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5.6 Impact of nitrogen deposition on species richness of calcareous grasslands in Europe - some preliminary results

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Abstract
- This paper seeks to determine whether N-deposition has a negative impact on the species richness of calcareous grasslands at a European wide scale.
- 100 calcareous grasslands across the Atlantic region of Europe were sampled in one season. Species composition and richness of vegetation communities were compared to some key environmental drivers (climate and N deposition) indirectly estimated via surrogates (latitude, longitude, N concentration in bryophyte tissue).
- There are marked differences in species composition across the calcareous grasslands of the Atlantic biogeographic zone within Europe. Contrasts in mean species richness between regions are also detectable at a European wide scale. These natural gradients may mask any footprint of N deposition on vegetation at a European-wide scale.
- For grasslands located along the western range of distribution, there are indications of a decline in species richness as N concentration in moss increases. This suggests that N deposition may be reducing biodiversity in calcareous grasslands at a wide scale, but that this impact can only be detected at the regional, rather than cross-European, level.
- Further research is needed to investigate the impact of N deposition on calcareous grasslands, particularly through the direct assessment of potential drivers as well as the characterisation of variations in species pools at the European scale.

5.6.1 Introduction
The increase of atmospheric deposition of nitrogen (N) in recent decades, due to fertilizer application and fuel consumption, represent nowadays a major threat for biodiversity in ecosystems (Langan 1999, Phoenix *et al*., 2006). In Europe, where this trend has been particularly documented, the

effects of N deposition have been studied through empirical or experimental approaches (Bobbink 1998, Stevens *et al*., 2004). Among several effects, N deposition affects nutrient availability, which is a major driver of plant community composition and species richness (Tilman and Pacala, 1993). As a consequence, the impact of N deposition is of major concern for those species-rich ecosystems which are strictly associated with nutrient-poor soils such as Natura 2000 grasslands and heathlands. Recent comparative studies, based on either spatial gradients or on time series analyses, have shown clear evidences of an impact of N deposition on acidic grasslands, leading to a decrease of plant species richness and a loss of species associated with less fertile conditions (Dupré *et al*., 2009, Stevens *et al*., 2004). Such evidence is also found, at local or national level, for other species-rich habitats such as calcareous grasslands or heathlands (Maskell *et al*., 2009), or from experimental results (Bobbink, 1991, Willems and van Nieuwstadt, 1996). Whether such results are generally applicable is of particular importance because calcareous grasslands are of major interest for the conservation of biodiversity in Europe as they support communities of exceptional diversity and many rare and endangered species of plants, insects and birds (WalliesDeVries *et al*., 2002).

The lack of knowledge at a broader scale has motivated the BEGIN project (Biodiversity of European Grasslands – the Impact of Atmospheric Nitrogen Deposition) which seeks to determine whether N-deposition is impacting the species richness of grasslands on a European wide scale . Different approaches have been used to assess the loss of biodiversity associated with N deposition in acidic grasslands: historical analysis (Dupré *et al*., 2009), experimental and comparative surveys (Stevens *et al*., 2004, 2010). Another objective of BEGIN was to investigate whether a similar decrease in biodiversity is occurring in a contrasting grassland system. The most important grassland type across Europe in terms of biodiversity are the calcareous grasslands of the Mesobromion alliance (Koch, 1926). Compared to acid grasslands, these habitats have a much greater species richness and larger number of rare species, and are also presumed to be sensitive to N deposition through increasing nutrient availability. We thus hypothesised that N deposition may be significantly impacting these grasslands.

