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Stevens, C.J.[a,b*] , Maskell, L.C. [c], Smart, S.M.[c], Caporn, S.J.M.[b,] Dise, N.B.[b] and Gowing, D.J.G.[a].

[a] Department of Life Sciences, The Open University, Walton Hall, Milton Keynes MK7 6AA

[b] Department of Environmental and Geographical Sciences, Manchester Metropolitan University, Manchester M1 5GD.

[c] NERC Centre for Ecology and Hydrology, Library Avenue, Bailrigg, Lancaster LA1 4YQ

Corresponding author:

Dr Carly Stevens

Department of Life Sciences

The Open University

Walton Hall

Milton Keynes

MK7 6AA

Tel +44 (0)1908 653225

c.j.stevens@open.ac.uk

Abstract: Atmospheric deposition of nitrogen has become a serious concern for nature conservation managers and policy makers. It has the potential to reduce species richness, increase the graminoid component of the sward, encourage species typical of more fertile conditions and alter the soil biogeochemistry of grasslands. Calcifugous grasslands (grasslands found on acid soils) are among the most sensitive to N deposition due to their poorly buffered soils and species typical of nutrient poor environments.

Indicators have an important role to play in detecting the impact of nitrogen deposition on sites of conservation importance and assessing conservation status. This study investigates potential indicators of nitrogen deposition impacts that could be incorporated into site-condition monitoring programmes such as the UK Common Standards Monitoring.

Using two national surveys of calcifugous grasslands we examined the potential for using: the presence or absence of indicator species, the cover of indicator species, the species richness and richness of functional groups, and the cover of functional groups as indicators of N deposition impacts. Of all the potential indicators investigated, graminoid:forb ratio was found to be the best indicator of N deposition impacts. It showed a significant relationship to N deposition in both data sets and is quick and easy to assess in the field. Vegetation indicators must be used with caution as there is potential for vegetation management regime and nutrients from other sources to cause similar changes in species composition. Consideration must be given to these before attributing changes to nitrogen deposition.

Keywords: Acid grasslands, canonical correspondence analysis, countryside survey, indicator species, common standards monitoring.

Introduction

In recent years, atmospheric deposition of nitrogen (N) has become a serious concern for nature conservation managers and policy makers. It has the potential to alter species composition, species richness and soil chemistry in a range of habitats through acidification and enrichment. In grasslands, addition of nitrogen typically reduces species richness (e.g. Mountford et al., 1993; Jenkinson et al., 1994; Stevens et al., 2004; Clark and Tilman, 2008), increases productivity (e.g. Wilson et al., 1995), increases the graminoid component of the sward (e.g. Wilson et al., 1995; Mountford et al., 1993, Stevens et al., 2006), encourages species typical of more fertile conditions (Kirkham and Kent, 1997), reduces pH (e.g. Blake et al., 1999) and increases N turnover rates (e.g. Phoenix et al., 2003).

Grasslands differ in their sensitivity to N deposition as a result of their soils and species composition. Grasslands with base-rich soils are well buffered against changes in soil pH, but soils that have low concentrations of base cations and already have an acid pH are much more sensitive to further acidification (Skiba et al., 1989). There is clear evidence for acidification of soils and metal availability that can be linked with N deposition (e.g. Falkengren-Grerup et al., 1987; Jönsson et al., 2003; Skiba et al., 1989; Stevens et al., 2006; Stevens et al., 2009).

Nitrogen deposition also has the potential to increase the amount of nitrogen in the soil that is available to plants (Stevens et al., 2006). This nitrogen may be stored in the soil or used by plants for increased biomass production. Grasslands dominated by species typical of nutrient poor environments are more likely to be impacted by the enrichment of the soil by N deposition through changes in their species composition and an increase in competitive species (Smart et al., 2003).

