Scale-dependence of vegetation-environment relationships in semi-natural grasslands

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Abstract

Questions: Which environmental and management factors determine plant species composition in semi-natural grasslands within a local study area? Are vegetation and explanatory factors scale-dependent?

Location: Semi-natural grasslands in Lærdal, Sogn og Fjordane County, western Norway.

Methods: We recorded plant species composition and explanatory variables in six grassland sites using a hierarchically nested sampling design with three levels: plots randomly placed within blocks selected within sites. We evaluated vegetation-environment relationships at all three levels by means of DCA ordination and split-plot GLM analyses.

Results: The most important complex gradient determining variation in grassland species composition showed a broad-scale relationship with management. Soil moisture conditions were related to vegetation variation on block scale, whereas element concentrations in the soil were significantly related to variation in species composition on all spatial scales. Our results show that vegetation-environment relationships are dependent on the scale of observation. We suggest that scale-related (and therefore methodological) issues may explain the wide range of vegetation-environment relationships reported in the literature, for semi-natural grassland in particular but also for other ecosystems.

Conclusions: Interpretation of the variation in species composition of semi-natural grasslands requires consideration of the spatial scales on which important environmental variables vary.

Keywords: Complex gradient; Management; Nested design; Scale; Split-plot GLM.

Nomenclature: Lid & Lid (1994) except for *Betula pubescens* agg. (which includes *B. pubescens* and *B. verrucosa)* and *Hieracium* spp. (which includes all *Hieracium* species except *H. umbellatum).*

Abbreviations: CNR = Cutting with no grass removal: CR = Cutting with grass removal; HAG = Heavy autumn grazing; HI = Heat index; HSG = Heavy spring grazing; LAG = Light autumn grazing; LSG = Light spring grazing; MI = Management index.

Introduction

The sampling scale of a study, i.e. the extent and the grain of the observations, may profoundly affect the patterns displayed (Levin 1992). Thus, the plot size of a vegetation study determines the spatial levels of resolution. Variation in scales finer than the plot size will most likely not be revealed by such analyses (Wiens 1989). As the choice of plot size (the grain) may influence the range of detectable vegetation-environment relationships, plot size should reflect the scale at which variation in environmental variables takes place (Økland 1990; Levin 1992).

Variation on a hierarchy of scales is typical of semi-natural grasslands where management greatly influences the vegetation-environment relationships (Austrheim et al. 1999; Kahmen et al. 2005). Semi-natural grassland vegetation has developed by modification of natural ecological gradients and addition of new management related gradients through long histories of human use, e.g. cutting or grazing or both (Pärtel & Zobel 1999; Hansson & Fogelfors 2000).

Different complex gradients have been suggested as major determinants of variation in semi-natural grassland vegetation, ranging from soil element concentrations (Austrheim et al. 1999; Norderhaug et al. 2000; McCrea et al. 2001; Kahmen et al. 2005) and soil moisture condititons (Garcia 1992; Bratli & Myhre 1999) to regional-scale climatic variables (Losvik 1993; Yeo & Blackstock 2002). The inconsistency of these findings may reflect the high complexity of semi-natural vegetation, but may also reflect differences in grain and extent of studies, e.g. the variation in plot size from 0.25 m^2 (e.g. Garcia 1992) via 25 m^2 (e.g. Ejrnæs & Bruun 1995) to entire fields (Yeo & Blackstock 2002; Bratli et al. 2006).

Management regimes usually exhibit fine scaling both in time and space (Jantunen & Saarinen 2003; Alard et al. 2005). However, the details of management history may hardly ever be recovered for scales finer than the individual management units, i.e. the fields (Bratli & Myhre 1999; Vandvik & Birks 2002). Variables such as

soil chemical properties, on the other hand, vary at far finer scales (Robertson et al. 1988; Jackson & Caldwell 1993) and may easily be collected for small plots.

A broad analysis of vegetation-environmental relationships therefore requires comparisons on different scales. This means that the conventional methods for establishing relationships between vegetation (species composition) and recorded explanatory variables are unsuitable. These methods include multivariate (ordination) analyses with subsequent interpretation by standard correlation analyses, of which the latter are invalid for nested designs or when explanatory variables (as is inevitable in many studies) are sampled on different spatial scales (Crawley 2002; Økland 2007). In this paper we introduce the use of split-plot GLM for analyses of relationships between vegetation variation and explanatory variables recorded at different scales. These methods explicitly take the hierarchical error variance structure into account and use correct degrees of freedom in statistical tests, thus avoiding spatial pseudoreplication of data (Crawley 2002; Økland 2007).

