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Journal Article

How to cite:

Damgaard, Christian; Jensen, Lennarth; Frohn, Lise Marie; Borchsenius, Finn; Nielsen, Knud Erik; Ejrnæs, Rasmus and Stevens, Carly J. (2011). The effect of nitrogen deposition on the species richness of acid grasslands in Denmark: a comparison with a study performed on a European scale. Environmental Pollution, 159(7) pp. 1778–1782.

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Version: Accepted Manuscript
Link(s) to article on publisher’s website:
http://dx.doi.org/doi:10.1016/j.envpol.2011.04.003

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The effect of nitrogen deposition on the species richness of acid grasslands in Denmark: a comparison with a European study

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Motivation (letter to the Editor)

The aims of this paper are i) to investigate the relationship between the model calculated nitrogen deposition and the observed species richness in monitoring data from Danish acid grassland; ii) to compare the results obtained from this analysis with the results obtained in the European BEGIN project (Steven et al. 2010); and iii) to consider a likely synthesis of the effects of nitrogen deposition on acid grasslands based on the two large investigations.

The European survey and the Danish monitoring program did not use the same protocol for collecting biodiversity data, and in order to compare the results of the Danish monitoring with the European survey it was necessary to convert the data from the Danish monitoring to a form that allowed a comparison of the two data sets. However, the comparison of the two different protocols for sampling species richness in acid grasslands also allowed an analysis of the possible reasons for the differences between the two data sets and an interesting synthesis to be made which could not be made by either study on its own.

If the scientific ecological community is going to have a role in answering important ecological questions, then we have to advance ways of comparing analogous ecological data that are obtained by different sampling methods and make relevant ecological syntheses, and this study is an example of such an undertaking.

Stevens, C.J. et al. 2010 Nitrogen deposition threatens species richness of grasslands across Europe. Environmental Pollution 158, 2940-2945.
**Abstract**

The effect of atmospheric nitrogen deposition on the species richness in acid grasslands was investigated by combining data from a large Danish monitoring program with a large European data set, where a significant non-linear negative effect of nitrogen deposition on species richness already had been demonstrated (Stevens, et al., 2010a). The nitrogen deposition range in Denmark is relatively small and when only considering the Danish data a non-significant decrease in the species richness with nitrogen deposition was observed. However, when both data sets were combined, then the conclusion of the European survey was further corroborated by the results of the Danish monitoring. Furthermore, by combining the two data sets, which were sampled with different protocols, a more comprehensive picture of the threats to the biodiversity of acid grasslands emerge; i.e., species richness in remnant patches of acid grassland in intensively cultivated agricultural landscapes is under influence not only from nitrogen deposition, but also from current and historical land use.
Nitrogen deposition from the atmosphere, which in part results from energy and food production, is considered to be one of the main causes for the currently observed decline in the biodiversity of terrestrial ecosystems (Millennium_Ecosystem_Assessment, 2005). The negative effect of nitrogen deposition on biodiversity has been demonstrated in several plant communities (Bobbink, et al., 2010), including acid grasslands, which are one of the plant communities that have received the most attention in regards to nitrogen deposition. In 2004, Stevens et al. demonstrated a negative correlation between species richness of acid grasslands and atmospheric nitrogen deposition in the UK (Stevens, et al., 2004). This relationship has since been confirmed using other independent datasets from the UK (Stevens, et al., 2009; Maskell, et al., 2010), in different habitats, including heathlands (Maskell, et al., 2010) and on a much larger spatial scale across the Atlantic biogeographic zone of Europe (Stevens, et al., 2010a).