Calcifugous grasslands are among the most sensitive to N deposition. The soils are poorly buffered against changes in pH and toxic metals are commonly mobilised at low pH. In a number of these grasslands, especially the Violion caninae alliance (British NVC Community U4, dominated by grasses *Agrostis capillaris* and *Festuca rubra*), the majority of species are typical of infertile environments (Hill et al., 1999). Evidence using a national gradient (Stevens et al., 2004; Stevens et al., 2006) shows sensitivity to changes even at low levels of N deposition and evidence of acidification from S deposition. This demonstrates that these grasslands are vulnerable to N deposition. Long-term experimental N additions in the UK have also demonstrated changes in species composition and soil chemistry. A decline in mosses was observed after three years of N addition (Morecroft et al., 1994), this effect has continued with a reduction in bryophyte cover and a reduction in the cover of individual species (Carroll et al., 2000).

Indicators have an important role to play in detecting the impact of N deposition. The use of critical load exceedance (Achermann & Bobbink 2003) gives an indication of the likely impact of N deposition on plant communities but, on a specific site, critical load exceedance does not tell us what the actual damage is and indeed whether it has occurred at the site under investigation. For the protection of sites of nature conservation importance and to aid in management decisions, indicators of damage from N deposition would be a useful tool.

The Conservation of Natural Habitats and of Wild Fauna and Flora Directive (92/43/EEC) requires that member states maintain or restore listed habitats or species to ‘favourable condition status’. The conservation status of the natural habitats and species should be monitored and a report produced every 6 years. In the UK Common Standards Monitoring (CSM), together with other information, is used to inform assessment of the conservation status of designated site features. CSM provides a basic framework to ensure consistent monitoring of site condition in the UK. CSM assesses the condition of designated features (species, habitats, or geological features) by examining attributes chosen to reflect condition. For a habitat, this might include extent, vegetation structure and physical characteristics. CSM is not specifically designed to assess and attribute drivers of global environmental change, such as air pollution impacts, but The Habitats Directive considers the conservation status of a habitat in terms of the ‘sum of all influences acting on a natural habitat’, which clearly includes air pollution.

The aim of this investigation is to investigate indicators of N deposition impacts that could be incorporated into site condition monitoring programmes such as CSM. In order to do this, indicators need to be identified that are sensitive to N deposition, robust and, for rapid assessments such as these, quick to assess and easy for non-specialist surveyors to use. Here we focus on calcifugous grasslands, identifying potential indicators of N deposition impacts using national datasets and assessing their suitability for inclusion in a rapid assessment monitoring scheme. Calcifugous grasslands are an important component of UK upland habitats covering extensive areas, whilst in lowland areas ‘Lowland dry acid grassland’ is a is a UK Biodiversity Action Plan Priority Habitat (UK Biodiversity Group, 1998).

Materials and methods

Two national datasets were used to identify potential indicators of N deposition impacts in calcifugous grasslands. The first data used were a survey of 68 calcifugous grassland belonging to the British National Vegetation Classification (NVC) community U4 *Festuca ovina-Agrostis capillaris-Galium saxatile* (Rodwell, 1992) grassland selected by stratified random sampling where the stratification was by level of N deposition**.** These data were collected during 2002 and 2003 (Stevens *et al*., 2004; Stevens *et al*., 2006). This data set encompasses both upland and lowland U4 grasslands throughout England, Scotland and Wales with mixed grazing intensities and management regimes. Five randomly placed 2 x 2m quadrats were recorded at each of the 68 sites and all plants identified to species level. The data used in the analysis are mean percentage cover for each species (lichens are excluded).