Semi-natural grasslands are among the most species-rich habitats in Europe (e.g. Kull & Zobel 1991; Myklestad & Sætersdal 2003). The considerable reductions in area these ecosystems currently undergo are considered major threats to European biodiversity (Pykälä 2000). To sustainably manage semi-natural grassland ecosystems, we need to understand relationships between management regime, important environmental factors and the species composition (Chapin et al. 1996). The aim of our study is to contribute to such an understanding, by examining the relationships between vegetation and environment on different scales (grains and extents).

Material and Methods

Study site

We studied six semi-natural grassland sites, three pastures and three road verges in Lærdal, Sogn og Fjordane county, W Norway (61°04' N, 7°32'- 49' E). The sites were situated < 11 km from each other, at altitudes from 35 to 420 m a.s.l., all faced south (from SE to SW). Areas of the pastures varied from 2.8 to 7.5 ha while the road verges comprised narrow (3 - 5 m) strips of 50 - 100 m length. All study sites were situated on terraces formed by eroded glacifluvial deposits (Klakegg et al. 1989). The bedrock consisted of Precambrian gneisses, except for one site on gabbro (Klakegg et al. 1989). All sites were situated in the southern boreal, slightly continental region (Moen et al. 1999), had low annual precipitation (ca. 500 mm, Førland 1993) and annual mean temperature of ca. 5.9 °C (Aune 1993) for the normal period 1961-1990.

Vegetation recording

In each of the six study sites we subjectively placed seven blocks – 3 m × 5 m in road verges (2 m × 7.5 m in the narrow SR road verge) and 4 m × 4 m in pastures – to span apparent local environmental variation. Quadrats of 1 m × 1 m were placed at random within each block (avoiding contiguency), five in each road-verge block and three in each grassland block. One 0.5 × 0.5 m plot was systematically placed in the middle of each quadrat to ensure a minimum between-plot distance of 0.7 m. Potential positions for quadrats were rejected if stone/rock covered more than 25% of the central 0.25 m² plot and a substitute quadrat was then chosen from a fixed priority list of adjacent positions. We divided each 0.25-m² plot into 16 subplots of 0.0156 m², and used subplot shoot frequency (0 - 16) as a measure of abundance of vascular plant species in the plot. In the vegetation analyses carried out in June-July 2003 a total of 92 vascular plant species were recorded in the 168 plots.

Recording of explanatory variables

Twenty-one explanatory variables, potentially influencing the vegetational variation, were recorded for each of the 168 plots. Several soil chemical and physical variables were determined in soil collected in four soil sub-samples from the upper 5 cm of the soil in each quadrat. The sub-samples were mixed and dried at 25 °C for ten days in drying cabinets before sifting (2 mm mesh width). pH and loss on ignition were measured at the soil laboratory at the University College of Sogn og Fjordane in accordance with Krogstad (1992). The soil laboratory at Skogforsk, Ås conducted the remaining analyses. Total N was determined by the Kjeldahl method by flow injection analysis (Skogforsk method FIA41000.M). A NH_4NO_3 solution was used for extracting elements from soil (Stuanes et al. 1984). Exchangeable element concentrations were determined by ICP (Skogforsk method ICP43000.M). We used elements regarded as macronutrients, micronutrients, or toxic to plants (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn) for further analyses, and recalculated all elements as ppm (parts per million) in organic matter by multiplication with 1/Loss on ignition (Økland 1988).

Soil moisture was determined thermogravimetrically (Gardner et al. 2001) from two soil sub-samples collected from each quadrat on 6 August 2003, after three days without rainfall. The reported soil moisture values are considered to represent median soil moisture conditions. Soil depth was measured as the distance a steel rod (1 cm diameter) could be driven into the soil at eight fixed positions 10 cm outside each sample plot border, and plot soil depth was calculated as the median

of these measurements.

Aspect and slope (°, 0-360 scale) were measured by means of clinometer compass at a position representative for each plot. Aspect was converted to Aspect favourability (Asp) on a 0.00-2.00 scale according to Beers et al. (1966). Heat index (HI) was calculated from aspect and slope according to Heikkinen (1991).

Management history

In addition to the 21 explanatory variables recorded in each plot, we developed a management index (MI) for each of the six sites. The index was based on information from the landowners and the local road authorities. The three pasture sites – Molde pasture (MP), Stuvane pasture (SP) and Nese pasture (NP) – had experienced traditional management for centuries, including spring and autumn grazing (generally from late May to Mid-June and from late August till late September, respectively). MP and NP had also been mown in July, but cutting had ceased completely or had been accomplished only infrequently for the last 20 years. Fertiliser had never been added to the pastures except for the dung deposited by grazing animals.