5.6.2 Aims and objectives

- We aim to determine whether any significant variability in plant species richness in calcareous grasslands across Western Europe could be detected and related to any regional-scale evaluation of N atmospheric deposition.
- In 2008, we surveyed 100 calcareous grasslands belonging to the Mesobromion alliance on a transect across the Atlantic biogeographic zone of Europe. Site selection was performed through a composition criterion (required presence of five species among a predefined list of target species) and a management criterion, in order to avoid abandoned grasslands. For each site, five 1 m × 1 m replicates were recorded. In each square meter, the cover of all occurring plant species (vascular plants and bryophytes) was visually estimated. Each site is therefore characterised by a list of species with average abundance (calculated from the five quadrats) and an average richness per plot (n=5 replicates).
- In this preliminary study, we only used environmental surrogates to account for the major environmental drivers we identified. A major predictor of large scale species richness is latitude (Hillebrand, 2004). We used latitude and longitude as aggregate variables integrating distinct climate factors i.e. mainly temperature (North-South) and precipitation (West-East) gradients. Total nitrogen concentration in bryophytes was used as a surrogate to estimate total N deposition at a high resolution (Harmens *et al*., 2008). Because species-specific differences are expected, we considered only the sites where the same moss species (i.e. Ctenidium molluscum) was collected and analysed. This accounted for about half of the sampled sites (51 sites for the 100 sites of this study).

- We performed a correspondence analysis (CA) and a hierarchical clustering of the [100 sites x 161 species] data table in order to provide an ordination and classification of plant communities. The table was obtained after removal of species occurring in less than 5 per cent of the records in the initial table [100 sites x 225 spp]. Similar analysis was performed on the sub-set of 51 sites where some environmental surrogates were available. Simple regressions were performed, after data normality was tested (Shapiro-Wilk test), to assess correlations between environmental surrogates and species richness (i.e. mean species number for the five replicates) and species composition (floristic gradients from the CA). All analyses were performed with R free software (2007).

5.6.3 Results and discussion

The Correspondence Analysis shows that gradients in species composition are well correlated to the geographical distribution of the sites (Figure5.8a,b). Latitude and longitude are highly significantly correlated with respectively axis one ($n=100$, $r^2=0.52$, $p<0.001$), and axis two ($r^2=0.63$, $p<0.001$) of the CA. A hierarchical clustering (Ward method) performed on the output of this CA (Figure 5.8c)

gives a very similar result to clusters defined on a national basis. Three main types of plant communities can be defined (Table 5.3), related to sites from France (FR), United Kingdom and Eire (UK+IRL), and Germany (GER). Sites from north central Europe (Belgium, Netherlands, Denmark) are distributed within these three clusters, while Norway sites form a specific sub-cluster.

Looking for pattern of variation of community species richness along these floristic/geographic gradients of the CA, we found no evidence of a correlation with species richness for any of the CA axes. However, when considering the different clusters from the CA, species richness appear significantly different between some regions (Figure 5.9 - one way ANOVA; $F= 3.01$, $df=99$, $p<0.05$). As we avoided abandoned sites, these differences could not be due to management contrasts but rather to differences in species pool size, depending on regional specificity (soil, climate, history).

We analysed the sub-set of 51 sites to test whether species composition gradients and species richness variations could be correlated to N deposition, estimated via the N surrogate (N per cent in the moss C. molluscum). The 51 sites were distributed in the three main clusters-regions: 27/27 sites of the South-West (SW) of Europe (CL2), 13/36 sites of the North-West (NW) of Europe (CL3) and 11/32 sites of the Est (E) of Europe (CL1). These sites were also regularly distributed along CA axes. We then performed a new Correspondence Analysis (CA2) on these 51 sites, to built floristic gradients on this specific data set. Patterns were similar to the first CA, the CA2 axes being even more correlated to latitude and longitude ($n=51$, $r^2=0.56$, $p<0.001$ for axis 1; $r^2=0.82$, $p<0.001$ for axis 2). N per cent in bryophyte tissue was correlated only with the axis three of this second correspondence analysis ($r^2=0.10$, $p<0.05$), suggesting at least that N deposition could be correlated to gradient of species composition in this data sub-set. We did not find any correlation

Table 5.3:

Cluster	Region	Countries	Some differential species
CL1	E	GER, (B), (NL)	*Silene vulgaris, Inula conyza, Poa angustifolia*
(CL1bis)	E	Norway	*Viola canina, Deschampsia flexuosa, Alchemilla filicaulis*
CL2	SW	FR, (B)	*Teucrium montanum, Gaudinia fragilis, Seseli montanum, Thesium humifusum*
CL3	NW	UK, IRL, DK, (B), (NL)	*Carex humilis, Festuca arundinacea, Ranunculus repens*