The second data set was taken from the Countryside Survey of Great Britain (see Smart et al., 2003). This is a nationwide survey of natural resources in the UK countryside which has been conducted at regular intervals since 1978. It uses 1 km squares based on a random sample stratified by the ITE Land Classification, which is a set of 32 groups of all 1km squares in Britain defined by having internally similar combinations of soils, geology and climate (Bunce et al., 1996). Within each 1 km square all of the habitats present are mapped and a number of different plot types are sampled to record species present and their percentage cover. Some of these are randomly placed nested plots (X plots), of which the inner nest was used for this analysis; some are stratified random plots located in particular habitat types (U plots) and some are targeted on semi-natural habitat patches missed by the other plots in each square (see Smart et al., 2003 for further details). All sampling units were 2 x 2 m in size. For this analysis only plots from 1998 were used. They were all allocated to an NVC community (Rodwell, 1992 et seq.). To select calcifugous grassland, plots were selected by broad habitat type as identified by the land-cover mapping carried out in each square, then within this a subset of grasslands that were classified to an acid grassland NVC category with a Jaccard coefficient of greater than 0.5 was selected.

The two datasets provide complementary perspectives on the relationship between species abundance and N deposition across Britain. The Stevens et al. dataset maximised sensitivity to detection of species x driver relationships because it was designed to sample the entirety of the atmospheric deposition gradient in GB with even replication and also minimised other sources of noise by targeting the same specific kind of plant community along the gradient. The Countryside Survey dataset reflects representative random sampling of British ecosystems. It is optimal for detecting the signal of N deposition in an unbiased sample of British vegetation but this also means that noise to signal ratio is likely to be high because sampled vegetation maybe heterogenous whilst reflecting the impacts of a range of other drivers and land-use histories.

Modelled N deposition data, provided by CEH Edinburgh for the period 1998 and 2000, were used. The N deposition estimates were extracted from 5 x 5 km maps for the UK and comprise measured wet deposition including orographic enhancement, and dry deposition of NH_3, NO_2 and HNO_3 from measured concentration fields and a dry deposition model (described in Smith et al., 2000; NEGTAP, 2001).

Data were analysed using regression analysis in excel to identify relationships between N deposition and changes in species richness, functional group richness and cover, and the cover of individual species. To identify individual species as positive or negative indicators canonical correspondence analysis (CCA) was used. CCA is a multivariate ordination technique for direct gradient analysis. Species composition is directly related to measured environmental variables (Palmer, 1993). It assumes that species have unimodal distributions along environmental gradients. The resultant ordination diagram conveys large amounts of information regarding the environmental variables and their relations to species. CCA distributes individual species in the ordination diagram in a position that reflects their net tolerance to the environmental factors based on their cover and frequency. CCA and Monte Carlo Permutation tests were carried out using CANOCO 4.5 (ter Braak and Smilauer, 2002) with species percentage cover data; species that occurred in less than 10 percent of quadrats were removed from the ordination. Default settings were used.

Results

Individual species that could potentially be used as indicators of N deposition impacts we identified using CCA. Data from Stevens et al. (2004) and the Countryside Survey (CS) (Smart et al., 2003) were combined into one ordination. N deposition at the sites ranged from 6 to 36 kg N ha^{-1} yr^{-1}. The centroids of the two datasets are shown on the ordination diagram (Figure 1), the differences between the two datasets explains the greatest amount of variation in the combined dataset. N deposition was significantly related to species composition (Monte Carlo Permutation test $F=2.63$; $p<0.01$). Species associated with high N deposition were *Hypnum cupressiforme* agg.

(*H. jutlandicum* and *H. cupressiforme* were not differentiated), *Carex panicea* and *Carex pilulifera*. Species associated with low levels of N deposition were *Lotus corniculatus*, *Hylocomium splendens*, *Plantago lanceolata*, *Campanula rotundifolia* and *Thuidium tamariscinum* (Figure 1). Examining these species individually against N deposition shows that although all except *L. corniculatus* show a significant ($p<0.05$) relationship with N deposition (*C. rotundifolia* $r^2=0.05$, *H. Splendens* $r^2=0.02$, *P. Lanceolata* $r^2=0.05$, *T. Tamariscinum* $r^2=0.03$, *C. panacea* $r^2=0.03$, *C. pilulifera* $r^2= 0.01$and H. *Cupressiforme* $r^2=0.16$) they all occur across the range of N deposition (Figure 2).