Two of the road-verge sites, Molde road verge (MR) and Stuvane road verge (SR), lay close to the MP and SP sites, respectively, and before road improvement in these areas (in 1950), they actually shared management with the respective pasture site. The vegetation adjacent to these verges was dry grassland. The Nese road verge (NR) was established during widening of the road in 1964 and had no former history of grazing or mowing. This verge bordered an encroached grassland dominated by young *Alnus incana*. The distance between NV and NP was ca. 1 km. The traffic intensity varied from low at the MR site to high at the two other sites. Since 1990, the road-verge sites have been cut annually, either by the road authorities or by Lærdal municipality. Grass has not been removed but instead left *in situ* for decomposition.

The MI incorporated the type and duration of management activities over four time periods from 1931 until 2003. We differentiated two cutting regimes, cutting with (CR) and without (CNR) hay removal, and four grazing regimes, light spring grazing (LSG), light autumn grazing (LAG), heavy spring grazing (HSG) and heavy autumn grazing (HAG). Light and heavy grazing differed in animal (mostly sheep) density, light grazing corresponding to less (usually much less) and heavy grazing to more than 40 sheep per ha. The cutting and grazing regimes were weighted differently. The two heavy grazing regimes (HSG and HAG) were given a weight of 3 as we assumed stronger impact on vegetation composition through removal of vegetation, gap creation through trampling and fertilizing effect of the dung (Wahlman & Milberg 2002). Light spring grazing (LSG) was given weight 2 and light autumn grazing (LAG) weight 1 as we assumed that grazing in spring would have greater impact than autumn grazing (Kahmen et al. 2005). Cutting with grass removal (CR) was given weight 2, while the modern cutting regime of road verges (late cutting with no grass removal, CNR) was given weight 1. Annual contributions to the management index $f(i)$ were calculated for every year in each of the six sites according to the following equation:

$$f(i) = 2CR+CNR+3HSG+3HAG+2LSG+LAG \quad (1)$$

Both present-day and historical management were included in the index, reflecting that past use also may influence present species composition (Norderhaug et al. 2000; Lindborg & Eriksson 2004; Cousins 2006). We gave higher weights to recent periods than to former as we assumed recent management to have stronger impact on present species composition: 1991-2003 was weighted 0.7 per year, 1971-1990 0.5 per year, 1951-1970 0.2 per year, and 1931-1950 0.1 per year. The management index (MI) for the whole time period since 1931 for each site was then calculated as:

$$M = 0.7\sum_{1991}^{2003}f(i) + 0.5\sum_{1971}^{1990}f(i) + 0.2\sum_{1951}^{1970}f(i) + 0.1\sum_{1931}^{1950}f(i) \quad (2)$$

The three pasture sites achieved the highest MI values; indeed much higher than the three road-verge sites (NP 183, SP 130, MP 82, MR 19, SR 15 and NR 9).

Statistical analyses

Ordination was performed by Detrended Correspondence Analysis (DCA, Hill 1979) using CANOCO for Windows, version 4.5 (ter Braak & Šmilauer 2002) with standard options. Prior to ordination, species with frequency less than the median frequency were downweighted in proportion to their frequency (Eilertsen et al. 1990).

Principal component analysis (PCA, Pearson 1901; ter Braak & Prentice 1988) was used to summarise relationships between abiotic explanatory variables in the dataset. PCA was run on a correlation matrix of data after standardization to zero skewness as recommended by Økland et al. (2001). The management index was left out of the PCA as it was calculated for sites only. The PCA was supplemented by calculations of Kendall's nonparametric rank correlation coefficients τ between all variable pairs to identify strong correlations ($|\tau| > 0.34$). This τ limit corresponded to $p \leq 0.001$ in a standard test of deviation of τ from zero, under the assumption of independence of observations. Due to the nested data structure, however, the test was used for indication rather

than as a formal statistical test.

