (number of quadrats the species was found in) above 17 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The lichen *Cladonia portentosa* was also consistently negatively associated with N deposition in both upland and lowland heath sites and the bog survey.

Task 3 showed that changes in overall diversity were often driven by changes in specific functional groups. Within upland heaths the reductions were apparent in total species richness, moss and lichens; in lowland heaths total species richness only; sand dunes moss species richness was strongly affected; in bogs forbs and lichens reduced and in acid grasslands forbs showed the strongest response. The lower plants, where present in significant numbers, are often the most sensitive to N. Of these, the moss *Hylocomium splendens* showed a remarkably consistent negative

### 7.3 Deciduous broadleaf woodland

Elevated nitrogen deposition has driven strong biogeochemical responses in woodlands with many authors documenting reductions in soil CN, acidification and increased nitrate leaching (Dise and Wright, 1995; Emmett *et al.*, 1998; Dise *et al.*, 2009). However, the impact of N deposition on vegetation composition is poorly understood partly due to the strong influence that tree canopy structure places on ground flora through interception of light, rainfall and pollution and the effect of woodland management and nitrogen deposition upon this structure.

Nevertheless, work has demonstrated that understory plants such as bryophytes, lichens and forbs can be negatively affected by N. Studies of mixed woodlands around four Scottish intensive livestock units showed marked changes in species composition within 300 m downwind of the units (Pitcairn *et al.*, 1998; Pitcairn *et al.*, 2009), the grasses *Deschampsia flexuosa* and *Holcus lanatus* increased in abundance close to the units as did the shrub *Rubus idaeus* and the forb *Urtica dioica*. Mosses in general were found to decrease in abundance downwind of the farm as did the forbs *Oxalis acetosella*, *Galium odoratum*, *Potentilla erecta* and *Dactylorhiza fuchsii*. A much broader scale survey of 103 woodlands in 1971 and revisited in 2001 found that overall species richness was unaffected by N but changes in composition were found with some species responding positively to N (*Poa nemoralis/trivialis*, *Galium aparine*, *Allium ursinum*, *Athyrium filix-femina*, *Carex pendula*, *Urtica dioica*) and others negatively (*Deschampsia flexuosa*, *Agrostis capillaris*, *Ajuga reptans*, *Holcus lanatus*, *Pteridium aquilinum*, *Vaccinium myrtillus*). The lack of an overall response in species richness was attributed to three main reasons: 1) much woodland ground flora tends towards the upper and middle of the Ellenberg spectrum; 2) impacts on woodlands may be from localised ammonia sources and unapparent over a national N gradient and 3) the interaction of canopy shading damping responses of the lower plants to N (Kirby *et al.*, 2005).

Experimental work in Sweden well summarised by Cunha *et al.* (2002) found that N addition altered the composition of species towards *Deschampsia flexuosa* and ruderal species. In

addition bryophyte abundance changed with increases in some *Brachythecium* species and reductions in others including *Hylocomium splendens*. These effects are similar to those found in the datasets studied in this report. Other studies have shown detrimental effects of N deposition on growth of bryophyte species *Isothecium myosuroides*, *Dicranum scoparium*, *Frullania tamarisci* in transplant experiments between Atlantic Oak woods (Mitchell *et al.*, 2004) and shifts in species composition of epiphytic lichens between nitrophytes and acidophytes (Sutton *et al.*, 2009).

A recent study has attempted identify the contribution of N deposition to vegetative change in addition to changes in woodland structure and age. Verheyen *et al.* (2012) studied data from over 1200 vegetation plots and found a shift towards shade tolerant and nutrient demanding species, however, N deposition did not explain these responses. Verheyen *et al.* (2012) concluded that the effects of N deposition could be obscured by changes in the tree canopy and highlighted a potential N 'time bomb' which could explode when the canopy opens up again.

Some of the species responses highlighted by the above studies showed a significant response in the survey datasets in this report including the grass *Deschampsia flexuosa* which increased in cover in upland heaths supporting the findings by Pitcairn *et al.* (1998) but contradictory to Kirby *et al.*, (2005), and the mosses *H. splendens* and *Brachythecium* species which showed respective decreased and increased in presence. This lack of an overall relationship between species richness and N deposition makes it difficult to assume a dose-response relationship to broad-scale N deposition in woodlands over a national gradient, however, it seems likely that the edges of the woodlands are likely to be more strongly affected by a nearby pollutant source such as an intensive livestock farm (Kirby *et al.*, 2005).

## 7.4 Vegetated shingle

Vegetated shingle is an important habitat for conservation in the UK (Natural England, 2011) yet there is limited knowledge of its responses to N deposition and no experimental data from this community is available for analysis. Vegetated shingle community species composition shifts from pioneer species close to the sea shore able to tolerate high salinity and sea spray to more stable gravel communities consisting of grassland with important moss and lichen assemblages (UK BAP, 2008). In the survey datasets studied in this report the closest analogue are the acidic dune grasslands (sites with pH <6.5) which share their dry, acidic nature with the gravel communities of vegetated shingle.

Results from analysis of the acidic dune grasslands in this report suggests that nitrogen deposition (in the form dry-oxidised N) alongside climate plays an important role in determining species composition but no overall relationship between species richness and N deposition was found. This lack of a relationship between N and species richness is likely due to the limited number of acidic-dune sites that were surveyed (only 9). However, a recent study of 19 coastal dunes around the Baltic Sea on acid soils found that high N deposition increased growth of the sedge *Carex arenaria* and reduced the species richness of lichen, grass and forb species (Remke *et al.*, 2009). These responses were strongest in the sites with the lowest pH suggesting the influence of acidification on species richness and the ability for *C. arenaria* to dominate under these conditions. The acidic-dune grassland survey data within this report showed that cover of *C. arenaria* appears to increase with N deposition although the number of data points is limited and the relationship non-significant. Moss species richness was most strongly correlated with Sulphur (S) deposition (in dunes with pH <6.5) and a correlation with N deposition was almost significant; in general a greater number of moss species were present at lower levels of N deposition. As discussed elsewhere in this report it is difficult to ascertain if this relationship with S is ecologically

significant, however, both N and S deposition are likely to increase acidity and may be responsible for the decline in moss species richness and concurrent increases in *C. arenaria*.

Whilst caution should therefore be applied in attempting to extrapolate relationships between species richness and N deposition from the sand dune survey data presented in this report, it would seem reasonable to assume that the widespread reductions in species richness observed across all habitats, particularly in lower plant species, would also occur in vegetated shingle, especially above the recommended critical load range of 8-15 kg ha<sup>-1</sup> yr<sup>-1</sup>.

## 7.5 Fens

Fen nutrient budgets are characterized by inputs and outputs of nutrients via groundwater and surface water, and are tightly linked with local hydrology. The extent to which these systems receive and lose nutrients with in- and out-flowing water determines for a large part their sensitivity to excess N (ECE, 2010). Open wetland ecosystems such as floodplain fens would be expected to show little sensitivity to deposition of atmospheric N, whereas the impact on fens with a closed N cycle is likely to be much more significant. Fens with lowered water tables, as a result of drainage or over-abstraction, may also exhibit increased nutrient availability through decomposition of surface layers following drying, so knowledge of hydrological status of sites is important in assessing impact and risk.

Fens are found across a wide range of base cation and nutrient levels, from acid to strongly alkaline, and from oligotrophic to eutrophic. In all of these fen types, elevated N deposition has been found to increase cover of vascular plants including graminoids and dwarf shrubs and reduce cover of bryophytes (ECE, 2010). The vegetation of acidic, oligotrophic fens closely resembles the bog habitat studied in this report however; the critical load for this habitat has been estimated to be higher at 10-15 kg N ha<sup>-1</sup> yr<sup>-1</sup> compared with 5-10 kg N ha<sup>-1</sup> yr<sup>-1</sup> for bogs, due to the assumed higher buffering capacity of these minerotrophic fens. It has been recommended that the upper end of this range is used for soligenous, acidic valley mires, while the lower end of this range is used for quaking bogs and transition mires (ECE, 2010), which are in many cases functionally ombrotrophic systems.

Experimental work on a mesotrophic fen in Ireland (Verhoeven *et al.*, 2011) has demonstrated significant responses to N additions of 35 and 70 kg ha<sup>-1</sup> yr<sup>-1</sup> on top of a low background N of 4-8 kg N ha<sup>-1</sup> yr<sup>-1</sup> and particularly to reduced-nitrogen. In these plots, vascular plant biomass increased strongly (from 170 g m<sup>-2</sup> to 340 g m<sup>-2</sup>) and bryophyte cover reduced considerably (from 350 g m<sup>-2</sup> to 60 g m<sup>-2</sup>) as did bryophyte species richness (from an average of 7 to 4). The authors attributed these changes in bryophytes to a combination of increased vascular plant cover and the direct toxic effect of ammonia. Other studies have also found N related increases in vascular plant biomass and shifts in vegetation composition in both mesotrophic and oligotrophic base-rich Dutch fens (Verhoeven and Schmitz, 1991; Verhoeven *et al.*, 1996; Paulissen *et al.*, 2004). Only limited evidence is available for the impacts of N on base-rich fens and the recommended critical load spans a wide range (15-30 kg N ha<sup>-1</sup> yr<sup>-1</sup>). The lower end of this range is recommended for oligotrophic types, such as montane fens,

These responses found in fens closely mirror those found in the bogs habitat survey and by the Whim Bog experiment discussed earlier in this report. It would seem reasonable therefore, given strong similarities in vegetation composition, soil type and hydrological regime to apply the relationships found in the bog survey data also to oligotrophic and mesotrophic fens, particularly in reference to changes in bryophyte, lichen and sedge cover and species richness around intensive livestock units with the caveat that the slightly higher

pH may offer marginally greater protection from acidification, however, not from eutrophication.

## 8. Discussion and overall conclusion

The objective of this report was to determine the effects of incremental increases in long-term nitrogen deposition on species composition and richness for a range of different habitat types. This was carried out by examining recent vegetation survey data in order to understand the relationships that exist between species composition and richness and nitrogen (N) deposition.

Tasks 1 and 2 studied the available datasets and sought other supporting data that existed from N addition experiments and in recent published literature. The survey data chosen consisted of data from 226 sites, across 8 surveys and 5 UK priority habitats encompassing the Terrestrial Umbrella (TU) 2009 multi-habitat survey, the BEGIN UK Acid Grassland dataset, the 2006 TU Moorland Regional Survey (MRS) and a 2002 Sand dune survey.

Task 3 then used statistical techniques to understand the ecological responses within the survey data for each habitat, be it changes in overall species composition, species richness, functional groups or individual species abundance, and related these to key environmental variables including climate and pollutant deposition data. Table 23 below summarises the responses variables that were significantly related to nitrogen deposition resulting from this task.

Table 23: Summary of the response variables found during the statistical analysis of vegetation datasets in Task 3 that were significantly related to atmospheric nitrogen deposition. MRS = Moorland Regional Survey.

Response variable	Acid grassland	Bog	Upland heath	Lowland heath	Sand dune
Species composition	#	#	#	#	#
Total species richness	#	#	#	#	#
Bryophyte species richness			# (MRS only)	#	#
Lichen species richness		#	#		
Forb species richness	#	#			#
Graminoid species richness			#	#	
Graminoid cover		#	#	#	#

Task 4 examined the nature of the relationship between total species richness, the relevant functional groups and key individual species and N deposition. In many cases a strong, curvi-linear relationship existed between N and the response variables. This means that sites located in areas of low background N deposition responded to additional nitrogen with a greater fall in species richness than sites located in more polluted parts of the country. However, contrasting this, graminoid<sup>2</sup> cover *increased* with increasing N deposition in bog, heath and sand dune habitats. Depending on the specific graminoid species affected, and the balance between graminoids and other functional groups, this could have a negative effect on the condition of the site and prevent the site achieving its conservation objectives. This task also examined the data for levels or ranges of pollution over which an ecosystem appeared to be more sensitive and whilst these results are difficult to interpret it was

<sup>2</sup> Graminoid functional group includes species from the grass, sedge and rush families.

apparent that much ecological change occurred between approximately 14 to 23 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

Task 5 used the relationships developed within Task 4 and quantified them in terms of change per x kg long-term N. This information could be used to understand the likely effect of additional increments in long-term nitrogen deposition (e.g. from a new pollution source) and assist with decisions making under the planning and/or environmental permitting regimes. It was apparent that even though a curvi-linear response dominates the relationship between the response variable and N deposition, significant falls in species richness were modelled for increases in long-term N deposition levels above the upper end of the critical load. Similarly, the positive relationship between graminoid cover and N in the sand dune, heath and bog habitats was reflected by marked increases in cover of grasses and sedges at the higher levels of long-term N. These simultaneous events may be interactive, with graminoids (and shrubs to some degree) enjoying a competitive advantage as N increases and shading out forbs and lower plants, or be a direct toxic effect of N on the lower and less nutrient-tolerant plants.