The majority of the decline in the species richness of acid grasslands was accounted for by forb species, which showed a steep decline in their species richness and cover with increasing nitrogen deposition (Stevens, et al., 2006). There was a slight decrease in grass species richness, but an increase in grass cover. In the UK, bryophyte richness and cover showed no significant relationship with nitrogen deposition (Stevens, et al., 2006), but in the larger European survey a weak, but significant, negative relationship was apparent (Stevens, et al., 2010a). Dupre et al. (2010) used data gathered from vegetation surveys collected over a span of 70 years in the UK, Germany and the Netherlands to demonstrate that temporal declines in species richness in relation to cumulative N deposition were also apparent.
The mechanisms for a decline in species richness are not known, though they are most likely due to acidification and a reduction in the number of species that are tolerant of low pH and consequent metal mobilisation (Stevens, et al., 2009), or enrichment of the soil leading to increased productivity and competition for other resources. Experimental additions of N over ten years to an acid grassland in northern England have shown signs of soil acidification and consequent base cation depletion as well as enrichment (Horswill, et al., 2008). Results from the same experiment also showed a stimulation of N mineralisation (Morecroft, et al., 1994; Carroll, et al., 2003), although the majority of N added in the experiment was retained in the soil with leaching only representing an important loss of N at the highest treatment levels (140 kg N ha\(^{-1}\) yr\(^{-1}\)) (Phoenix, et al., 2003). Examination of Ellenberg values (Ellenberg, 1979; Hill, et al., 1999) and Grime’s C-S-R strategies for data from the UK gradient of deposition indicated that in this narrowly defined community acidification may be the main driver of species loss (Stevens, et al., 2010b).

In Denmark, an ambitious monitoring programme of natural habitats (NOVANA, Svendsen, et al., 2005) has been initiated as a direct response to the European Habitat Directive (1992), and one of the main objectives of the terrestrial monitoring program has been to assess the effect of nitrogen deposition on biodiversity in light-open natural habitats and suggest criteria for Favourable Conservation Status of the different habitat types (Søgaard, et al., 2007). The aims of this paper are i) to investigate the relationship between the model calculated nitrogen deposition and the observed species richness in monitoring data from Danish acid grassland; ii) to compare the results obtained from this analysis with the results obtained in the European BEGIN project (Stevens, et al., 2010a); and iii) consider a likely synthesis of the effects of nitrogen deposition on acid grasslands based on the two large investigations.
The European survey and the Danish monitoring program did not use the same protocol for collecting biodiversity data, and in order to compare the results of the Danish monitoring with the European survey it was necessary to convert the data from the Danish monitoring to a form that allowed a comparison of the two data sets. Unlike genetic data, which naturally come in a standardized form, ecological information may be sampled in different ways that all are more or less relevant for a specific scientific question, and different ecological studies typically use different sampling methods. However, if the scientific ecological community is going to have a role in answering important ecological questions, then we have to advance ways of comparing analogous ecological data that are obtained by different sampling methods and make relevant ecological syntheses, and this study is an example of such an undertaking.

The average annual nitrogen deposition in Denmark in 2008 was 14 kg/ha, with a geographic variation from 6 to 19 kg/ha on a regional scale and significantly larger variation on local scale (Ellermann, et al., 2010). One of the reasons for the large variations is the dependence of the deposition on the local emissions of ammonia, and thereby on the local agricultural activity level. Another variable that affects the deposition significantly is the precipitation amount. In general, the highest deposition values in Denmark are obtained in the southern part of Jutland where the agricultural production is intense and the amount of precipitation is large. Correspondingly, the lowest deposition values are seen in the northern part of Zeeland and on some of the smaller islands, where the distance to source areas is large and the amount of precipitation is low. The deposition process also depends on the atmospheric stability, the concentration of other gases and aerosols in the atmosphere and the characteristics of the surface. Regarding the latter, the deposition in general increases with surface roughness. As a consequence, deposition to e.g. a forest is typically larger than deposition to e.g. a meadow or a grassland area.
The nitrogen compounds which deposit from the atmosphere can be divided into two main groups: reduced (NH₃) and oxidised (NOₓ) nitrogen. NH₃ consists of gas phase ammonia (NH₃) and particulate ammonium (NH₄⁺) and the dominant source of these components in the atmosphere is agriculture. NOₓ consists mainly of gas phase nitrogen oxides (NO and NO₂) and particulate nitrates (NO₃⁻) and the origin of these components is primarily combustion processes. NH₃ dry-deposits relatively quickly compared to the other nitrogen components, and NH₃, therefore, often constitutes the largest part of the atmospheric nitrogen deposition close to agricultural sources. A significant part of the nitrogen deposition in background areas is related to wet deposition of particulate ammonium and nitrate. This is due to the fact that the diameter of the particulate nitrogen compounds is within the size range that has the smallest dry deposition velocity, and the primary removal pathway for the particulates is, thus, wet deposition.