We interpreted each of the four main vegetation gradients by using the DCA plot score as response variable and one zero-skewness transformed explanatory variable in turn as predictor in a split-plot GLM (generalised linear models, McCullagh & Nelder 1989; Venables & Ripley 2002) analysis, specifying error components at three hierarchical levels (Crawley 2002). Thus, statistical inference was obtained by considering plots as nested within blocks (168 plots within 42 blocks), and blocks as nested within sites (42 blocks within 6 sites). For the MI only site level was addressed while for all other explanatory variables the relationship was evaluated for all three nested levels. For each DCA axis, we calculated the overall fraction of total variation explained (FVE) at each of the three grain levels as the level sum of squares (SS_{level}) divided by the total sum of squares (SS_{total}) for the axis. The variation explained for each variable on each grain level was computed as SS for the variable ($SS_{variable}$) divided by the SS for the three different grain levels ($SS_{grain\ level}$). For each model the sign of the coefficient c (positive or negative) indicated the sign of significant relationships. As numerous tests were performed, we mainly restricted interpretation to strongly significant relationships (p-values of 0.01 or lower) to avoid making Type I errors, as recommended by Crawley (2002). Split-plot GLM with identity link function and normal distribution of errors (family = Gaussian) was performed by R Version 2.3.1 (Anon. 2006).
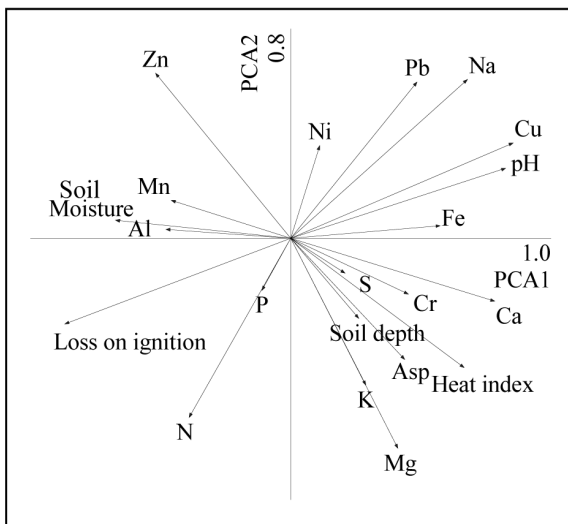

Fig. 1. PCA ordination of 21 abiotic variables measured at plot level ($\lambda = 0.353$ for axis 1 and $\lambda = 0.176$ for axis 2).

Results

Relationships between explanatory variables

The PCA analysis revealed several loose groups of correlated explanatory variables (Fig. 1). Loss on ignition was positively correlated with N and moisture ($|\tau| > 0.50$). Mg and Ca were strongly positively correlated with one another and with the topographic variables HI and Asp that express microclimatic favourability (Fig. 1). Mg was also strongly positively correlated with K and Cr. Moisture and loss on ignition were strongly negatively correlated with HI and Asp. Thus, plots were dispersed along a gradient from higher to lower humus content, soil moisture, and N content, from lower to higher pH and availability of base cations.

Relationships between variation in vegetation and explanatory variables

The main gradient in species composition as revealed by DCA ordination, axis 1 (eigenvalue (λ) = 0.422 (total inertia 4.033), gradient length = 3.34 SD units), spanned from vegetation characterised by species such as *Galium boreale* and *Plantago media* (low scores) to vegetation in which *Betula pubescens* and *Tanacetum vulgare* occurred frequently (Fig. 2b). The majority of variation in DCA axis 1 was explained at site level (FVE = 0.87; Table 1). The site with the lowest MI value (NR) was separated from the other sites at the high-score end of the axis, plots from the other two road verge sites obtained intermediate scores (Fig. 2a) and plots from pastures (which had high MI) were positioned near the low-score end of the axis. Along DCA-axis 1 plots showed a significantly negatively relationship (p = 0.009, Table 1) with the Management index (MI) at the site level while no other relationship (at any level) was significant at the α = 0.01 level. Although several soil base cations (Ca, Na, Fe) also explain much of the main vegetation variation (0.67 - 0.72) at site level, the few degrees of freedom at this level keep p values low. The explained relative vegetation variation (FVE) at finer scales than the site was negligible (FVE = 0.06 and 0.07, Table 1). Thus, we interpreted the main vegetational variation in the data set as related to the difference between sites with long history of traditional management (pastures) versus the road verge sites with their differing management histories.

The second most important gradient in species composition (DCA axis 2: $\lambda = 0.242$, gradient length = 2.95 SD units) ran from plots in which *Phleum pratense* and *Trifolium medium* were common, to vegetation characterised by, among others, *Campanula*

rotundifolia and *Ranunculus acris* (Fig. 2). As for DCA axis 1, several variables (K, Mg and Soil depth) explained much of the variation at site level (FVE = 0.59, Table 1), again with low p values. At the block level, however, soil depth ($p = 0.001$), soil moisture ($p = 0.006$) and N ($p = 0.001$) were related negatively to plot scores. Many variables showed significant relationship to DCA axis 2 on block and plot levels (Table 1), both negative (e.g. Mg) and positive (e.g. Mn and Zn). These two grain levels jointly explained 0.41 of the total variation along DCA axis 2. Therefore, we interpreted the second most important vegetation gradient as related to variation in soil base cation concentrations, soil depth, soil moisture and N over a span of scales within sites.