Consequently, the data suggests that habitat quality is affected across the UK N deposition range. The decline in species richness commences at the low end of the N deposition range and by upper end of the critical load range a substantial loss has already occurred. This reduces the inherent biodiversity of habitats through the loss of more sensitive and rare species. At higher loads of long-term N deposition beyond the critical load range, the integrity of sites may be threatened by graminoid domination and structural change to the habitat. Furthermore, all of the data sets examined as well as the reviewed published studies, show that at high rates of long-term N deposition, above for example 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> (i.e. beyond the upper range of all critical loads of the communities addressed in this report) there is significant decline in species richness. Table 24 below summarises the relationships found in the vegetation datasets in Task 4 and indicates where these findings are supported by N addition experiments and the literature reviewed in this report. It should be highlighted that many of these responses are also supported by the broader scientific literature.

Table 24: Summary of the key findings from the analysis of the vegetation datasets and supporting reviews of literature and experimental site work. <sup>1</sup>Details of the UKREATE experiments in this report is presented in Tasks 2, 3 and 4, summarised in Phoenix *et al* (2012) and additionally in the literature cited in the table below. <sup>2</sup>For details of studies included in the literature review see Tasks 2 and 3 and the references in the table below.

Habitat	Response curve shape from data analysis in this report	Direction of change	Supported by UKREATE experiments <sup>1</sup>	Literature <sup>2</sup>
<b>All habitats</b>				
Total species richness (SR)	linear	↓	#	#
<b>Upland heath</b>				
Total SR	mild curvilinear	↓	<i>lichens and bryophytes only</i>	Edmondson <i>et al</i> , 2010; Stevens <i>et al</i> , 2009; Maskell <i>et al</i> , 2010; Payne <i>et al</i> , 2014
Lichen SR	mild curvilinear	↓	Carroll <i>et al</i> , 1999; Pilkington <i>et al</i> , 2007	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014
Graminoid SR	mild curvilinear	↓	<i>no</i>	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014
Graminoid cover	mild curvilinear	↑	<i>no</i>	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014



Habitat	Response curve shape from data analysis in this report	Direction of change	Supported by UKREATE experiments <sup>1</sup>	Literature <sup>2</sup>
<b>Lowland heath</b>				
Total SR	mild curvilinear	↓	no	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014
Moss SR	mild curvilinear	↓	no (but lichen cover reduced)	
Graminoid SR	mild curvilinear	↓	no	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014
Graminoid cover	mild curvilinear	↑	Barker <i>et al</i> , 2004	Southon <i>et al</i> , 2013; Field <i>et al</i> , 2014
<b>Bog</b>				
Total SR	linear			
Lichen SR	mild curvilinear	↓	cover only (Sheppard <i>et al</i> , 2011)	Field <i>et al</i> , 2014
Forb SR	mild curvilinear	↓		
Graminoid cover	linear	↑	(Sheppard <i>et al</i> , 2011)	Field <i>et al</i> , 2014
<b>Sand dune</b>				
Total SR	mild curvilinear	↓	no	Jones <i>et al</i> , 2004; Field <i>et al</i> , 2014
Moss SR	strong curvilinear	↓	biomass only (Plassman <i>et al</i> , 2009)	Remke <i>et al</i> , 2009; Field <i>et al</i> , 2014
Forb SR	mild curvilinear	↓	no	Remke <i>et al</i> , 2009; Field <i>et al</i> , 2014
Graminoid cover	mild curvilinear	↑	no	Jones <i>et al</i> , 2004; Remke <i>et al</i> , 2009
<b>Acid grassland</b>				
Total SR	curvi-linear	↓	no	Stevens <i>et al</i> , 2004; Maskell <i>et al</i> , 2010; Stevens <i>et al</i> , 2010
Forb SR	linear	↓	no	Stevens <i>et al</i> , 2006

Some individual species appeared to show a response at a specific point in the N deposition range, for example presence of *Hylocomium splendens* declined sharply above 17 kg N ha<sup>-1</sup> yr<sup>-1</sup>, whilst overall species richness changed more gradually. It is possible that many more species have a range of N pollution over which they can thrive up to a particular deposition load, however, the relatively small datasets used in this analysis has meant that some of the rarer species within each habitat are present at only a small number of locations. In these cases it was not possible to develop a mathematical relationship with N but each individual species contributed to an overall change in species composition and gradual fall in species richness. The relatively small datasets mean that caution should be applied when drawing conclusions on site integrity based on the presence or absence of individual species and that this information be used in conjunction with changes in species richness and composition.

Equally, some species groups responded in certain habitats (see Table 21) but not others. The reasons for these differences are not immediately obvious as many factors interact to govern how a habitat or species responds. In some cases the effect of a pollutant may be direct, for example a particular plant may be intolerant of N, and in others it could be a competitive interaction as discussed above. In addition to eutrophication and its fertilisation effect, N deposition can also acidify the soil. This could cause a response in rooted plants (and less in bryophytes and lichens), particularly in the poorly-buffered acidic habitats and less so in well-buffered calcareous habitats.

Due to a lack of root structure the lower plants are more directly responsive to current N and are likely to respond quickly to increases in pollution, whilst changes in the dominant species present within the vascular plant community are likely to occur more slowly in response to changes in soil N. Management interaction such as grazing can also alter the competitive interaction between lower plants and faster growing higher plants through competition for light. The relationships determined in this report were based upon modelled recent annual nitrogen deposition, and no attempt was made to consider long-term cumulative N deposition which occurs over many years. Whilst current day N deposition can be used as a good proxy for long-term deposition and gives a strong indication of changes with regard to N deposition, it would be advised to further consider pollution over the longer-term. However, the overall findings of this report would not be expected to change dramatically if cumulative N deposition is used instead of present day N deposition.

These alternative mechanisms for change mean that species richness is a better indicator of N deposition in some habitats and changes in species composition more suitable in others. An example of the latter is the Calcareous Grassland habitat where existing survey work (Van den Berg *et al.*, 2010) has suggested that species richness is not affected by increasing N but found changes in species composition and the less presence of rare or scarce species as N increases. New data studying the Calcareous Grassland habitat has been obtained as part of recent survey work by the BEGIN project and this should be reviewed to develop understanding further.

Whilst the general trend in reducing species richness as N increases holds true across the habitats that were studied in this report, not all of the habitats behaved in the same way. The bog habitat is probably affected more strongly by site hydrology whilst in sand dunes, precipitation and decalcification were the more dominant drivers. For bogs, this means that the species richness response to N is buffered by the hydrological status and the response curve is shallower per unit N than the habitats that are more freely drained. The approach used to predict the response to incremental N can still be used, however, sites with lower rainfall may be more sensitive to N and the relationships expressed in this report for the bog habitat should be regarded as conservative. For sand dunes, site location and rainfall drive acidity with the more acidic sites being less biodiverse. This situation is complicated by the fact that N (and sulphur) deposition can also acidify a habitat. The relationships found in Task 5 can still be used to understand the likely impact, however, limited data is available for sites with a pH less than 6.5 and a conservative approach would be to use the relationships obtained for the largest datasets. These are the TU 2009 all site dataset and the combined TU 2009 and 2002 surveys.

Task 6 used the relationships developed from the survey data and considered if they may be reasonably applied to habitats where significant data is not currently available. Many UK ecosystems share similarities in species and soil type and it seems likely that where this is the case similar responses to those found within this report would occur. However, local site conditions, management interactions affecting canopy structure, and natural variation within a habitat should be carefully considered when applying these relationships to a new habitat. Gaps in data do exist and mean that there remain large habitat types in the UK where the impact of N deposition is not fully understood, for example, woodlands, fens, vegetated shingle and mesotrophic grasslands and further work. Experimentally work and gradient studies should be performed to understand the changes that occur with increases in N. The incremental effect of changes in pollutant concentration is also not fully understood as the differing effects between concentration and deposition over time are unclear. High pollutant concentrations in particular may be very damaging, especially for lower plants and these should be further researched experimentally. Further work on the datasets used in this report could also be carried out to study the evidence of response to changes in NH<sub>3</sub> concentration and deposition based upon current experimental work.

The overall goal in the management of pollution deposition levels should be one of reduction but it should be stressed that lowering current N deposition may not reverse the declining trend in species richness. Indeed it is likely that lowering current N deposition may only slow the decline in species richness. Considerable N remains stored within the soil and management such as cutting or burning may only remove small amounts of the N pool. However, as the rate of decline may be slowed by a reduction in N, management may help tip the competitive balance in favour of lower plants that are not directly affected by the pollutant.

The effects of N deposition extend beyond its impact on biodiversity and species composition. Many experiments have demonstrated an impact on soil biodiversity, ecosystem services and the onset of leaching. Depending upon the ecosystem studied and the timescale of deposition, these responses can have broader implications upon water quality and aquatic systems. The focus of this report has been the study of botanical datasets, further work should be carried out to quantify the effects on incremental N on soils and biogeochemistry. The relationship between N concentration and ecosystem response is poorly understood and further work should be carried out experimentally to develop our knowledge.

The main weaknesses of the analyses performed in this report fall in two main areas. Firstly, due to the time-consuming survey methods used to obtain the data, and with the possible exception of the acid grassland habitat, the size of the datasets is inevitably limited. However, the fact that N deposition still comes through the analysis reflects its strength as a driver of change. Secondly, across the large north-south pollution gradient that exists in the UK a similar climatic gradient also occurs, with cooler, wetter sites in the north and warmer, drier sites in the south. The design of the surveys and choice of site locations attempted to cross these climatic boundaries as much as possible within a given habitat type by also choosing cooler, dryer sites and warmer, wetter sites. However, in a gradient survey it is difficult to completely separate the simultaneous and interactive effects of climate and pollutant.

Overall, in all of the analysis of species composition and species richness, N comes through as a strong driver of change and in many cases is the strongest driver. The fact that this does happen adds considerable assurance to the belief that N is driving considerable change within our semi-natural habitats. This is supported with many years of experimental manipulation of N with climatic change controlled for within the main habitats studied. This provides confidence in using the data presented in Task 5 of this report to understand the relative effects of incremental increases in N over the long-term from new or existing sources at different background levels of N deposition.

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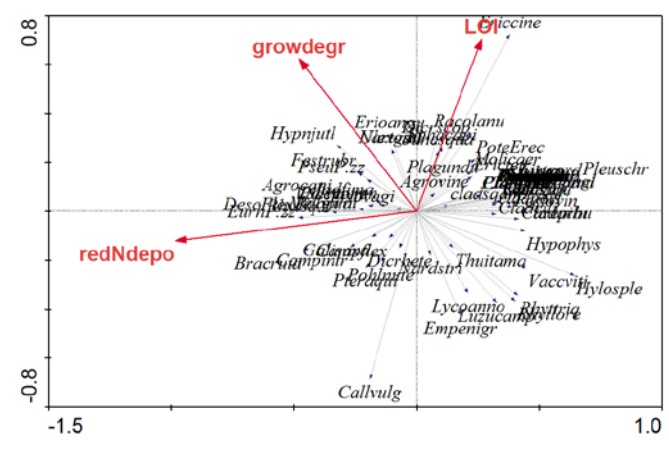
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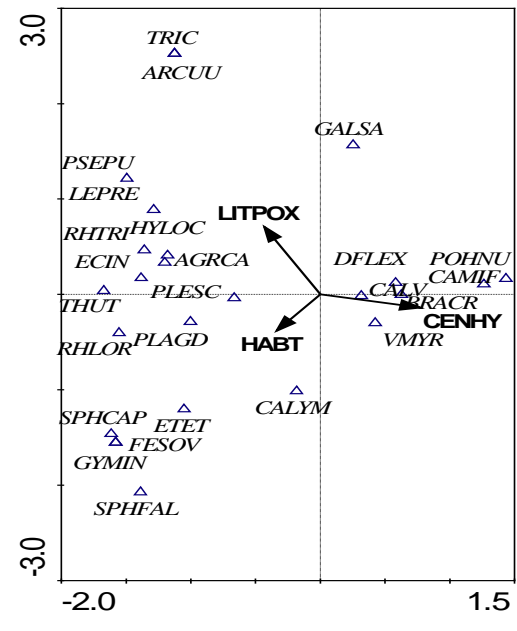
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# Appendix 1: Key ordination plots

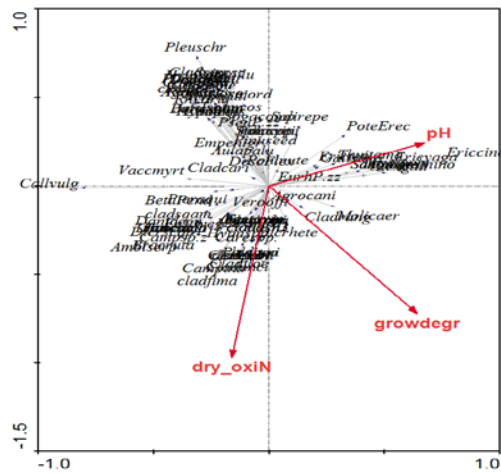
## Upland heaths (TU 2009): RDA ordination plot