**Materials and methods**

**Observed species richness**

The data for species richness in grasslands used in this study come from the Danish National habitat monitoring program, NOVANA, where the biodiversity of plant communities has been monitored since 2004 (Fredshavn, et al., 2009). This program records species composition in some 800 Danish sites forming part of the European Natura 2000 network. For each site, vegetation structure is recorded using a random stratified sampling process involving 20, 40 or 60 sample plots per locality, depending on its size. Sites are divided into intensively monitored stations, censused every year, and extensively monitored stations, censused only once per six years. For each sample plot, vegetation data is recorded by pin-point measure (n = 16) in a 0.25 m² quadrate plus a complete species list in a 78.5 m² circle (radius = 5 m) centered on the sample quadrate. Data used in this
study are the species lists from the 78.5 m$^2$ circles from intensively monitored stations. Only sample plots classified as habitat type 6230, "Species-rich Nardus grasslands", were included. In the Danish interpretation of the European habitats directive, this type includes semi natural grassland with low pH, often dominated by species such as Deschampsia flexuosa, Festuca ovina and Carex pilulifera.

Data for a total of 1669 sample plots distributed on 120 sites were used. Species richness was calculated as the average species richness recorded for the years 2004-2007, as records from 2008 and 2009 were not completely entered into the database at the time of data extraction (March 20, 2009).

To link species richness of sample plots established according to the Danish NOVANA sampling protocol to species richness data sampled according to the European BEGIN protocol, during the period 6. July – 17. July 2009 we re-sampled species diversity following the BEGIN protocol (Stevens, et al., 2010a) in 55 acid grassland sample plots at 14 sites. The sites were selected to be representative of the Danish nitrogen deposition gradient. Additionally, a minimum of 50 % of the sample plots at the station should be habitat type 6230. The spatial position of the 55 sample plots are known with GPS accuracy (<2 meter), and the species richness of each plot had been sampled several times in the period from 2004 – 2007 using the Danish protocol.

The European BEGIN protocol involved sampling five 4 m$^2$ plots at random locations within 1 hectare avoiding areas which could be identified as belonging to different vegetation communities (e.g. wet areas) animal tracks or paths, animal feeding areas or field boundaries. All species (vascular plants and bryophytes) were identified to a species level and mean species richness for the site was calculated.
The observed species richness using the European protocol (Stevens, et al., 2010a) was regressed in a linear model on species richness using the Danish protocol (average of the available data in the period 2004 – 2007). The residual variation of the observed species richness using the European protocol was assumed to be normally distributed, and this assumption was checked by plotting the residuals. Afterwards, the linear model was used to predict the expected species richness in the 120 Danish acid grassland sites if they had been sampled according to the European protocol.

**Nitrogen deposition**

In the Danish National Monitoring and Assessment Programme (NOVANA), the nitrogen deposition is calculated using a regional scale model - the Danish Eulerian Hemispheric Model (DEHM, Christensen, 1997; Frohn, et al., 2001; Frohn, 2004) as well as a local scale model - the Operational Model for Air Quality (OML-DEP, Sommer, et al., 2009).

In the present study, a data set consisting of depositions calculated with the DEHM model for the year 2006 and reported within the NOVANA programme (Ellermann, et al., 2007), has been utilised. Data have been extracted from depositions calculated to all land surfaces in Denmark and consist of separate values of dry and wet deposition of NH$_3$, NH$_4^+$ and NO$_3^-$ to grass surfaces for all plots.