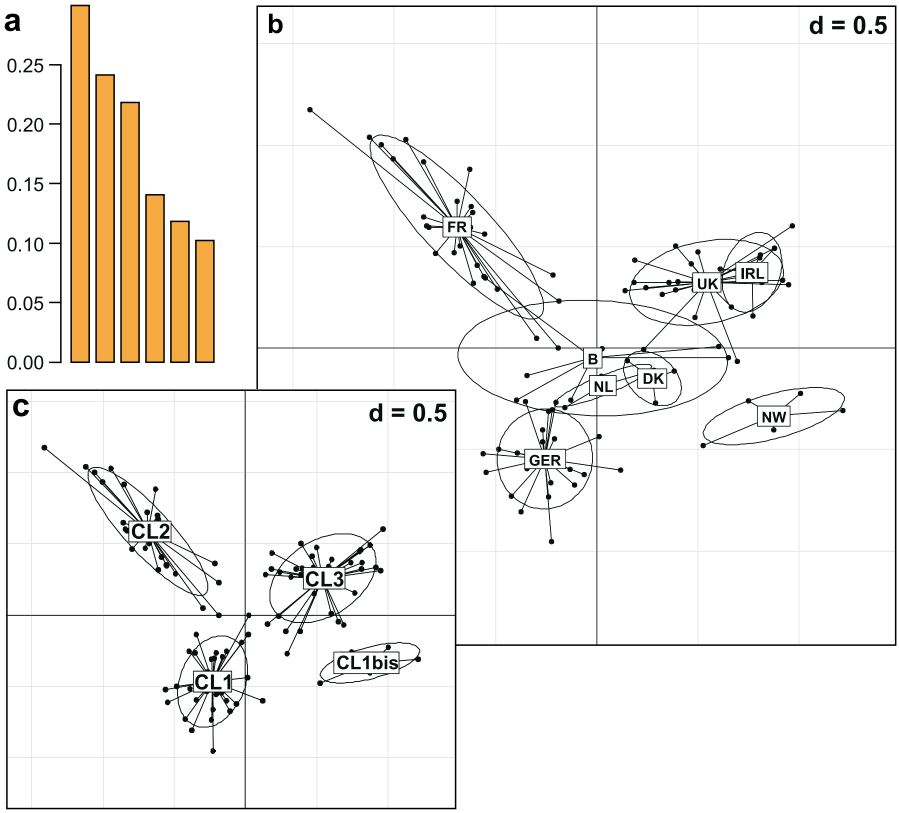

Figure5.8: Correspondence analysis of the [100 relevés x 161 species] data table for the analysis of composition gradients in calcareous grasslands. a) Eigenvalues; b) F1×F2 plane showing distributions of relevés in the national surveys, c) F1×F2 plane with clusters performed from hierarchical clustering (Ward method) on relevés coordinates.

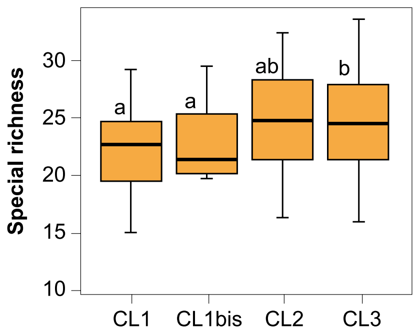

Fig.5.9: Box plots showing species richness (mean SR.m-²; n=5 replicates) variations in the four clusters from the CA. see text for details. Boxes sharing the same letter are not statistically different (P < 0.05, Tukey's HSD comparing all clusters).

between N surrogate and species richness of plant communities at a broad scale (Figure5.10a; n=51, p=0.18). However, when we performed regressions at the regional level (Figure 5.10 b,c,d), correlation was significant for SW region (Cluster 2, n=27, $r^2=0.20$, $p<0.05$), while no trend was detected for other

clusters. When significant, correlation shows a decline of species richness at the highest levels of N concentration (deposition). The processes responsible for this decline may be found in the effects of N enrichment, resulting in changes in vegetation structure and species interactions to the benefit of competitive tall grasses (Bobbink, 1991, Liancourt *et al*., 2005).