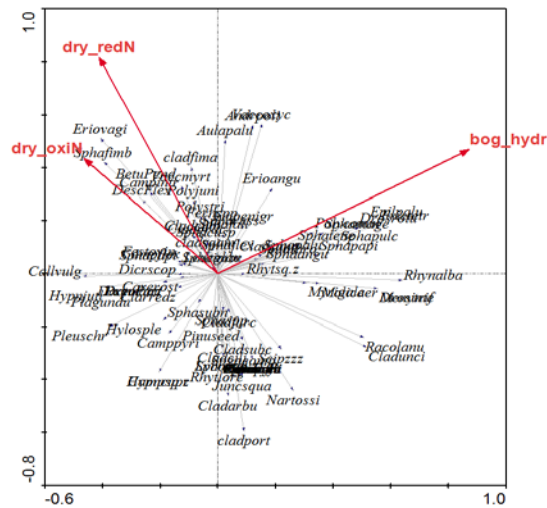
## Upland heaths (MRS): CCA ordination plot



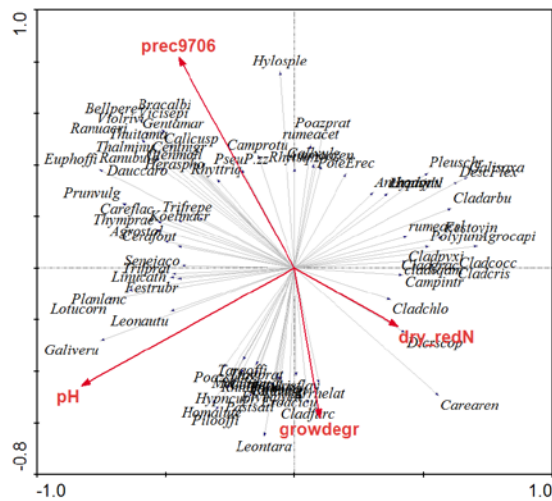
### Lowland heaths (TU 2009): RDA ordination plot



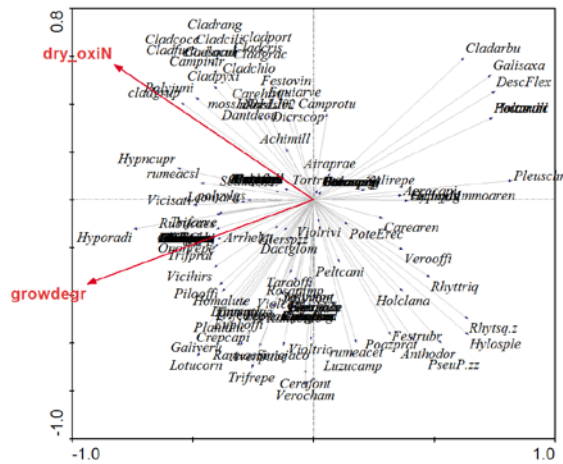
### Bog (TU 2009): RDA ordination plot



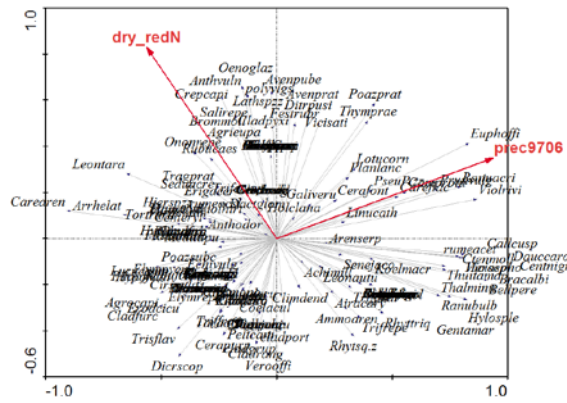
### Sand dune (TU 2009 all pH): RDA ordination plot showing response of species with 15% minimum fit to axes



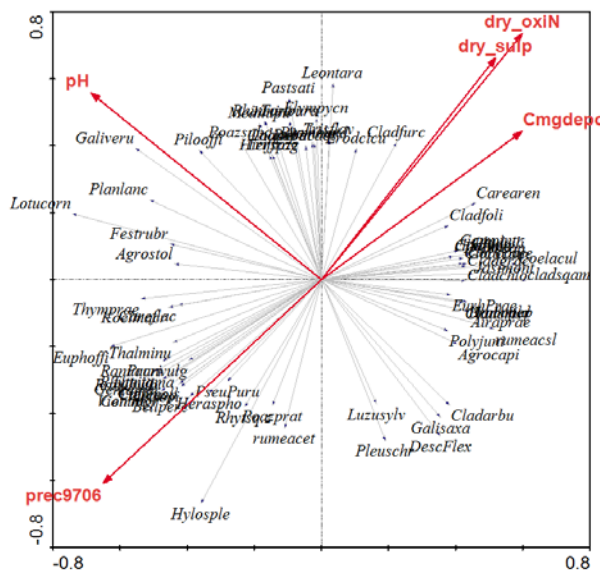
### Sand dune (TU 2009 pH < 6.5): RDA ordination plot



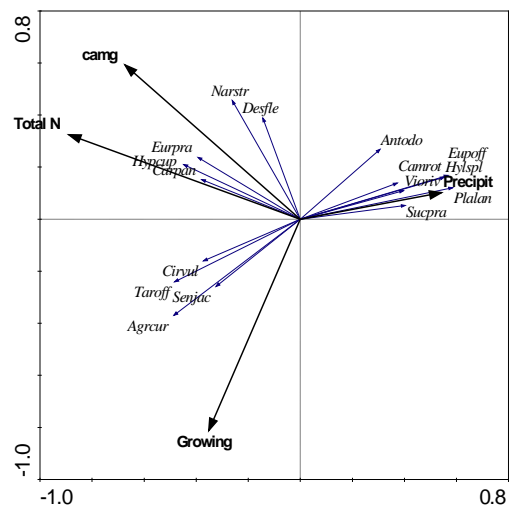
### Sand dune (TU 2009 pH ≥ 6.5): RDA ordination plot



### Sand dune (TU 2009 all pH plus 2002 fixed-dune grasslands): RDA ordination plot showing response of species with 15% minimum fit to axes



# Acid grasslands (BEGIN): RDA ordination plot

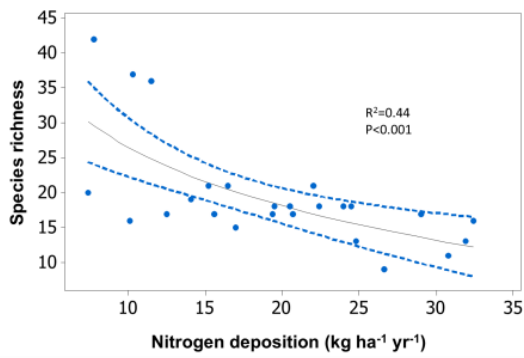


## Appendix 2: Species richness nitrogen response curves.

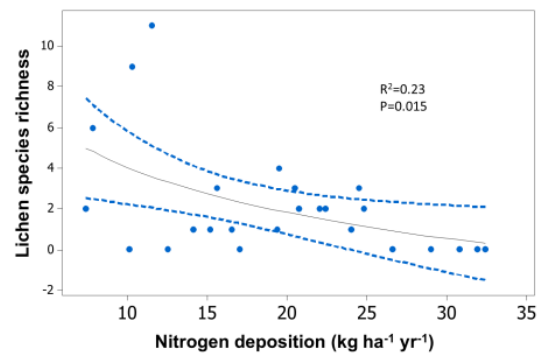
Only those with a significant relationship to nitrogen deposition are shown.

### Upland heath: TU Survey 2009

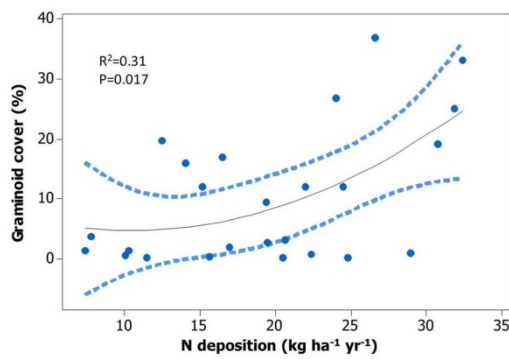
Upland heath: total species richness



Upland heath: lichen species richness

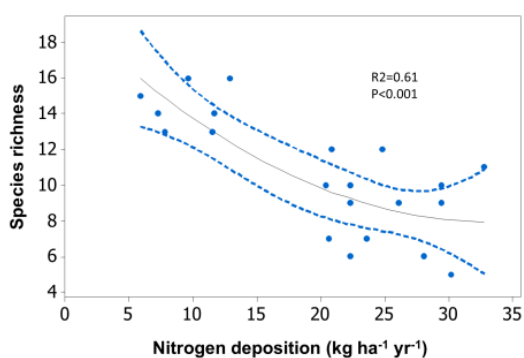


Upland heath: graminoid cover

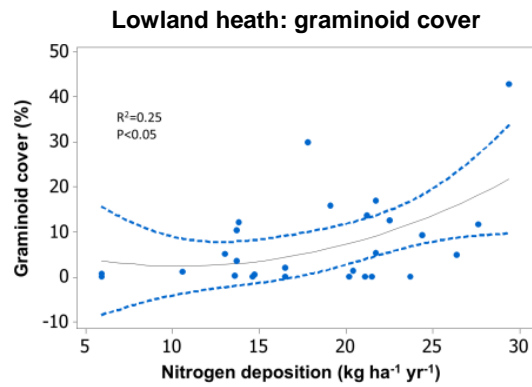
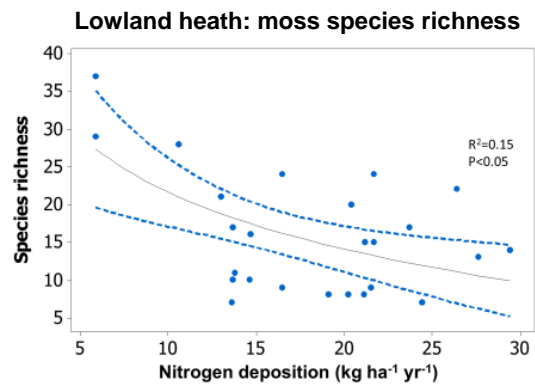
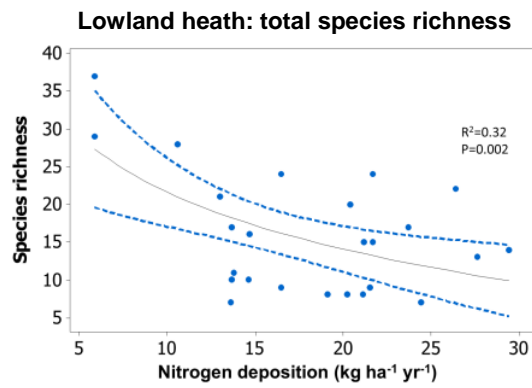


### Upland heath: MRS

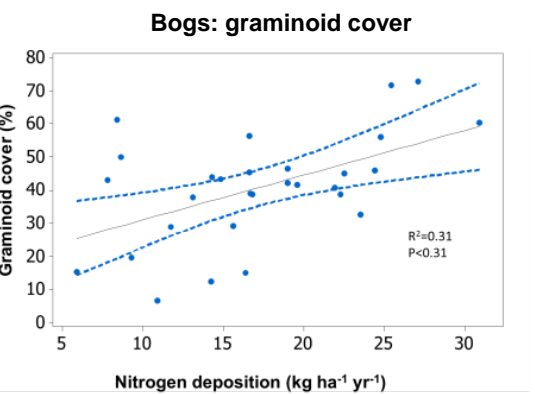
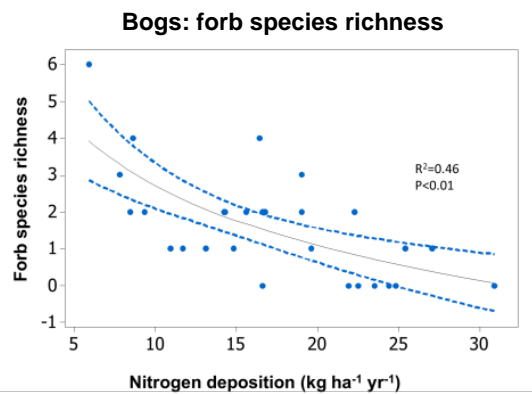
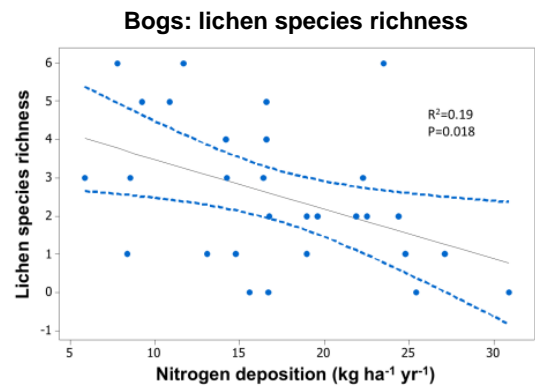
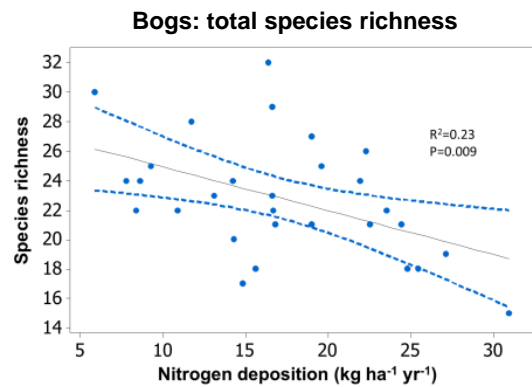
Upland heath: total species richness