The resolution of the DEHM calculations is 16.67 km x 16.67 km and the dry deposition scheme implemented in the models has been adapted from the EMEP model (Simpson, et al., 2003). With the aid of this scheme, the dry deposition of all relevant nitrogen components is calculated to ten generalised surface classes using among other parameters, information of leaf area index, length of growth season and average height of typical vegetation.
Statistical analysis

As suggested by Stevens et al. (2010a), the relationship between observed species richness and the calculated nitrogen deposition in acid grasslands was investigated in an exponential function,

$$S = a \text{ Exp}(-b \text{ Ndep})$$

(1),

where $S$ is the observed species richness and $Ndep$ is the calculated total nitrogen deposition. The observed species richness of the two “surveys” was compared at variable nitrogen deposition rates using the model,

$$S = a \text{ Exp}(-b \text{ Ndep}) + c \text{ Survey}$$

(2),

where Survey is a coded variable for either the European survey (coded zero) or the Danish monitoring (coded one).

In both models (1) and (2), the residual variation of the observed species richness was assumed to be normally distributed, and this assumption was checked by plotting the residuals.

Results

The relationship between the observed species richness using the European protocol and the average of the available data of species richness from the plot using the Danish protocol in the period 2004 – 2007 was approximately linear (Fig. 1), and 44% of the variation was explained by a linear model. Consequently, it was concluded that the linear model was adequate for predicting the
expected species richness in the 120 Danish acid grassland sites if they had been sampled according to the European protocol.

The Danish monitoring data displayed a non-significant decrease of species richness with nitrogen deposition when fitted to an exponential model (P = 0.16), contrasting the European data where a significant negative response of richness to nitrogen deposition was found (P < 0.00001, Stevens, et al., 2010a). The likelihood of demonstrating a negative effect of nitrogen deposition on the species richness of higher plants in the Danish monitoring is smaller given the narrow range of calculated nitrogen deposition between 8.1 kg ha\(^{-1}\) yr\(^{-1}\) and 17.2 kg ha\(^{-1}\) yr\(^{-1}\) compared to the European survey, which is between 2.4 kg ha\(^{-1}\) yr\(^{-1}\) and 43.5 kg ha\(^{-1}\) yr\(^{-1}\) (Fig. 2). The correlation in the European data set remains significant however when tested within the Danish range in nitrogen deposition (P = 0.00048).

The species richness in Danish acid grassland is estimated to be 2.5 species lower (when measured in a 4 * 4 meter plot) than expected in acid grasslands with a similar nitrogen deposition sampled from across Europe (parameter \(c\) in Table 1) indicating that differences between the two samples of acid grassland should be taken into consideration in the interpretation of the results.

Discussion

The main aim of the Danish monitoring program is to evaluate the conservation status of species and habitats targeted by the habitats directive by means of their status and trends. Nitrogen deposition is one amongst a number of current threats to the conservation status of acid grassland (habitat type 6230*) as shown by e.g. Stevens et al.(2010a).
In this study we tested the relationship between modelled nitrogen deposition and species richness from 120 monitoring sites and found a non-significant declining trend (P=0.16). Stevens et al. (2010a) found a similar, but significant decline in richness along a European gradient in nitrogen deposition. Furthermore comparisons between the two data sets revealed that the Danish sites had lower mean species richness than the European sites when the effect of nitrogen deposition was taken into account (Table 1).

The Danish data set differs from the European data in several respects: First the range in deposition values for Denmark is much narrower than for Europe (Fig. 2). Secondly, in the BEGIN survey only localities with a narrow community definition and current management by grazing or mowing were included (Stevens, et al., 2010a). In the Danish monitoring program, a slightly broader community concept was used, including species-poor grass heaths and dry acid grasslands, and possibly also sites with a management and land use history including current or historical abandonment and/or old ex-arable fields (Fredshavn, et al., 2009). Thus the Danish monitoring data has a relative narrow nitrogen deposition gradient but a relative large variation in soil types, management and land use history compared to the European survey, and this may explain why the observed relationship between nitrogen deposition and species richness was insignificant in Denmark.