In the Stevens data, there was a statistically significant negative correlation between N deposition and species richness ($r^2=0.52$; $p<0.01$). This relationship is discussed in detail by Stevens et al. (2004). Mean annual precipitation and altitude were found to explain additional significant variation (Stevens et al., 2004). CS data also showed a significant negative correlation between N deposition and species richness ($r^2=0.09$, $p<0.001$) with a similar line of best fit from the two surveys and although this relationship was much weaker, given the wider range of grasslands included, this is not surprising. Combining the two data sets maintains a significant negative relationship ($r^2=0.04$, $p<0.001$), although the r^2 is low, the trend is still apparent (Figure 3).

Examining the richness of functional groups reveals further trends. Although less of the variation in forb richness is explained by N deposition the relationship between forb richness and N deposition is highly significant in the Stevens data set ($r^2=0.38$, $p<0.001$). Forb richness also shows a significant relationship in the CS data ($r^2=0.04$,

$p<0.001$). Richness of pleurocarpous mosses also showed a significant negative relationship with N deposition in the Stevens data set, however, this relationship was not as strong as with either forb or species richness ($r^2=0.10$, $p<0.01$). Richness of grasses, sedges, rushes and acrocarpous mosses did not show significant relationships with N deposition.

Functional group abundance provides an alternative to species richness. In the Stevens data set, forb cover showed a significant negative relationship with N deposition ($r^2=0.42$, $p<0.001$). Forb cover did not show a significant relationship in the CS data ($r^2=0.002$, $p<0.36$) suggesting it is not a robust indicator. Using percentage cover of other groups (grasses, sedges, rushes, shrubs, all mosses, pleurocarpous mosses, acrocarpous mosses) did not reveal any significant trends. Ratios (based on percentage cover) were also examined and revealed several potentially interesting relationships. Grass:forb ratio (based on cover) shows a significant positive relationship ($p<0.05$) with N deposition in the Stevens data set. For the Stevens data, this relationship shows that at the highest levels of N deposition, the grass:forb ratio increases sharply, suggesting that this may be an indicator of the sites most severely affected by N deposition. Fitting an exponential relationship gives an r^2 of 0.33, but this relationship is strongly driven by a sudden increase in the ratio at 32kg N ha^{-1} yr^{-1}, a level of deposition which few of the surveyed sites exceeded. Grass:forb ratio also showed a significant relationship ($r^2=0.05$, $p<0.001$) with N deposition in CS data (Figure 4a). An alternative to a grass:forb ratio is a gramnoid:forb ratio where the cover of grasses and sedges are included together. Although cover of sedges may not show a response to N deposition alone, in most cases they are a small component of the vegetation so the combined cover shows a very similar pattern to the grass:forb ratio in both data sets (Figure 4b).

Discussion and Conclusions

The presence or absence of indicator species is commonly used in CSM assessment of site condition in the UK. However, all of the species identified as potential indicators in this study were found across the range of N deposition in the UK (Figure 2). This means that the presence or absence of indicator species is not suitable for use as an indicator of N deposition impacts. The percentage cover of individual species shows more promise as an indicator. Species identified as potential indicators of positive site condition in response to N deposition were *Lotus corniculatus*, *Campanula rotundifolia*, *Hylocomium splendens, Plantago lanceolata* and *Thuidium tamariscinum*. *L. corniculatus* is a leguminous species and may have a competitive advantage at low N deposition though this trend is not strong (Figure 2E) *C. rotundifolia* is a species of infertile habitats (Preston et al., 2002; Hill et al., 1999) and is described as intolerant of competition with vigorous grasses (Sinker et al., 1991). It is also a species that is increasingly rare at low soil pH (Grime et al., 2007) so may be reacting to acidification of the soil. The decline in *Hylocomium splendens* in response to air pollution was noted by Porley & Hodgetts (2005) and experiments in Europe have confirmed a strong link with N eutrophication (Dirkse & Martakis, 1992; Hallingbick, 1992; Strengbom et al., 2001; Koranda *et al*., 2007). *P. lanceolata* is a stress-tolerant species (Grime et al., 2007) and is very common in a wide range of grasslands. N addition experiments at Tadham Moor, Somerset, on an unimproved hay meadow, showed a significant decline in percent cover of *P. lanceolata* with low N additions (25 kg N ha^{-1} yr^{-1}) (Mountford et al., 1993; Mountford et al., 1994;