The third most important gradient in species composition, DCA axis 3 ($\lambda = 0.185$, gradient length = 2.69 S.D. units) ran from plots with high frequency of species like *Festuca ovina* and *Achillea millefolium* to vegetation characterised by, among others, *Anthriscus sylvestris* and *Vicia cracca*. DCA axis 3 showed significant relationships with few explanatory variables, and the block and plot levels shared few variables significantly related to the axis (Table 1). At the block level (at which 0.47 of the variation along this axis was explained), elements that may be toxic for plants in high concentrations (Al, Ni, and Cu) showed significant relationships. The strongest relationships to the axis on plot level (explaining 0.22 of the variation) was observed for N ($p < 0.001$), followed by Zn, Ca and pH ($p \leq 0.003$). Thus, the vegetational variation along DCA axis 3 may be interpreted as a fine-scale gradient related to N.

DCA axis 4 ($\lambda = 0.120$, gradient length = 2.03 S.D. units) ran from plots with species like *Linaria vulgaris* and *Sedum acre* to plots with high frequency of *Hieracium umbellatum* and *Deschampsia flexuosa*. Few explanatory variables were significantly related to DCA axis 4 and no single variable was significantly related to the axis on both block and plot levels (Table 2). The majority of the axis' variation was explained at these two grain levels (FVE = 0.57 and 0.38, respectively). Mg was the best predictor of DCA-axis 4 plot score at the block level, Ca and P at the plot level.
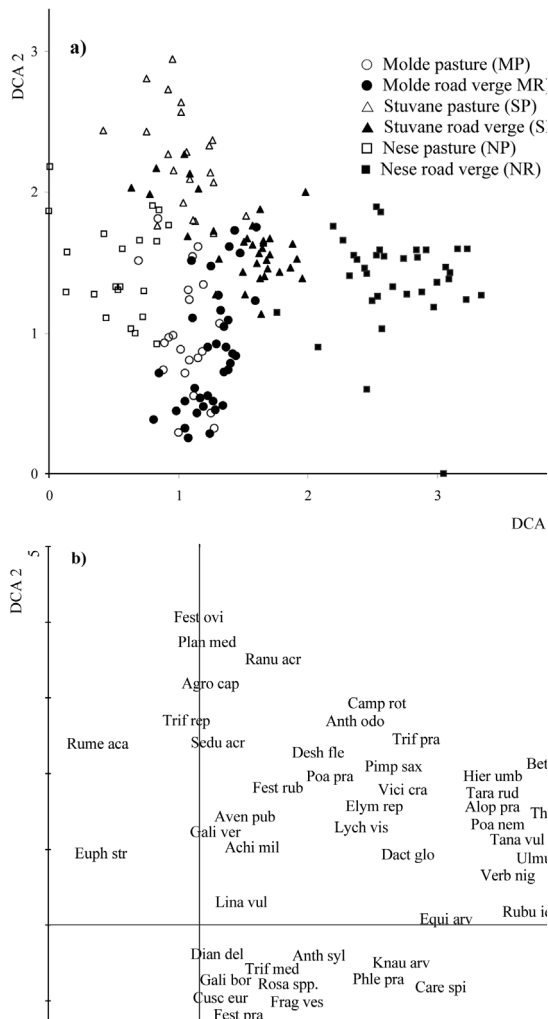

Fig. 2. DCA ordination of the 168 plots, axes 1 and 2. **a.** Site affiliation of plots. **b.** Species plot showing the 45 species occurring in > 3% of the plots. All 92 species are listed in App. 1, along with explanations of the species abbreviations used in the figure.

Discussion

Our results highlight the importance of fine-tuning the sampling unit size (grain) to the scale of variation in vegetation-environment studies. We found profound scale differences in vegetation-environmental relationships between 'coarse' (between sites), 'intermediate' (between blocks) and 'fine' (between plots) scales. At the coarse scale, the most prominent vegetation gradient was significantly related to management. At the intermediate scale soil depth and moisture were strongly related to variation in species composition. As to the two finer scales there were both common patterns and differences. Species composition was related to concentrations of base cations and N on both these scales, but variation related to P-availability occurred only at the finest scale. The split-plot GLM used in this study (see also Mathiassen & Økland 2007) does not only ensure a statistically appropriate handling of nested data but also strongly facilitate disentangling of complex multi-scale relationships.

Table 1. Relationships between vegetation gradients (DCA plot score; response variable) and the different zero-skewness transformed explanatory variables (predictor variable), evaluated at three grain levels (plot, block and site) by split-plot GLM (identity link, normal errors). For each of the four response variables and at all three grain levels, the total SS (sum of squares) and FVE (fraction of total variation explained) are given. Site level had 4 degrees of freedom, block level 35 and plot level 126. The variation explained ($SS_{expl}/SS_{grain\ level}$) by each explanatory variable is given for all three grain levels and four DCA axes. F^p gives F-value and p-level for significant ($p \geq 0.05$) relationships. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, $p < 0.01$ are given in bold. Model coefficients (c) show the sign of the relationship (+/-).