From our data, composition gradients in calcareous grasslands are marked at the European scale, even though we removed the less frequent species in the data set (i.e. with occurrence less than 5 per cent) which should attenuate contrasts between countries. This species turn-over is shown in phytosociological works (Royer 1985, Willems, 1982). Our survey confirms that climate gradients are likely to be the most important drivers of species turn-over in calcareous grasslands in Europe, as climate variables such as temperature and rainfall are known to be correlated with latitute and longitude (Ozenda 1994, Duckwoth *et al*., 2000). Similarly, our data suggest that regions in Europe could be characterised by species pools of different sizes. However, this has to be confirmed with species pool studies (e.g. Dupré, 2000), based on more complete phytosociological datasets.

Because of the strong climate-driven variation in species composition and richness, it is difficult to detect a separate signal of N deposition as a potential driver of calcareous grassland diversity on a cross-Europe scale. The use of N concentration in moss as a surrogate for N deposition can also introduce some potential artefacts. Besides differing among different species, this relationship might also depend on other factors such as N speciation, the ratio of wet/dry deposition in N deposition, and local climate (Harmens *et al*., 2008). Despite these limitations, there are some indications of an N-deposition signal on species composition at a European wide scale and on species richness for calcareous grasslands located at the western range of their distribution.

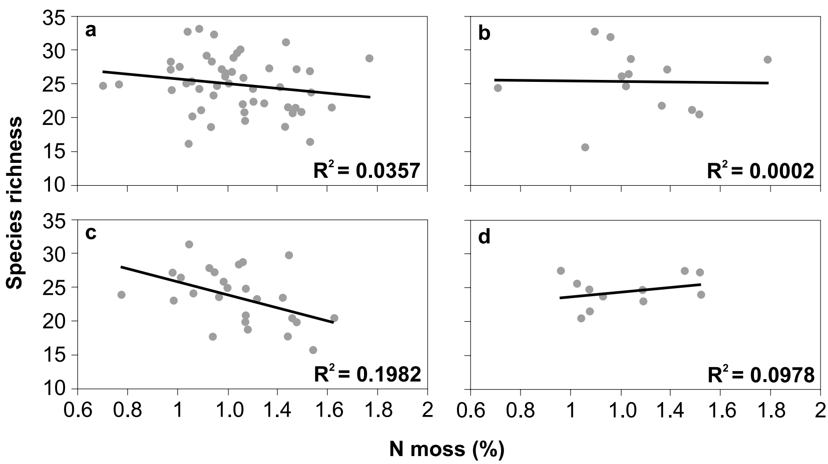

Fig.5.10: Regression plots between nitrogen concentration in the moss Ctenidium molluscum (N per cent dry weight) and species richness (mean SR.m-²; n=5 replicates) in calcareous grasslands according to different geographic ranges: a) subset covering the whole geographic range i.e. Atlantic Europe; n=51; b) subset from NW Atlantic Europe i.e. Cluster 3; c) subset from

5.6.4 Conclusions

- There are strong gradients of species composition in calcareous grasslands in western Europe. Contrasts in mean species richness between regions are also detectable at a European-wide scale. These gradients and contrasts appear to be driven primarily by climate.
- Because of these strong environmental responses, it is difficult to detect a clear influence of N deposition on species richness and composition at the European scale.
- However, when filtering the data at the regional to national scale, there are indications of an N-deposition signal on species richness for grasslands sampled in western regions (Atlantic coast). If real, regressions suggest a fairly strong decline in diversity with increasing N deposition for these sites
- These intimations of a N impact on calcareous grassland diversity strongly point to a need for targeted research, particularly through the direct assessment of potential drivers as well as the characterisation of natural variations in species pools at the European scale.
- The above have strong implications for conservation and pollution mitigation actions for management of calcareous grasslands (Calciura and Spinelli, 2008).

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5.7 Nitrogen critical load and butterflies

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Abstract
The biodiversity effect of nitrogen deposition is now well recorded and explored for vegetation but the effect on invertebrates representing most of biodiversity (by number of species) is unknown.

Biodiversity is defined and used as the product of a number of qualitative indices of a group of organisms (in this case butterflies).

Meta data analysis of seventeen years of survey data of butterflies in a wide range of habitats in the Netherlands is analysed along with nitrogen critical load exceedence (CLE) data calculated for the same survey sites.