Kirkham et al., 1996). As with *C. rotundifolia*, *P. lanceolata* becomes increasingly rare at low soil pH (Grime et al., 2007). In experimental N additions *Thuidium tamariscinum* was not found to be sensitive to N deposition (Koranda et al., 2007) and has even been shown to be associated with high deposition (Zechmeister et al., 2007) so it is surprising that this species was identified in our ordination. The negative association of *Thuidium tamariscinum* with N deposition in this study is mainly due to one outlier, a relatively low N deposition site with relatively high cover of this species (Figure 2D). Ellenberg values (Hill et al., 2007) for reaction (pH) and nutrients for this species also do not indicate that it is likely to be an indicator of low N deposition.

Potential indicators of negative site condition as a result of N deposition were *Carex panicea*, *C. pilulifera* and *Hypnum cupressiforme* agg.. There is no experimental evidence linking either *C. panicea* or *C. pilulifera* to high N deposition although sedges in general have been shown to increase in some habitats (e.g. van der Wal, 2003). Both species are more typical of nutrient poor environments, although both also tend to be found in acid soils (Hill et al., 1999), so may benefit from acidification. *H. cupressiforme* is a pollution-tolerant moss (Rodenkirchen, 1992), and has been found to persist in areas where other bryophytes have declined considerably due to N deposition (Hallingback, 1992). *H. jutlandicum* has been shown to increase in cover with the addition of N in both heathland (Gordon et al., 2001) and forest habitats (Zechmeister et al., 2007). This pollution tolerance would allow *H. cupressiforme* to compete well with more sensitive mosses.

Figure 2 shows that some species would make better indicators than others. An average cover of *C. rotundifolia* of over 0.5% would initially seem to be a good indicator of low N deposition. However, there are quite a few sites with low N deposition where this species has lower cover or where it is absent. It is also extremely difficult to estimate percentage covers accurately in this range. Most of the other species identified contain too many absences or very low cover values to be useful as an indicator. *H. cupressiforme* could potentially be used to identify sites with the highest levels of N deposition as cover of over 5% is only found at sites with the highest levels of deposition. Some high deposition sites are not identified by this; estimating the proportion of moss cover that is accounted for by *H. cupressiforme* may provide a better indicator, however, the work involved in assessing this in the field is likely to be prohibitive for a rapid assessment monitoring scheme.

Despite the potential of individual species to indicate high or low N deposition, using the cover of individual species is not a reliable indicator of N deposition impacts. The main reason for this is that individual species have less potential for detecting a signal of N deposition due to the inherent ubiquity or rarity of the species. Additionally, we rarely have sufficient knowledge of species niche requirements to determine the relative importance of N deposition compared to other environmental drivers in controlling how common or rare the species will be at a particular site.

Although the results presented describe indicators as functions of current levels of N deposition, they may actually express the net result of many years of cumulative N deposition (with the exception of communities that do not have a stable substrate), because current N deposition is a reasonable surrogate for the amount of past N deposition. A century or more of elevated N deposition means there is a high likelihood that major shifts in plant species composition have already taken place in sensitive vegetation types receiving elevated N deposition (Stevens et al. 2004, 2006). Therefore, just as the impact of a point application of N in field experiments needs to take into account the impact of cumulative background N deposition, the detection of local impacts of nitrogen deposition should also account for the expected starting point. Long-term monitoring or experimentation would be needed to determine how quickly these grasslands respond to changes in N deposition.