DCA1

	Explanatory variable	Site SS_{site}=79.21 FVE=0.87 SS_{expl}/SS_{site}	F^p	c	Block within site SS_{block}=5.87 FVE=0.06 SS_{expl}/SS_{block}	F^p	c	Plot within block SS_{plot}=6.81 FVE=0.07 SS_{expl}/SS_{plot}	F^p	c
1	Management index	**0.85**	21.98**	–	.	.	.	.	.	.
2	Soil depth	0.07			0.05			0.03	4.27*	+
3	Soil moisture	0.35			0.01			0.02		
4	pH	0.60			0.16	6.43*	+	0.00		
5	Loss on ignition	0.56			0.00			0.01		
6	Heat index	0.30			0.09			0.00		
7	Aspect favourability	0.33			0.02			0.00		
8	N	0.37			0.00			0.00		
9	Al	0.15			0.09			0.00		
10	Ca	0.72	10.29*	+	0.12	4.65*	+	0.01		
11	Cr	0.01			0.04			0.01		
12	Cu	0.60			0.00			0.00		
13	Fe	0.68	8.53*	+	0.05			0.00		
14	K	0.07			0.02			0.03		
15	Mg	0.02			0.03			0.00		
16	Mn	0.58			0.00			0.00		
17	Na	0.67	8.07*	+	0.01			0.00		
18	Ni	0.28			0.11	4.39*	–	0.00		
19	P	0.48			0.08			0.00		
20	S	0.01			0.02			0.00		
21	Zn	0.27			0.02			0.01		

DCA2

	Explanatory variable	Site SS_{site}=31.30 FVE=0.59 SS_{expl}/SS_{site}	F^p	c	Block within site SS_{block}=14.23 FVE=0.27 SS_{expl}/SS_{block}	F^p	c	Plot within block SS_{plot}=7.50 FVE=0.14 SS_{expl}/SS_{plot}	F^p	c
1	Management index	0.05			.	.	.	.	.	.
2	Soil depth	0.68	8.42*	–	**0.27**	12.94***	–	0.02		
3	Soil moisture	0.58			**0.19**	8.47**	–	0.00		
4	pH	0.06			**0.18**	7.67**	–	0.00		
5	Loss on ignition	0.20			0.13	5.33*	–	0.05	6.61*	–
6	Heat index	0.59			0.00			0.01		
7	Aspect favourability	0.38			0.00			0.01		
8	N	0.02			**0.26**	12.04**	–	0.03		
9	Al	0.10			**0.22**	9.77**	+	0.03		
10	Ca	0.25			0.02			0.02		
11	Cr	0.43			0.14	5.61*	–	0.05	5.94*	+
12	Cu	0.17			**0.19**	8.04**	+	**0.08**	10.90**	+
13	Fe	0.15			**0.31**	15.53***	+	0.00		
14	K	0.80	16.36*	–	0.10			0.00		
15	Mg	0.63			**0.32**	16.19***	–	**0.07**	10.16**	–
16	Mn	0.05			**0.34**	18.22***	+	**0.11**	16.15***	+
17	Na	0.00			0.00			**0.05**	7.33**	+
18	Ni	0.45			0.16	6.59*	+	0.05	6.32*	+
19	P	0.08			0.00			0.00		
20	S	0.46			0.00			0.01		
21	Zn	0.55			**0.29**	14.23***	+	0.06		

DCA3

	Explanatory variable	Site SS_{site}=13.94 FVE=0.31 SS_{expl}/SS_{site}	F^p	c	Block within site SS_{block}=20.79 FVE=0.47 SS_{expl}/SS_{block}	F^p	c	Plot within block SS_{plot}=9.74 FVE=0.22 SS_{expl}/SS_{plot}	F^p	c
1	Management	0.11			.	.	.	.	.	.
2	Soil depth	0.05			0.03			0.00		
3	Soil moisture	0.01			0.10			0.02		
4	pH	0.31			0.08			**0.07**	9.39**	–
5	Loss on ignition	0.09			0.16	6.80*	+	0.00		
6	Heat index	0.20			0.04			0.00		
7	Aspect favourability	0.25			0.02			0.00		
8	N	0.55			0.06			**0.12**	16.94***	–
9	Al	0.22			**0.29**	14.04***	–	0.02		
10	Ca	0.05			0.02			**0.07**	10.08**	–
11	Cr	0.10			0.07			0.02		
12	Cu	0.11			**0.22**	10.01**	–	0.00		
13	Fe	0.04			0.04			0.00		
14	K	0.07			0.01			0.01		
15	Mg	0.62			**0.21**	9.53**	+	**0.06**	7.55**	–
16	Mn	0.25			0.08			0.02		
17	Na	0.44			0.05			0.05	6.53*	+
18	Ni	0.07			**0.24**	11.09**	–	0.00		
19	P	0.00			0.04			0.05	6.07*	+
20	S	0.15			0.11	4.34*	+	0.00		
21	Zn	0.29			0.05			0.09		