## Lowland heath: TU Survey 2009

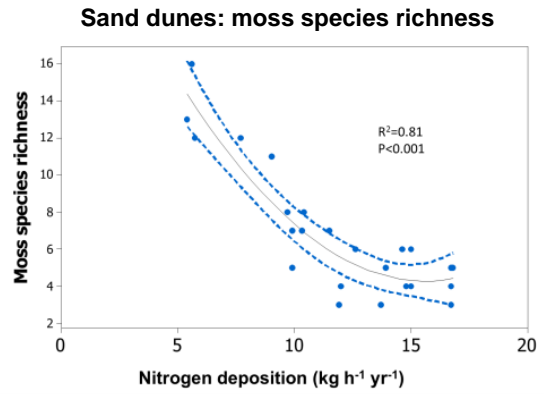
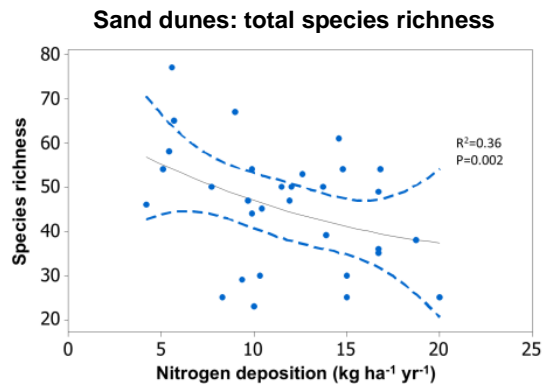


## Bog: TU Survey 2009

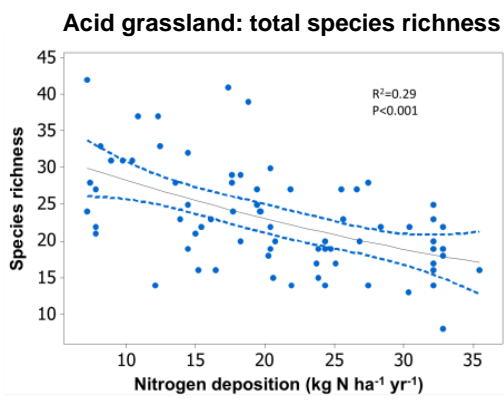




## Sand dunes (TU 2009 all pH)

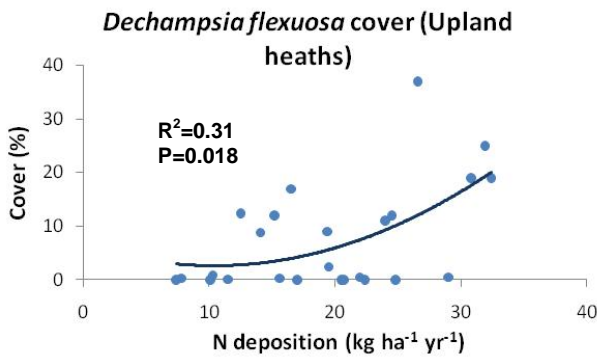
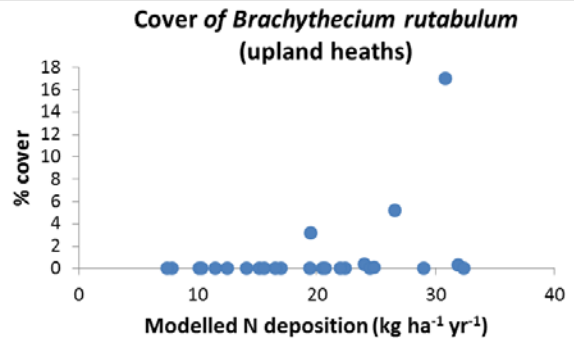
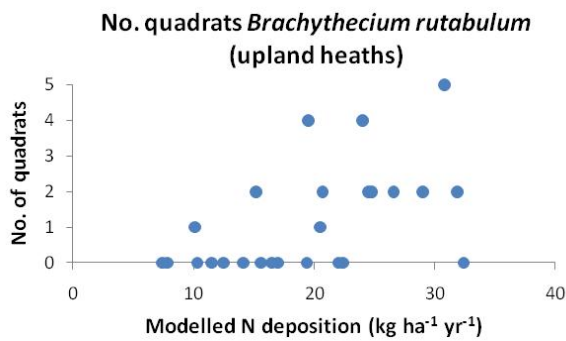
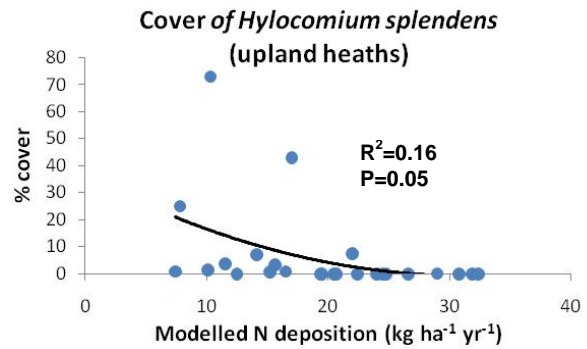
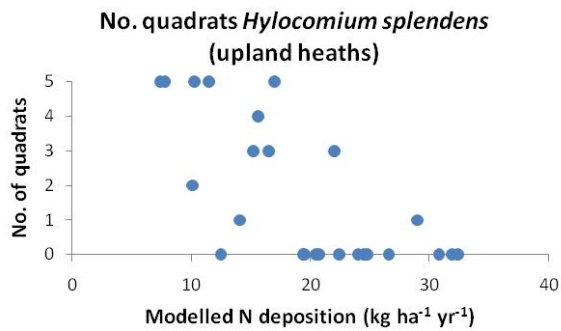


## Acid grasslands (BEGIN)

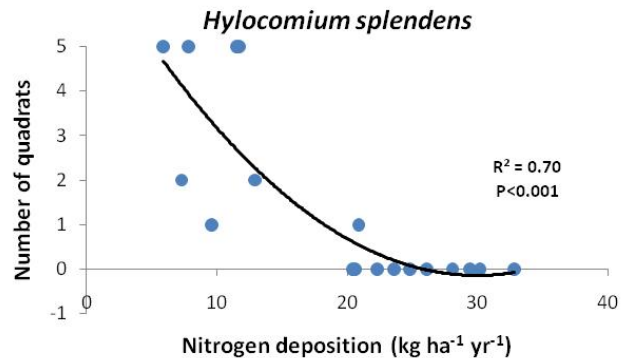
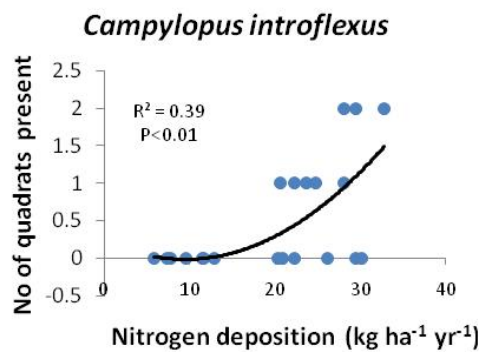


# Appendix 3: Individual species N response curves

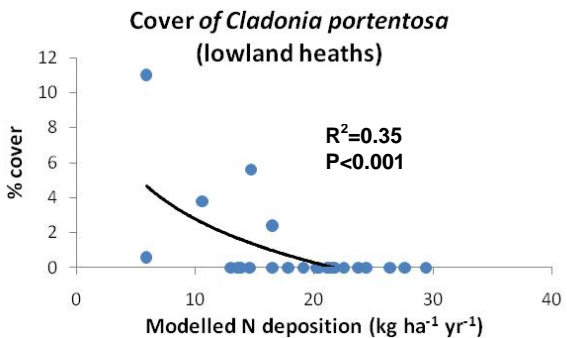
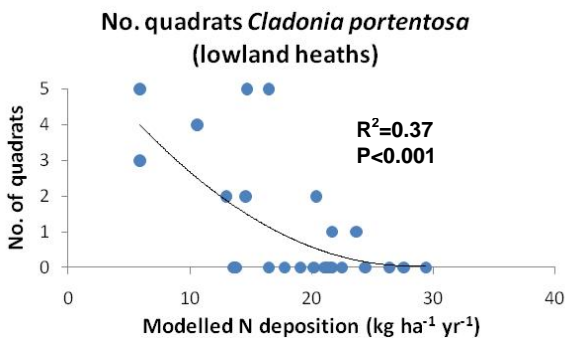
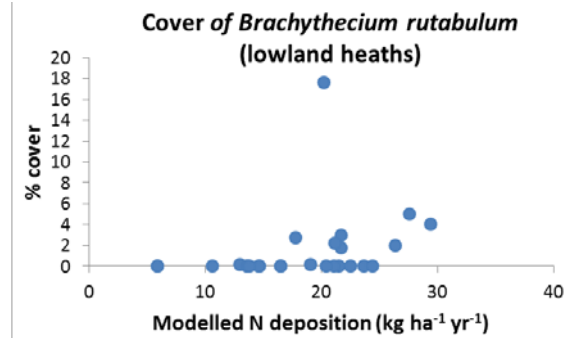
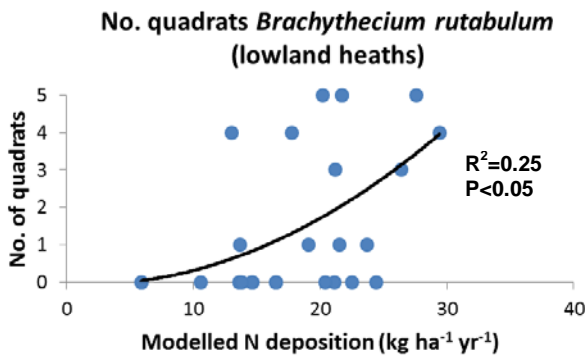
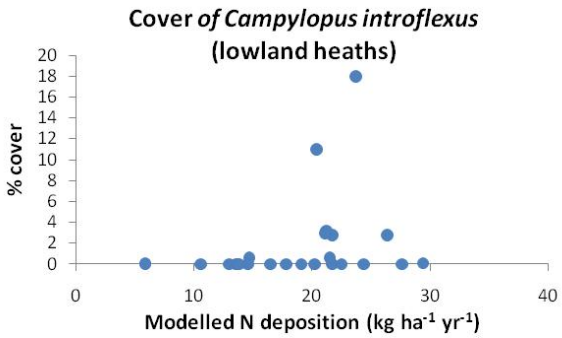
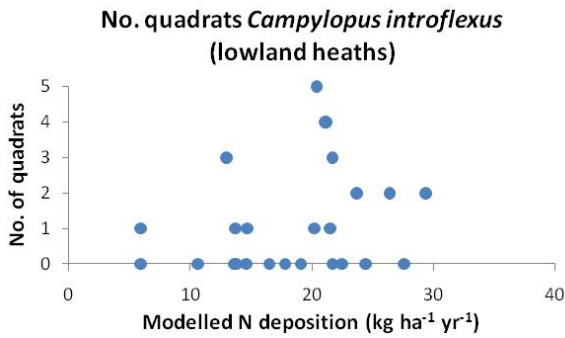
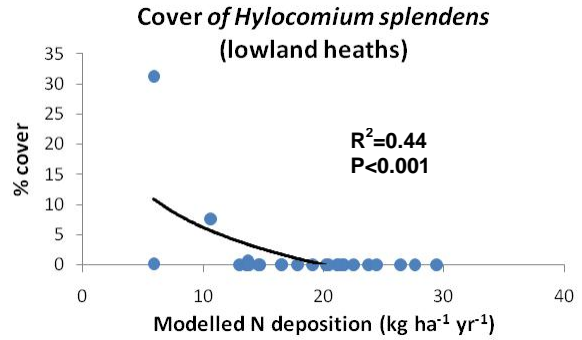
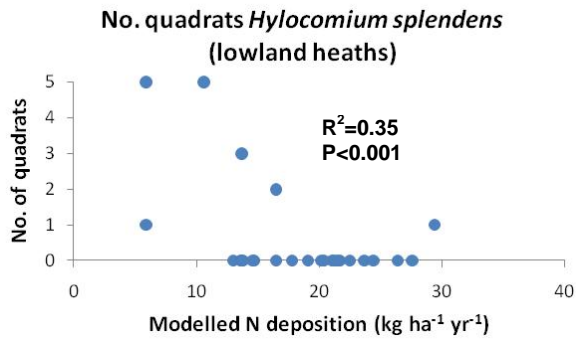
## Upland heaths: TU 2009

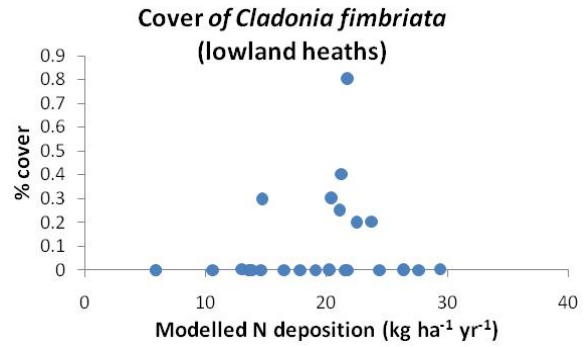
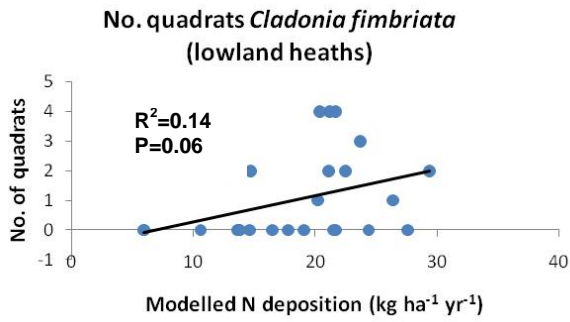