In this study species richness (Figure 3) would appear to be a good indicator but there are some drawbacks to using species richness as an indicator of N deposition impacts. Species richness is also governed by many different factors (including vegetation management), so care would need to be taken in interpreting the data. For example, species richness shows a typically unimodal relationship with the full length of the substrate productivity gradient so species richness may be increased initially by increasing the nutrient status of the soil but will fall at higher nutrient levels, hence the expected direction of change needs to be supported by carefully establishing the starting community type, i.e. being certain the monitored stand qualifies as acid grassland. It is also time consuming to collect species richness data and requires a trained botanist, especially in this typically grazed system. Species do not all need to be identified, but there is a need to distinguish all distinct species, including mosses and grasses. This probably makes it prohibitively time consuming for incorporation into a rapid assessment scheme, but the value of species richness as a potential indicator should be considered. Setting a level of species richness to indicate habitats that are negatively impacted by N deposition is hard with these trends, but a richness of below 10 species per 2 x 2m quadrat is consistently associated with higher N deposition in both of the data sets examined in this study.

The decline in forb richness accounted for the majority of the species loss in both data sets and a decline in pleurocarpous moss richness was also observed in the Stevens data. Neither of these relationships was as strong as species richness, yet they present the same difficulties. In addition to the problems of collecting richness data described above, finding mosses in a closed sward requires careful attention and specialist knowledge is needed to distinguish species.

Assessments of percent cover for functional groups offers an alternative to richness. Percent cover is quick and easy to assess, but cover of plant species is notoriously variable between surveyors and is also prone to considerable seasonal variability as the growing season progresses. Random error between surveyors will reduce the sensitivity of the measurements to potential drivers and increase scatter about the regression line, but should not alter its slope or intercept. Bias is more problematic because it could affect the apparent direction and strength of relationships. The danger is that if it is not avoided or controlled, users may not be able to separate real ecological relationships from bias-induced relationships. The best guidance for avoiding bias is to be vigilant to the possibility of seasonal or observer effects and take steps to increase replication by gathering data across the growing season or at least by starting in the south or in lowland sites, and recording more northerly or upland sites later in the year. Future surveys should then be carried out at the same time of year (or ideally the same phenological stage) as the baseline.

As forbs account for the main decline in species richness, possibly due to increased competition with grasses promoted by N deposition (Stevens et al., 2004) using a grass:forb ratio provides a good potential indicator of N deposition impacts in this data set. Both the Stevens and CS data sets showed significant relationships with N deposition ($p<0.001$). Although there is a less clear trend in the CS data, the absolute values of the grass:forb ratios are similar between the two data sets. In both data sets, a grass:forb ratio of above five could be taken as indicative of sites where N deposition is having a negative impact on species composition. Grass:forb ratio may vary considerably over a site and several estimates would need to be made to gain an average, it will also vary with time of year and should only be estimated during the summer as that is when the reference data were gathered.

Grass cover itself may be quite hard for a non-expert to reliably estimate as there are many other graminoid species (especially sedges) that look like grasses without close inspection. A graminoid:forb ratio, where the cover of grasses and sedges are included together, provides an alternative with less potential for error in estimation. Although sedges do not show a response to N deposition alone they are a small component of the vegetation in these grasslands so the combined cover shows a very similar pattern.