DCA4

	Explanatory variable	Site SS_{site}=1.21 FVE=0.05 SS_{expl}/SS_{site}	F^p	c	Block within site SS_{block}=13.12 FVE=0.57 SS_{expl}/SS_{block}	F^p	c	Plot within block SS_{plot}=8.81 FVE=0.38 SS_{expl}/SS_{plot}	F^p	c
1	Management	0.12			.	.	.	.	.	.
2	Soil depth	0.52			0.03			0.02		
3	Soil moisture	0.67	8.29*	+	0.01			0.04	5.58*	+
4	pH	0.13			0.02			**0.06**	7.75**	–
5	Loss on ignition	0.22			0.01			0.00		
7	Heat index	0.64			0.08			0.01		
6	Aspect favourability	0.50			0.00	0.00				
8	N	0.01			0.08			0.02		
9	Al	0.09			0.00			0.04	5.25*	+
10	Ca	0.45			0.09			**0.08**	11.56***	+
11	Cr	0.19			0.03			0.00		
12	Cu	0.21			0.02			0.00		
13	Fe	0.16			0.00			0.00		
14	K	0.31			0.04			0.03	4.41*	–
15	Mg	0.49			**0.29**	14.53***	+	0.00		
16	Mn	0.22			0.04			0.00		
17	Na	0.04			0.02			0.01		
18	Ni	0.35			0.02			0.01		
19	P	0.01			0.04			**0.08**	11.40**	–
20	S	0.42			0.07			0.00		
21	Zn	0.66	7.76*	+	**0.23**	10.20**	–	0.03		

The main gradient related to management

The highly significant relationship between plot scores along DCA axis 1 at the site level and the management index (MI) points to management as a main factor for vegetation variation in our study. Although such constructed indices can always be criticised for subjectivity in the weighting of different attributes, our MI seems fairly robust as it reflects major compositional differences as extracted on DCA axis 1. Changes of weights would have had to be rather large in order to e.g. change the ranking of the sites according to management intensity. Used with care, such indices may continue to prove useful for simplifying multifaceted variables like management impact (see also Jantunen & Saarinen 2003; Kahmen et al. 2005).

In most studies addressing the relative importance of management versus other explanatory variables for variation in species composition, data are analysed at the plot level without taking the hierarchical data structure into account. Conclusions based on such analyses might be flawed due to missing replication of management related variables (cf. Økland in press). The split-plot design applied in our study enables valid comparison of variables measured at different spatial scales (e.g. impact of management versus soil chemical variables such as N) without risking spatial pseudoreplication. Our study included few sites ($n = 6$), but the specification of error components at all three hierarchical levels (Crawley 2002) in the split-plot GLM nevertheless ensures that the inferred relationship between vegetation variation and management on site scale is valid.

We interpret the variation along DCA axis 1 as gradual variation from pastures with a long management history to grasslands with a shorter or different management history, e.g. road verges. Plots without a traditional management history (NR) differed from plots from all other sites with respect to species composition (Fig. 2a). On the other hand, even though the Molde and Stuvane road verges and the three pastures have received different management for at least 50 years, the vegetation of these sites still has many similarities. The pastures have experienced shifts in management through time. Former (spring and autumn grazed) hay meadows have been turned into pastures, often with gradually reduced grazing pressure. These changes of management practices have most likely given rise to shifts in species composition. Lindborg & Eriksson (2004) argue that slow response by many species to cessation of traditional management may lead to a species composition that reflects historical rather than present land use. This is likely to be the case for two of the road verges and all the pastures in this study, in which historical management regimes (expressed in the MI) still seems to influence species composition.

Secondary gradients: related to moisture conditions and level of soil base cations and N

The second most important gradient in species composition reflects variation mainly between blocks where topographic variation between shallow depressions and small elevations gives rise to an intermediate-scaled variation in soil depth and soil moisture. Kelly & Canham (1992) identified a similar pattern in old fields where moisture varied within patches of 220 cm extent. The levels of N and the soil base cation Mg parallel soil moisture conditions along DCA axis 2. Robertson et al. (1988) found a similar pattern for moisture and N-availability in a study of old field vegetation. Among several explanations offered by Giesler et al. (1998) to higher N-concentrations of moister patches are: influx of N from surrounding recharge areas and accumulation of N due to elevated soil microbial activity.