## Upland heaths (MRS)

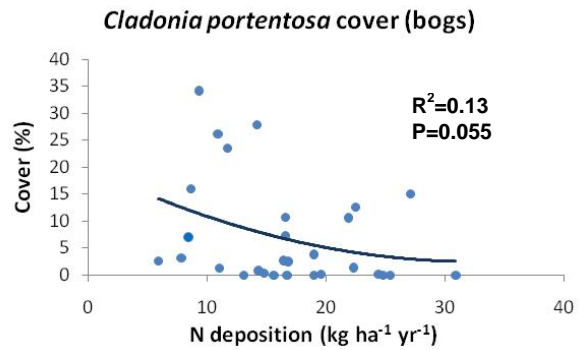
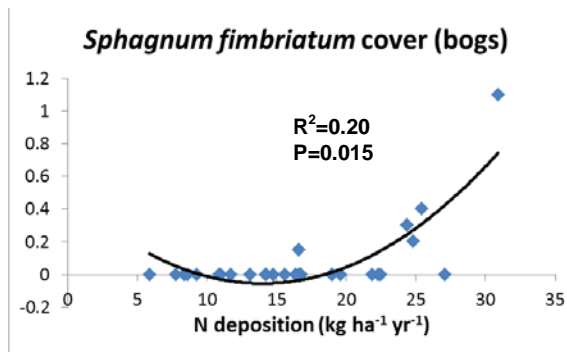


# Lowland heaths: TU 2009

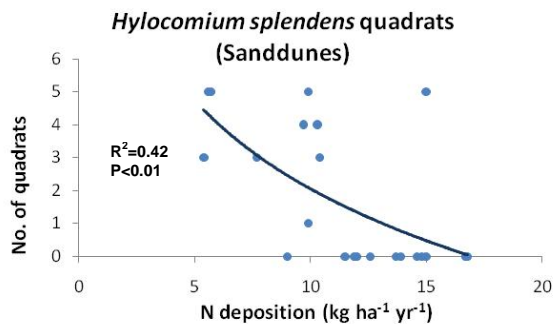
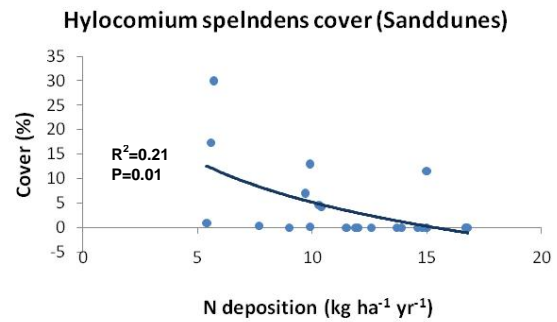
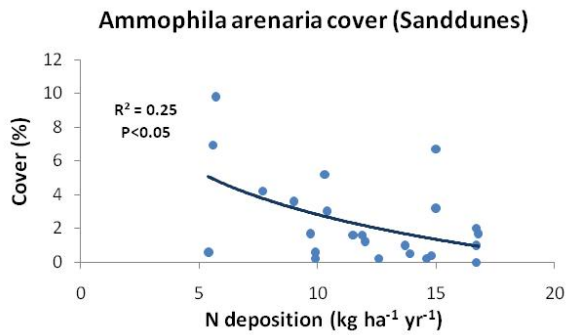




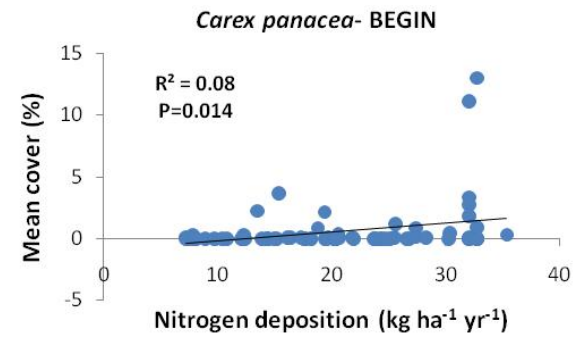
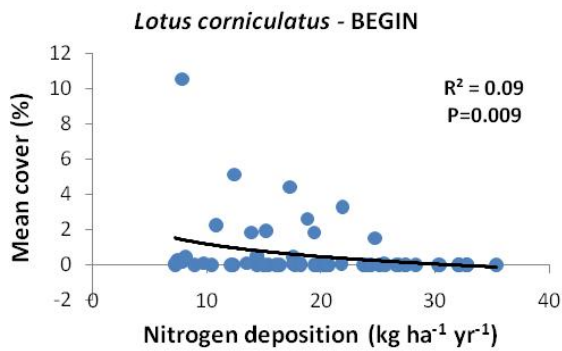
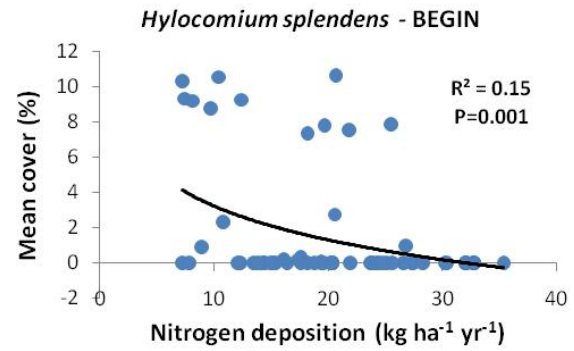
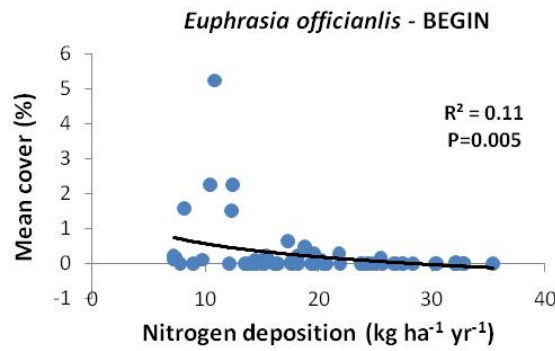
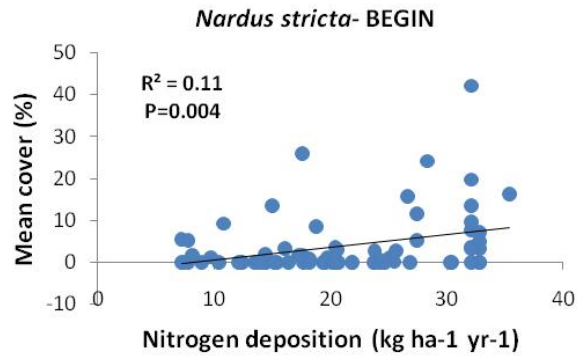
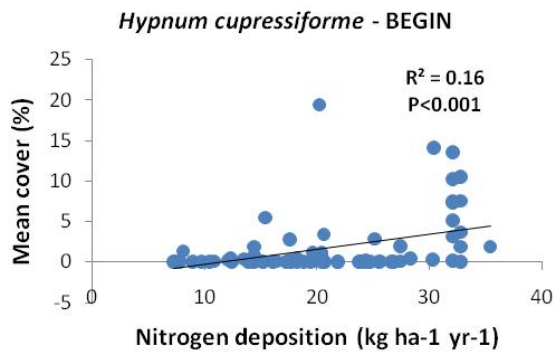
## Bogs: TU survey 2009



## Sand dunes (TU 2009 all sites)



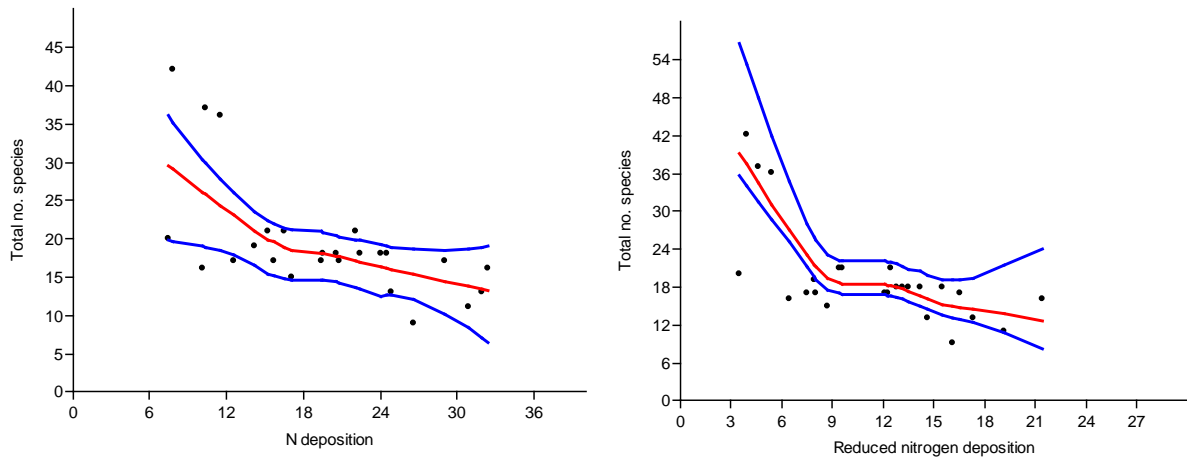
## Acid grasslands (BEGIN)



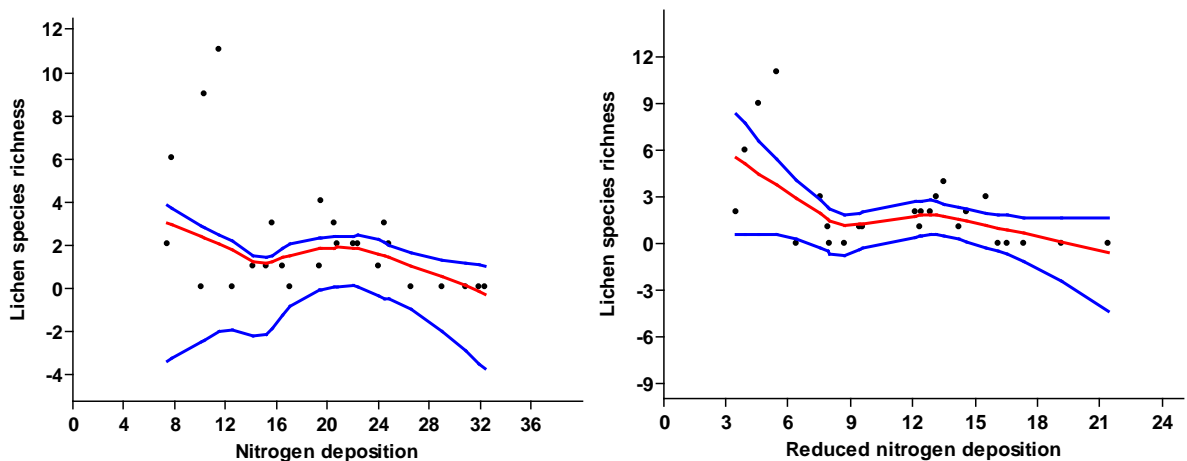
## Appendix 4: LOESS regression curves

Best fit to data line in red, 95% confidence limits shown in blue and fitted by bootstrapping.