The Danish sample spans the same range in species richness as the European sample, with sites reaching the observed European maximum of 25-30 species per site (Fig 2). It is therefore not the range, but rather the density of sites of low species richness that is causing the observed lower mean species richness in Denmark. In this way the scatter of Danish sites supports our notion that
differences in sampling protocols rather than different species pool sizes may explain the lower species richness of the Danish sample.

The variation in species richness of Swedish and Danish semi-natural grassland sites and adjacent ex-arable fields has been found to be under strong influence of land use history (Bruun, et al., 2001; Cousins, et al., 2009), and we find it likely that larger variation in land use history may explain the Danish bias towards sites of lower species richness as compared to the other European data. This bias reflects the character of the Danish landscape which is intensively cultivated and generally without natural constraints to cultivation such as rocky outcrops and extensive uplands and mountain areas. The larger variation in conservation value of the Danish data may thus explain the weakening of the response of species richness to the studied gradient in nitrogen deposition.

Experimental studies have shown considerable delays between nitrogen addition and resulting effects on species richness in grassland vegetation (Clark and Tilman, 2008) and it may be considered a weakness that our study only considers current gradients in nitrogen deposition. However, the ranking of the nitrogen deposition across the Danish sites has been relatively stable over the years (pers. com. Jesper Christensen, NERI, Aarhus University), so even though the fitted relationship between the calculated nitrogen deposition and the observed species richness may depend somewhat on the year the calculation was made, the qualitative relationship is expected to stay the same. The same is true if species richness actually depends on the cumulative deposition over several years.

If the Danish monitoring results had been analysed without considering the results of the European survey, then the likely conclusion would have been that: “nitrogen deposition has no significant
effect on the species richness in acid grasslands”. However, this conclusion cannot be maintained in the light of the results of the European study (Fig. 2), and instead we have to conclude that the observed decrease in species richness with nitrogen deposition in the Danish monitoring (Fig. 2), although not statistically significant when analysed on its own, is a genuine observation. Additionally, the conclusion of the European survey has been further corroborated by the results of the Danish monitoring. Furthermore, when the results of the Danish monitoring program are analysed together with data of the European survey is analysed together, then a more comprehensive picture of the threats to the biodiversity of acid grasslands emerge; i.e., species richness in remnant patches of acid grassland in intensively cultivated agricultural landscapes is under influence not only from nitrogen deposition, but also from current and historical land use.
Tables and Figures

Table 1. The estimated parameters in the model \( S = a \exp(-b \, Ndep) + c \, \text{Survey}, \) where \( S \) is species richness, \( Ndep \) is the calculated total nitrogen deposition, and \( \text{Survey} \) is a coded variable for either the European survey (coded zero) or the Danish monitoring (coded one).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t Statistic</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
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<td>( a )</td>
<td>24.80</td>
<td>1.061</td>
<td>23.37</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>( b )</td>
<td>0.0233</td>
<td>0.00248</td>
<td>9.37</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>( c )</td>
<td>-2.470</td>
<td>0.559</td>
<td>-4.42</td>
<td>0.000014</td>
</tr>
</tbody>
</table>
Fig. 1. The relationship between the observed species richness of the Danish sampling method (average of available data 2004 – 2007 from the plot) and the observed species richness of the European sampling method from the plot. Fitted linear model: $y = 3.83 + 0.4506 \times x$; $R^2 = 0.44$ (adjusted for the number of model parameters). The positions of the plots were determined with GPS accuracy.
Fig. 2. The relationship between calculated total nitrogen deposition and observed species richness in a European survey (blue points) and a Danish monitoring (red points). The species richness in the observed in the Danish monitoring program has been converted in order to allow a direct comparison with the European survey. The lines are the fitted exponential functions of the two data sets. The data of the European survey showed a significant decrease in species richness with nitrogen deposition ($P < 0.00001$), whereas the data of the Danish monitoring program showed a non-significant tendency of species richness decline with nitrogen deposition ($P = 0.16$).
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