Attributing the effects of nutrient enrichment and acidification to N deposition is very difficult because many of the effects are the same as those that would be seen from other forms of nutrient enrichment, such as the application of organic and inorganic fertilisers. Where species of unimproved habitats are used as positive site condition indicators, they will generally decline through the use of fertilisers as well as N deposition. To separate these effects using species composition alone is not possible. General observation of the surrounding area and identification of other nitrogen sources combined with the presence of negative site condition indicators and the absence of positive site condition indicators could be used to give a stronger indication of nitrogen source. This method could be applied to all habitat types, but as N deposition is expected to decline gradually across the UK (NEGTAP, 2001), relationships such as those described here need to be regularly validated to determine if species richness has rapidly responded to this change, or if it lags behind the change.

Considering whether a site is subject to other sources of enrichment by fertilisation (current or historic), the occurrence of flooding or the presence of flushes or springs should be established to identify whether atmospheric N deposition is responsible for site condition. In some managed habitats, failure to cut and remove herbage can have equivalent effects to N deposition. Reducing grazing pressure or changing the seasonality of management may also be factors in species loss or a change in species composition. If there are indicators of inappropriate or poor management, it may not be possible to determine the impact of N deposition. To confidently attribute N deposition as the cause of change in species composition would require some certainty that other factors such as management were optimal which may be difficult in practice. If the critical load for nutrient nitrogen is exceeded, this indicates that N deposition has a greater potential to have an impact. If there are point sources (such as intensive animal units where animals are housed, or manure stored) or diffuse sources (major roads and other areas of intensive vehicle use) in close proximity to the site, these will increase N deposition and the potential for damage from N deposition.

Of the variables investigated, the most promising as indicators of N deposition impacts were species richness, forb richness and graminoid:forb ratio (based on % cover). Of these variables, the graminoid:forb ratio is the easiest to apply and most reliable in a rapid assessment monitoring scheme. Although estimating the relative cover of these groups is not always easy in a species-rich grassland, it would be relatively easy to apply this indicator within the UK's current CSM framework, since cover of forbs is already estimated in the CSM guidance for upland acid grasslands (JNCC, 2004). As an indicator of N deposition impact there are other factors that could be responsible for such changes and this indicator should not be used without considering the proximity and potential impact of other current and historical sources of enrichment as well as site management history. This approach to identifying indicators could be used in other habitat types sensitive to N addition although data would need to be gathered to determine specific indicators.

Acknowledgements

This work was funded by the Joint Nature Conservation Committee (JNCC) and Natural England. We are grateful to staff from the JNCC, Natural England, Countryside Council for Wales and Scottish Natural Heritage for comments on the project report and to Clare Whitfield, Richard Jefferson, Zoe Masters, Ian Strachan and three anonymous referees for comments on this manuscript.

Countryside Survey is funded by a partnership of government funded bodies led by The Natural Environment Research Council (NERC) and the Department for Environment, Food and Rural Affairs (Defra). The other funding partners are: Natural England, Welsh Assembly Government, The Scottish Government, Northern Ireland

Environment Agency, the Forestry Commission, Countryside Council for Wales and Scottish Natural Heritage.

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Figure 1. CCA ordination diagram of species and total inorganic N deposition (Kg N ha^{-1} yr^{-1}) for Countryside Survey and Stevens datasets. Species △. For clarity labels for species are only shown for those either positively or negatively associated with N deposition. Survey (●) shown as a categorical variable.

Figure 2. Percentage cover against total inorganic N deposition (kg N ha^{-1} yr^{-1}) for A. *Campanula rotundifolia*, B. *Hylocomium splendens*, C. *Plantago lanceolata*, D. *Thuidium tamariscinum*, E. *Lotus corniculatus*, F. *Carex panacea*, G. *Carex pilulifera* and H. *Hypnum cupressiforme* for Countryside Survey and Stevens datasets.

Figure 3. Species richness per 2 x 2 metre quadrat against N deposition (Kg N ha^{-1} yr^{-1}) for Countryside survey (●) and Stevens survey (○).

Figure 4. A. grass:forb ratio and B. graminoid:forb ratio against total inorganic N deposition for Countryside survey (●) and Stevens survey (○).