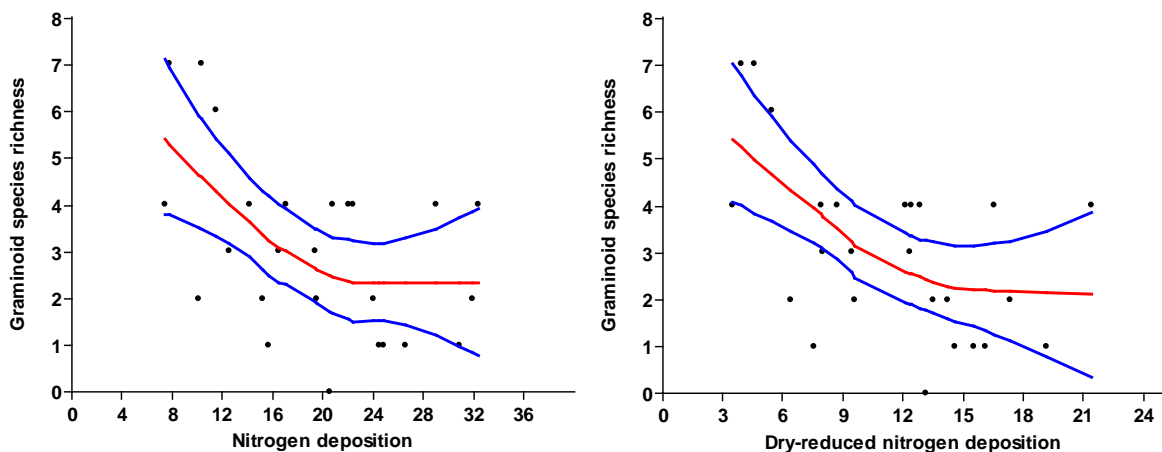
### Upland heaths (TU): total species richness



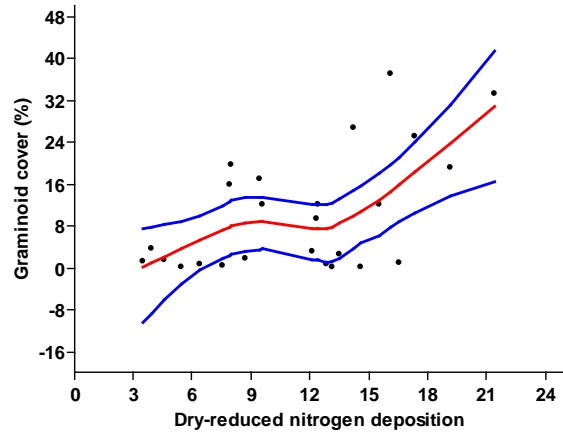
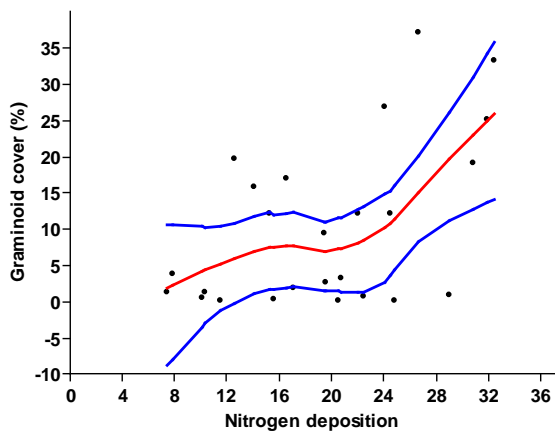
### Upland heaths (TU): lichen species richness



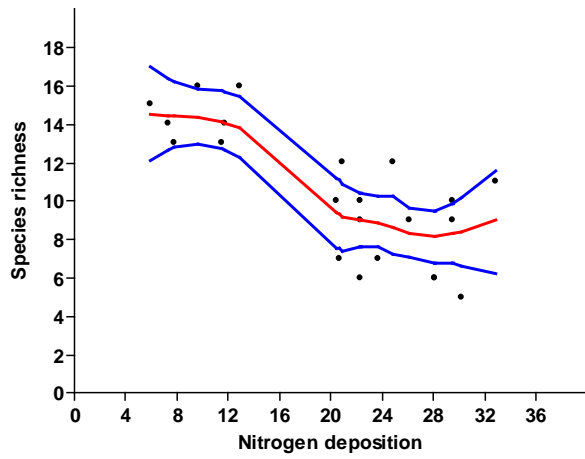
### Upland heaths (TU): graminoid species richness



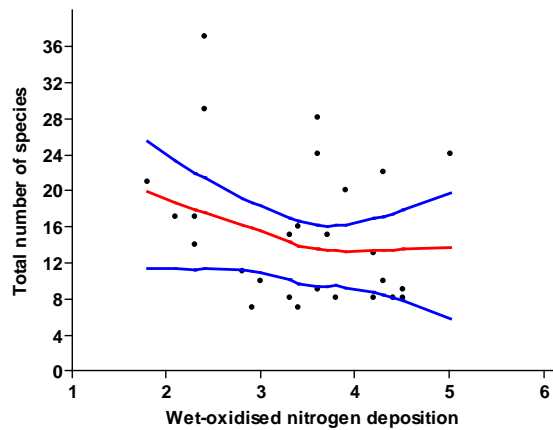
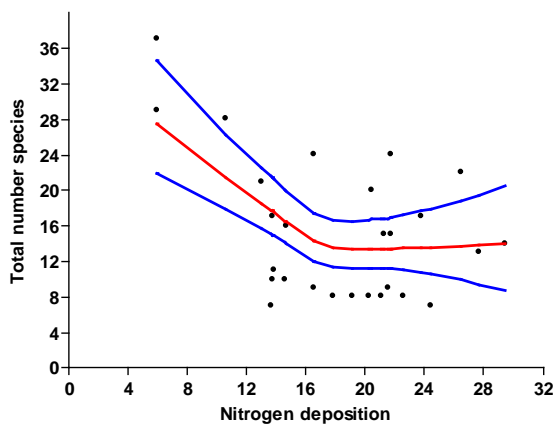
## Upland heaths (TU): graminoid cover



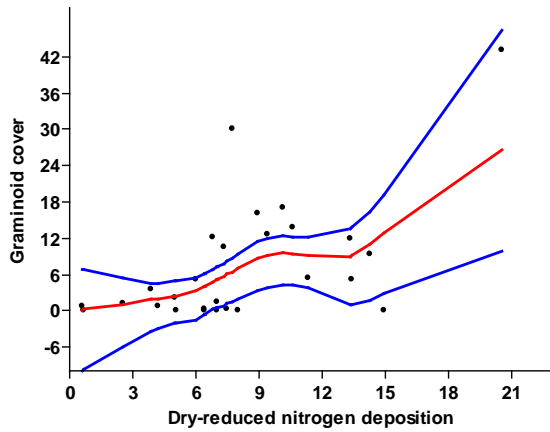
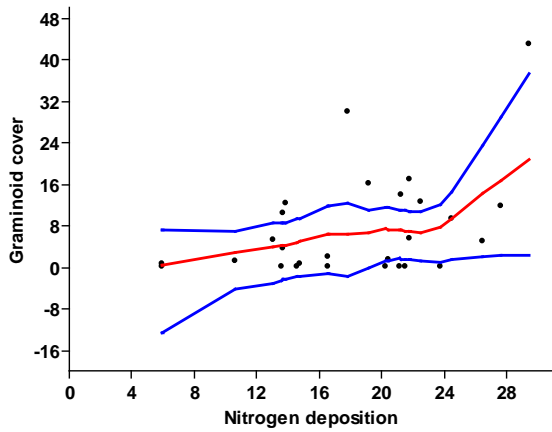
## Upland heaths (MRS): total species richness



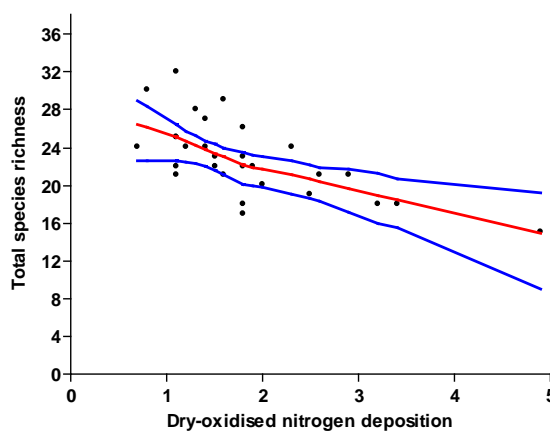
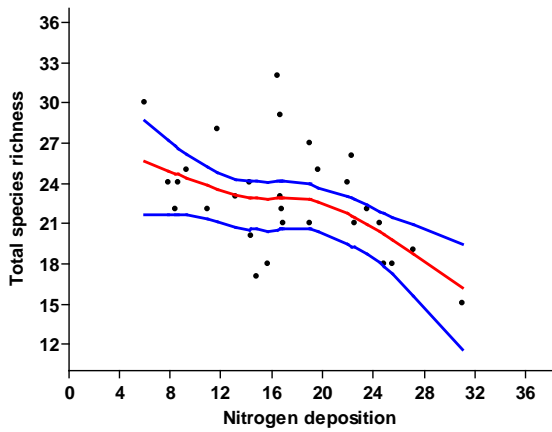
## Lowland heaths: total number species



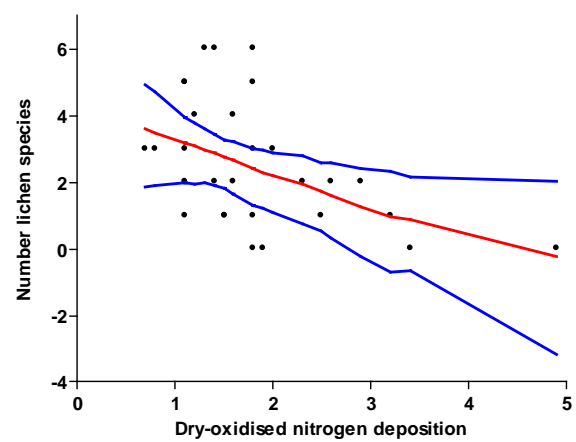
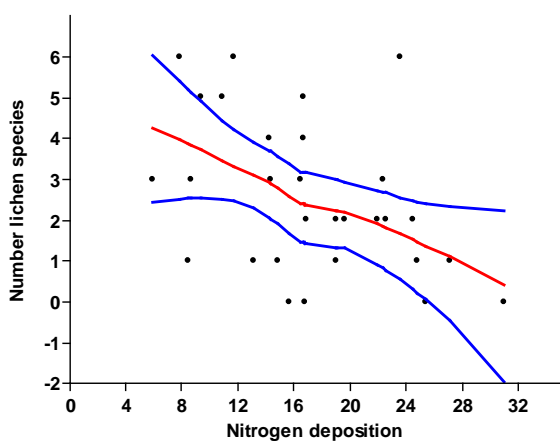
## Lowland heaths: Graminoid cover



## Bogs: total species richness

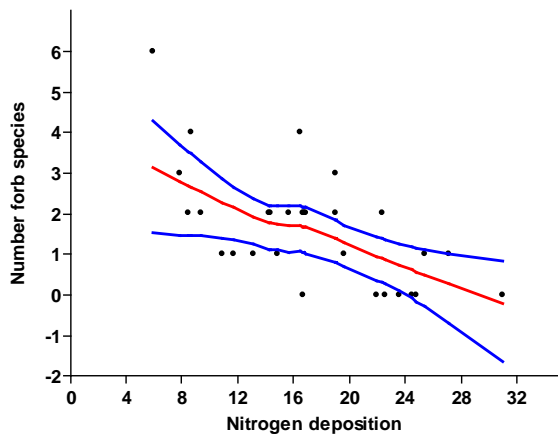


## Bogs: lichen species richness

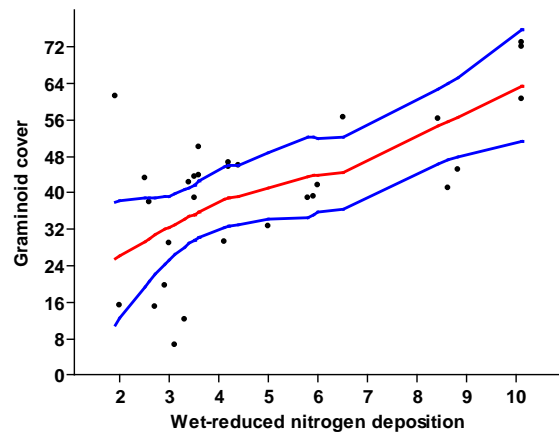
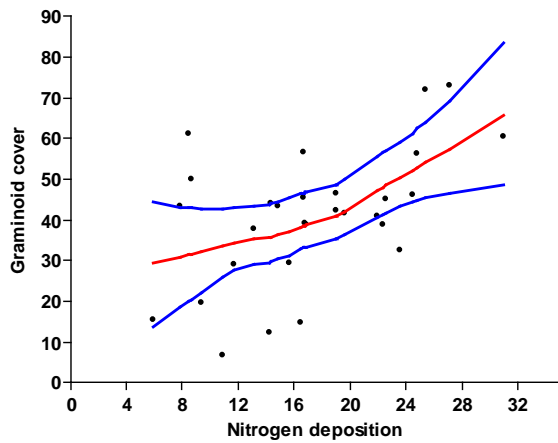




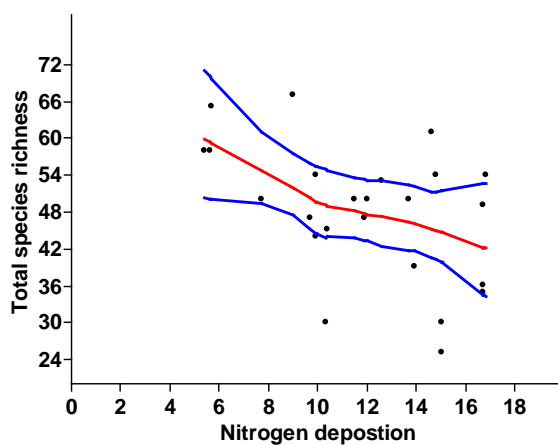
### Bogs: Forb species richness



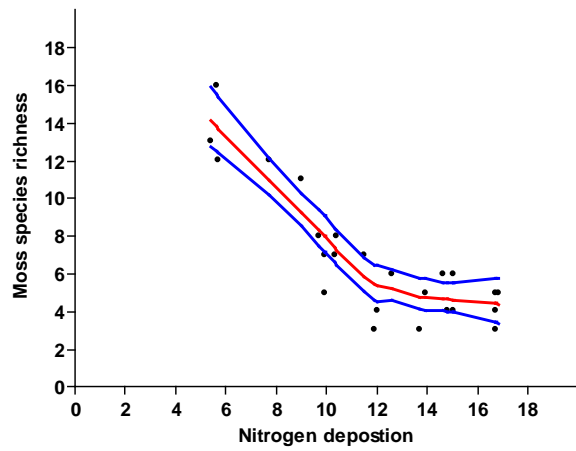
### Bogs: Graminoid cover



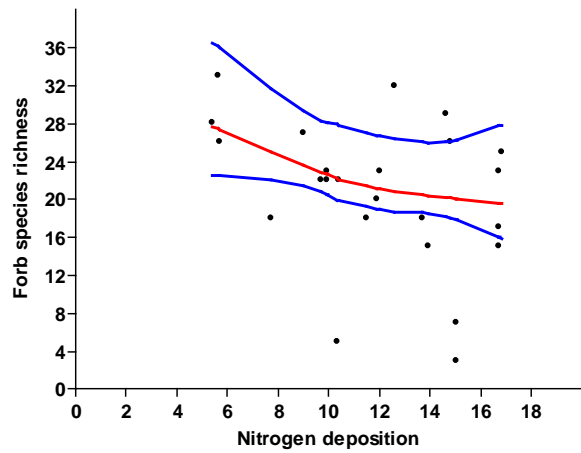
### Sand dunes (all TU 2009): Total species richness



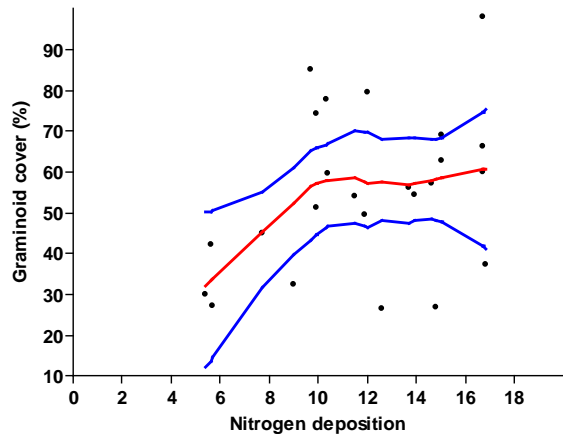
**Sand dunes (all TU 2009):  
Moss species richness**



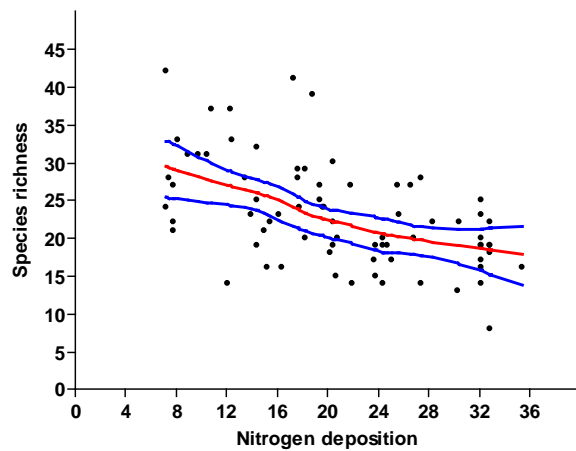
**Forb species richness**



**Sand dunes (all TU 2009): Graminoid cover**



**Acid Grasslands (BEGIN): Total species richness**



## Appendix 5: Effect of incremental increases in N deposition upon species richness

Summary of relationships between nitrogen deposition and species richness by habitat expressed as a percentage of the maximum in a habitat. Change in cover expresses as an absolute percentage. Incremental effect of a **0.3 kg** increase in N deposition shown.

Survey/ Habitat/	Max. species richness	Habitat/species critical load	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 0.3 kg increase in N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total Species Richness (SR)	77 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.5 % of maximum number of species					
<b>Upland heath (TU 2009 )</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.7%	-0.9%	-0.6%	-0.4%	-0.3%	-0.3%
Lichen SR	11 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.7%	-0.9%	-0.6%	-0.4%	-0.3%	-0.3%
Graminoid SR	7 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.9%	-1.0%	-0.6%	-0.5%	-0.4%	-0.3%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.13%	-0.01%	+0.11%	+0.24%	+0.37%	+0.5%
<b>Upland heath (MRS)*</b>								
Total SR	16 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.1%	-0.9%	-0.7%	-0.5%	-0.3%	-0.1%
<b>Lowland heath (TU 2009)</b>								
Total SR	37 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.8%	-0.9%	-0.6%	-0.5%	-0.4%	-0.3%
Moss SR	12 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.6%	-0.8%	-0.5%	-0.4%	-0.3%	-0.3%
Graminoid SR	9 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.9%	-1.3%	-0.6%	-0.3%	-0.2%	-0.1%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	n/a	-0.04%	+0.11%	+0.26%	+0.41%	+0.56%
<b>Bog (TU 2009 )</b>								
Total SR	32 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-0.3%		
Lichen SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-0.6%		
Forb SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.3%	-1.1%	-0.8%	-0.6%	-0.5%	-0.4%
Graminoid cover	-	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				+0.41%		
<b>Sand dunes (TU 2009, all sites)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.9%	-0.7%	-0.3%	-0.2%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-6.0%	-1.5%	-0.7%	-0.4%	-	-
Graminoid cover	n/a	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+2.43%	+0.63%	+0.28%	-0.16%	-	-
Forb SR	33 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.9%	-0.7%	-0.3%	-0.2%	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.7%	-0.9%	-0.6%	-0.4%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.5%	-1.3%	-0.9%	-0.6%	-	-
<b>Acid grasslands (BEGIN)</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.4%	-0.4%	-0.4%	-0.3%	-0.3%	-0.3%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

Summary of relationships between nitrogen deposition and species richness by habitat expressed as a percentage of the maximum in a habitat. Change in cover expresses as an absolute percentage. Incremental effect of a **0.5 kg** increase in N deposition shown.

Survey/ Habitat/	Max. species richness	Habitat/species critical load	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 0.5 kg increase in N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total SR	77 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.8 % of maximum number of species					
<b>Upland heath (TU 2009)</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.7%	-1.4%	-0.9%	-0.7%	-0.6%	-0.5%
Lichen SR	11 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.8%	-1.4%	-0.9%	-0.7%	-0.6%	-0.5%
Graminoid SR	7 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-3.1%	-1.6%	-1.1%	-0.8%	-0.6%	-0.5%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.22%	-0.01%	+0.20%	+0.41%	+0.62%	+0.83%
<b>Upland heath (MRS)*</b>								
Total SR	16 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.9%	-1.5%	-1.2%	-0.8%	-0.5%	-0.1%
<b>Lowland heath (TU 2009)</b>								
Total SR	37 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.9%	-1.5%	-1.0%	-0.8%	-0.6%	-0.2%
Moss SR	12 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.6%	-1.3%	-0.9%	-0.7%	-0.5%	-0.2%
Graminoid SR	9 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-7.9%	-2.1%	-0.9%	-0.5%	-0.3%	-0.2%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.31	-0.06%	+0.19%	+0.44%	+0.69%	+0.94%
<b>Bog (TU 2009)</b>								
Total SR	32 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-0.5%		
Lichen SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-1.1%		
Forb SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-3.7%	-1.9%	-1.3%	-1.0%	-0.8%	-0.6%
Graminoid cover	-	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				+0.68%		
<b>Sand dunes (TU 2009, all sites)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.6%	-1.2%	-0.5%	-0.3%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-9.6%	-2.5%	-1.1%	-0.6%	-	-
Graminoid cover	n/a	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+3.91%	+1.02%	+0.46%	+0.26%	-	-
Forb SR	33 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.6%	-1.2%	-0.5%	-0.3%	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.8%	-1.4%	-1.0%	-0.7%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.2%	-2.1%	-1.4%	-1.1%	-	-
<b>Acid grasslands (BEGIN)</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.7%	-0.7%	-0.6%	-0.6%	-0.5%	-0.4%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

Summary of relationships between nitrogen deposition and species richness/cover by habitat expressed as a percentage of the maximum in a habitat. Incremental effect of 1 kg increase in N deposition shown.

Survey/ Habitat/	Max. species richness	Habitat/species critical load	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 1 kg increase in N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total species richness (SR)	77 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.6 % of maximum number of species/kg N increase					
<b>Upland heath (TU 2009 )</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-5.7 %	-2.9 %	-2.0 %	-1.4 %	-1.2 %	-1.0 %
Lichen SR	11 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-5.4 %	-2.7 %	-1.8 %	-1.8 %	-1.0 %	-1.0 %
Graminoid SR	7 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-7.0 %	-2.9 %	-2.9 %	-1.4 %	-1.4 %	-1.0 %
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.5 %	no change	+0.4 %	+0.8 %	+1.2 %	+1.6 %
<b>Upland heath (MRS)*</b>								
Total SR	16 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-3.4 %	-3.1 %	-2.5 %	-1.9 %	-1.3 %	-0.3 %
<b>Lowland heath (TU 2009)</b>								
Total SR	37 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-6.2 %	-3.5 %	-2.2 %	-1.6 %	-1.4 %	-1.0 %
Moss SR	12 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-5.8 %	-2.5 %	-1.7 %	-1.7 %	-1.7 %	-0.9 %
Graminoid SR	9 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-17.8%	-4.4 %	-2.2 %	-1.1 %	-1.1 %	-0.5 %
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.6 %	no change	+0.5 %	+1.05 %	+1.6 %	+2.2 %
<b>Bog (TU 2009 )</b>								
Total SR	32 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.9 % of maximum number of species/kg N increase					
Lichen SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.7 %					
Forb SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-7.7 %	-3.9 %	-2.6 %	-1.9 %	-1.6 %	-1.3%
Graminoid cover	-	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+1.5 % cover/kg N increase					
<b>Sand dunes (TU 2009, all sites)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-10.1%	-2.6 %	-1.2 %	-0.6 %	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-21.3%	-5.0 %	-2.5 %	-1.3 %	-	-
Graminoid cover	n/a	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+ 8.6 %	+ 2.2 %	+ 1.0 %	+ 0.5 %	-	-
Forb SR	33 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-10.3%	-2.4 %	-1.2 %	-0.6 %	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.4 %	-2.2 %	-1.4 %	-1.0 %	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-21.3%	-5.6 %	-2.5 %	-1.3 %	-	-
<b>Sand dunes TU 2009 + 2002 (Fixed dune grasslands)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-4.4 %	-2.2 %	-1.4 %	-1.0 %	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-8.9 %	-4.4 %	-3.1 %	-2.5 %	-	-
<b>Acid grasslands (BEGIN)</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.5 %	-1.4 %	-1.2 %	-1.1 %	-1.0%	-0.9%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

Summary of relationships between nitrogen deposition and species richness by habitat expressed as a percentage of the maximum in a habitat. Change in cover expresses as an absolute percentage. Incremental effect of a **2 kg** increase in N deposition shown.

Survey/ Habitat/	Max. species richness	Habitat/species critical load	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 2.0 kg increase in N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total Species Richness (SR)	77 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-3.2 % of maximum number of species					
<b>Upland heath (TU 2009 )</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-9.7%	-5.3%	-3.6%	-2.7%	-2.2%	-1.9%
Lichen SR	11 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-9.7%	-5.3%	-3.6%	-2.8%	-2.2%	-1.9%
Graminoid SR	7 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-11.0%	-5.9%	-4.1%	-3.1%	-2.5%	-2.1%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-0.8%	+0.1%	+0.9%	+1.8%	+2.6%	+3.5%
<b>Upland heath (MRS)*</b>								
Total SR	16 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-7.2%	-5.8%	-4.5%	-3.1%	-1.7%	-0.3%
<b>Lowland heath (TU 2009)</b>								
Total SR	37 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-10.2%	-5.5%	-3.8%	-2.9%	-2.3%	-2.0%
Moss SR	12 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-9.2%	-5.0%	-3.4%	-2.6%	-2.1%	-1.8%
Graminoid SR	9 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-24.8%	-7.2%	-3.4%	-2.0%	-1.3%	-0.9%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.1%	-0.1%	+0.9%	+1.9%	+2.9%	+3.9%
<b>Bog (TU 2009 )</b>								
Total SR	32 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-1.9%		
Lichen SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-4.3%		
Forb SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-13.1%	-7.1%	-4.9%	-3.7%	-3.0%	-2.5%
Graminoid cover	-	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				+2.7%		
<b>Sand dunes (TU 2009, all sites)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-14.4%	-4.2%	-2.0%	-1.1%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-30.1%	-8.8%	-4.1%	-2.4%	-	-
Graminoid cover	n/a	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+12.3%	+3.6%	+1.7%	+1.0%	-	-
Forb SR	33 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-14.6%	-4.2%	-2.0%	-1.2%	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-9.9%	-5.4%	-3.7%	-2.8%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-14.7%	-8.0%	-5.5%	-4.2%	-	-
<b>Acid grasslands (BEGIN)</b>								
Total species richness	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.9%	-2.7%	-2.4%	-2.2%	-2.0%	-1.7%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

Summary of relationships between nitrogen deposition and species richness by habitat expressed as a percentage of the maximum in a habitat. Change in cover expresses as an absolute percentage. Incremental effect of a **5.0 kg** increase in N deposition shown.

Survey/ Habitat/	Max. species richness	Habitat/species critical load	Change in species richness expressed as a % of maximum species richness recorded in habitat with a 5.0 kg increase in N deposition at different background N deposition levels					
			5 kg N	10 kg N	15 kg N	20 kg N	25 kg N	30 kg N
<b>All habitats (TU 2009)</b>								
Total species richness (SR)	77 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-8 % of maximum number of species					
<b>Upland heath (TU 2009 )</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-20.0%	-11.7%	-8.3%	-6.4%	-5.3%	-4.4%
Lichen SR	11 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-20.0%	-11.7%	-8.3%	-6.5%	-5.3%	-4.5%
Graminoid SR	7 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-22.6%	-13.2%	-9.4%	-7.3%	-5.9%	-5.0%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-1.3%	+0.9%	+3.0%	+5.1%	+7.2%	+9.3%
<b>Upland heath (MRS)*</b>								
Total SR	16 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-17.0%	-13.6%	-10.1%	-6.7%	-3.3%	-
<b>Lowland heath (TU 2009)</b>								
Total SR	37 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-21.1%	-12.3%	-8.7%	-6.8%	-5.5%	-4.7%
Moss SR	12 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-19.0%	-11.1%	-7.9%	-6.1%	-5.0%	-4.2%
Graminoid SR	9 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-43.3%	-14.4%	-7.2%	-4.3%	-2.9%	-2.1%
Graminoid cover	n/a	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-2.0%	+0.5%	+3.0%	+5.5%	+8.0%	+10.5%
<b>Bog (TU 2009 )</b>								
Total SR	32 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-4.7%		
Lichen SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				-10.8%		
Forb SR	6 spp.	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-26.9%	-15.7%	-11.2%	-8.7%	-7.1%	-6.0%
Graminoid cover	-	5-10 kg N ha <sup>-1</sup> yr <sup>-1</sup>				+6.8%		
<b>Sand dunes (TU 2009, all sites)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-25.3%	-8.4%	-4.2%	-2.5%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-52.8%	-17.6%	-8.8%	-5.3%	-	-
Graminoid cover	n/a	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	+21.5%	+7.2%	+3.6%	+2.2%	-	-
Forb SR	33 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-25.5%	-8.5%	-4.2%	-2.5%	-	-
<b>Sand dunes TU 2009 (pH ≥6.5)</b>								
Total SR	77 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-20.3%	-11.9%	-8.4%	-6.5%	-	-
Moss SR	16 spp.	8-15 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-30.2%	-17.7%	-12.6%	-9.7%	-	-
<b>Acid grasslands (BEGIN)</b>								
Total SR	42 spp.	10-20 kg N ha <sup>-1</sup> yr <sup>-1</sup>	-7.2%	-6.5%	-5.9%	-5.3%	-4.7%	-4.1%

\* in the upland heath MRS survey quadrat size was 0.5 x 0.5 m. This produced different results than the other surveys which used 2 x 2 m quadrats.

## Appendix 6: Current critical loads for all habitats taken from ECE Empirical critical loads and dose-response relationships

Overview of empirical critical loads for nitrogen deposition ( $\text{kg N ha}^{-1} \text{ year}^{-1}$ ) to natural and seminatural ecosystems (column 1), arranged according to EUNIS class and level (column 2), as originally established in 2002 and reported in 2003 (column 3) and as revised in 2010 (column 4). The reliability is expressed in qualitative terms: ## reliable; # quite reliable; and (#) expert judgement (column 5). Column 6 provides a selection of effects that can occur when critical load are exceeded. Changes with respect to values of 2003 are indicated in bold.) For more information see Defra Report AQ801 Hall *et al.* (2011).

<i>Ecosystem type</i>	<i>EUNIS code</i>	<i>2003 kg N ha<sup>-1</sup> year<sup>-1</sup> and reliability</i>	<i>2010 kg N ha<sup>-1</sup> year<sup>-1</sup></i>	<i>2010 reliability</i>	<i>Indication of exceedance</i>
Marine habitats (A)					
Mid-upper salt-marshes	A2.53		20–30	(#)	Increase in dominance of graminoids
Pioneer and low-mid salt-marshes	A2.54 and A2.55	30–40 (#)	20–30	(#)	Increase in late-successional species, increase in productivity
Coastal habitat (B)					
Shifting coastal dunes	B1.3	10–20 (#)	10–20	(#)	Biomass increase, increase N leaching
Coastal stable dune grasslands (grey dunes)	B1.4 <sup>e</sup>	10–20 #	8–15	#	Increase in tall graminoids, decrease in prostrate plants, increased N leaching, soil acidification, loss of typical lichen species
Coastal dune heaths	B1.5	10–20 (#)	10–20	(#)	Increase in plant production, increase in N leaching, accelerated succession
Moist-to-wet dune slacks	B1.8 <sup>b</sup>	10–25 (#)	10–20	(#)	Increased biomass and tall graminoids
Inland surface water habitats (C)					
Soft-water lakes (permanent oligotrophic waters)	C1.1 <sup>e</sup>	5–10 ##	3–10	##	Change in the species composition of macrophyte communities, increased algal productivity and a shift in nutrient limitation of phytoplankton from N to phosphorous (P)
Dune slack pools (permanent oligotrophic waters)	C1.16	10–20 (#)	10–20	(#)	Increased biomass and rate of succession
Permanent dystrophic lakes, ponds and pools	C1.4 <sup>d</sup>		3–10	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P



<i>Ecosystem type</i>	<i>EUNIS code</i>	<i>2003 kg N ha<sup>-1</sup> year<sup>-1</sup> and reliability</i>	<i>2010 kg N ha<sup>-1</sup> year<sup>-1</sup></i>	<i>2010 reliability</i>	<i>Indication of exceedance</i>
<b>Mire, bog and fen habitats (D)</b>					
Raised and blanket bogs	D1 <sup>e</sup>	5–10 ##	5–10	##	Increase in vascular plants, altered growth and species composition of bryophytes, increased N in peat and peat water
Valley mires, poor fens and transition mires	D2 <sup>f</sup>	10–20 #	10–15	#	Increase in sedges and vascular plants, negative effects on bryophytes
Rich fens	D4.1 <sup>g</sup>	15–35 (#)	15–30	(#)	Increase in tall graminoids, decrease in bryophytes
Montane rich fens	D4.2 <sup>g</sup>	15–25 (#)	15–25	(#)	Increase in vascular plants, decrease in bryophytes
<b>Grasslands and tall forb habitats (E)</b>					
Subatlantic semi-dry calcareous grassland	E1.26	15–25 ##	15–25	##	Increase in tall grasses, decline in diversity, increased mineralization, N leaching, surface acidification
Mediterranean xeric grasslands	E1.3		15–25	(#)	Increased production, dominance by graminoids
Non-Mediterranean dry acid and neutral closed grassland	E1.7 <sup>b</sup>	10–20 #	10–15	##	Increase in graminoids, decline of typical species, decrease in total species richness
Inland dune pioneer grasslands	E1.94 <sup>b</sup>	10–20 (#)	8–15	(#)	Decrease in lichens, increase in biomass
Inland dune siliceous grasslands	E1.95 <sup>b</sup>	10–20 (#)	8–15	(#)	Decrease in lichens, increase in biomass, increased succession
Low and medium altitude hay meadows	E2.2	20–30 (#)	20–30	(#)	Increase in tall grasses, decrease in diversity
Mountain hay meadows	E2.3	10–20 (#)	10–20	(#)	Increase in nitrophilous graminoids, changes in diversity
Moist and wet oligotrophic grasslands					
• <i>Molinia caerulea</i> meadows	E3.51	15–25 (#)	15–25	(#)	Increase in tall graminoids, decreased diversity, decrease of bryophytes
• Heath ( <i>Juncus</i> ) meadows and humid ( <i>Nardus stricta</i> ) swards	E3.52	10–20 #	10–20	#	Increase in tall graminoids, decreased diversity, decrease of bryophytes
Moss- and lichen-dominated mountain summits	E4.2	5–10 #	5–10	#	Effects upon bryophytes or lichens
Alpine and subalpine acid grasslands	E4.3		5–10	#	Changes in species composition; increase in plant production
Alpine and subalpine calcareous grasslands	E4.4		5–10	#	Changes in species composition; increase in plant production

<i>Ecosystem type</i>	<i>EUNIS code</i>	<i>2003 kg N ha<sup>-1</sup> year<sup>-1</sup> and reliability</i>	<i>2010 kg N ha<sup>-1</sup> year<sup>-1</sup></i>	<i>2010 reliability</i>	<i>Indication of exceedance</i>
Heathland, scrub and tundra habitats (F)					
Tundra	F1	5–10 #	3–5	#	Changes in biomass, physiological effects, changes in species composition in bryophyte layer, decrease in lichens
Arctic, alpine and subalpine scrub habitats	F2	5–15 (#)	5–15	#	Decline in lichens, bryophytes and evergreen shrubs
Northern wet heath	F4.11				
• “U” Calluna-dominated wet heath (upland moorland)	F4.11 <sup>a,h</sup>	10–20 (#)	10–20	#	Decreased heather dominance, decline in lichens and mosses, increased N leaching
• “L” Erica tetralix-dominated wet heath (lowland)	F4.11 <sup>e,h</sup>	10–25 (#)	10–20	(#)	Transition from heather to grass dominance
Dry heaths	F4.2 <sup>e,h</sup>	10–20 ###	10–20	##	Transition from heather to grass dominance, decline in lichens, changes in plant biochemistry, increased sensitivity to abiotic stress
Mediterranean scrub	F5		20–30	(#)	Change in plant species richness and community composition

<i>Ecosystem type</i>	<i>EUNIS code</i>	<i>2003 kg N ha<sup>-1</sup> year<sup>-1</sup> and reliability</i>	<i>2010 kg N ha<sup>-1</sup> year<sup>-1</sup></i>	<i>2010 reliability</i>	<i>Indication of exceedance</i>
Forest habitats (G)					
Fagus woodland	G1.6		10–20	(#)	Changes in ground vegetation and mycorrhiza, nutrient imbalance, changes soil fauna
Acidophilous Quercus-dominated woodland	G1.8		10–15	(#)	Decrease in mycorrhiza, loss of epiphytic lichens and bryophytes, changes in ground vegetation
Meso- and eutrophic Quercus woodland	G1.A		15–20	(#)	Changes in ground vegetation
Mediterranean evergreen (Quercus) woodland	G2.1		3–7	(#)	Changes in epiphytic lichens
Abies and Picea woodland	G3.1		10–15	(#)	Decreased biomass of fine roots, nutrient imbalance, decrease in mycorrhiza, changed soil fauna
Pinus sylvestris woodland south of the taiga	G3.4		5–15	#	Changes in ground vegetation and mycorrhiza, nutrient imbalances, increased N <sub>2</sub> O and NO emissions
Pinus nigra woodland	G3.5		15	(#)	Ammonium accumulation
Mediterranean Pinus woodland	G3.7		3–15	(#)	Reduction in fine root biomass, shift in lichen community
Spruce taiga woodland	G3.A <sup>f</sup>	10–20 #	5–10	##	Changes in ground vegetation, decrease in mycorrhiza, increase in free algae
Pine taiga woodland	G3.B <sup>i</sup>	10–20 #	5–10	#	Changes in ground vegetation and in mycorrhiza, increase occurrence of free algae
Mixed taiga woodland with Betula	G4.2		5–8	(#)	Increased algal cover
Mixed Abies-Picea Fagus woodland	G4. <sup>0</sup>		10–20	(#)	
Overall					
Broadleaved deciduous woodland	G1 <sup>k)</sup>	10–20 #	10–20	##	Changes in soil processes, nutrient imbalance, altered composition mycorrhiza and ground vegetation
Coniferous woodland	G3 <sup>k,l</sup>	10–20 #	5–15	##	Changes in soil processes, nutrient imbalance, altered composition mycorrhiza and ground vegetation

<sup>a</sup> For acid dunes, use the 8–10 kg N ha<sup>-1</sup> year<sup>-1</sup> range, for calcareous dunes use the 10–15 kg ha<sup>-1</sup> year<sup>-1</sup> range.

<sup>b</sup> Use the lower end of the range with low base cation availability. Use the higher end of the range with high base cation availability.

<sup>c</sup> This critical load should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other human inputs. Use the lower end of the range for boreal and alpine lakes, use the higher end of the range for Atlantic softwaters.

<sup>d</sup> This critical load should only be applied to waters with low alkalinity with no significant agricultural or other direct human inputs. Use the lower end of the range for boreal and alpine dystrophic lakes.

<sup>e</sup> Use the high end of the range with high precipitation and the low end of the range with low precipitation. Use the low end of the range for systems with a low water table, and the high end of the range for systems with a high water table. Note, that water table can be modified by management.

<sup>f</sup> For D2.1 (quaking fens and transition mires) use lower end of the range (#).

<sup>g</sup> For high latitude systems use lower end of the range.

<sup>h</sup> Use the high end of the range when sod cutting has been practiced; use the lower end of the range with low intensity management.

<sup>i</sup> In 2003 presented as overall value for boreal forests.

<sup>j</sup> Included in studies which were classified into G1.6 and G3.1.

<sup>k</sup> In 2003 presented as overall value for temperate forests.

<sup>l</sup> For application at broad geographical scales.