Nitrogen is generally regarded a major determinant of species composition (Tilman 1988), repeatedly contributing (together with base cation concentrations) to complex gradients in grasslands (Bratli & Myhre 1999; Critchley et al. 2002; Vandvik & Birks 2002). However, in other grassland studies N-concentration relates weakly to vegetational variation (e.g. Ejrnæs & Bruun 1995; Austrheim et al. 1999). The fine-scaled variation in N-availability, observed in old fields on scales < 100 cm (Robertson et al. 1988; Kelly & Canham 1992), is hypothesised to affect plant species composition (Turkington & Harper 1979). The correspondence between N and species composition may, however, be masked if plot size is larger than the spatial scale at which N varies. This probably also explains why vegetational variation shows stronger relationship to N at plot than at block level in our study.

Phosphorus is significantly related to vegetational variation at plot level along the minor DCA axis 4. Although we cannot decide if this reflects a causal relationship, it is noteworthy that the concentrations of P and Ca are strongly and inversely related to this axis. A similar relationship is described from calcium-rich but infertile mires in which phosphorus is immobilised during the process of calcite formation (Boyer & Wheeler 1989). Gough & Marrs (1990) identified P as a controlling factor for variation in grassland vegetation, and Critchley et al. (2002) found that P-level successfully indicated suitable soil conditions for mesotropic grassland maintenance. Soil P-availability has also been hypothesised to limit the uptake of N in P-poor grasslands (Janssens et al. 1998). However, Austrheim et al. (1999) and Myklestad (2004) rejected P-availability as a significant predictor of vegetational variation in low-fertilised grasslands, e.g. meadows and pastures. The larger plots employed in these two studies might have concealed variability, as

our study indicated a significant relationship only along a low-order ordination axis, and only on very fine plot (0.25 m^2) scales. Also Jackson & Caldwell (1993) found that P showed spatial autocorrelation only at spatial scales of less than 1 m.

Scale: the importance of grain and extent to vegetation-environment relationships in grasslands

Our results emphasised, as indicated by Wiens (1989) and Økland et al. (2001) and discussed by Otýpková & Chytrý (2006), that the choice of resolution grain (plot size) strongly influences the results of vegetation-environment studies by ordination. Thus, the aim of a study should guide the choice of grain, rather than evaluating plot-size appropriateness by means of ordination results. We therefore disagree with the approach as well as the assessments made by Chytrý & Otýpková (2003) and Otýpková & Chytrý (2006) who recommend a plot size of 16 m^2 for analyses of most grasslands (Chytrý & Otýpková 2003). They argue that since small plots (i.e. ≤ 4 m^2) contain fewer species than larger plots, stochastic variation may produce large between-plot floristic dissimilarities in the fine-scale data sets that in turn may generate less stable ordination patterns. Poor performance of ordination methods due to low representativity of individual plots does, however, usually not occur before the number of species per plot drops below about five (Økland 1990). Thus, we found no outlier in the DCA ordination despite a plot size of 0.25 m^2. The high species richness even within small areas typical of semi-natural grasslands (Norderhaug et al. 2000) suggests that ordination methods generally perform well even when very small plots are used. In this study we demonstrate that also fine scales have to be addressed to understand vegetation-environment relationships in semi-natural grasslands. Thus, interpretation of the variation in species composition of semi-natural grasslands requires consideration of the spatial scales on which important environmental variables actually vary.

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App. 1. Species found in the 168 plots with a presence of > 3 % (shown in Fig. 2b) listed with corresponding abbreviations.

Achillea millefolium (Achi mil)
Agrostis capillaris (Agro cap)
Alopecurus pratensis (Alop pra)
Anthoxanthum odoratum (Anth odo)
Anthriscus sylvestris (Anth syl)
Avenula pubescens (Aven pub)
Betula pubescens agg. (Betu pub)
Campanula rotundifolia (Camp rot)
Carex spicata (Care spi)
Cuscuta europaea (Cusc eur)
Dactylis glomerata (Dact glo)
Deschampsia flexuosa (Desh fle)
Dianthus deltoides (Dian del)
Elymus repens (Elym rep)
Equisetum arvense (Equi arv)
Euphrasia stricta (Euph str)
Festuca ovina (Fest ovi)
Festuca pratensis (Fest pra)
Festuca rubra (Fest rub)
Fragaria vesca (Frag ves)
Galium boreale (Gali bor)
Galium verum (Gali ver)
Hieracium umbellatum (Hier umb)
Knautia arvensis (Knau arv)
Linaria vulgaris (Lina vul)
Lychnis viscaria (Lych vis)
Phleum pratense (Phle pra)
Pimpinella saxifraga (Pimp sax)
Plantago media (Plan med)
Poa nemoralis (Poa nem)
Poa pratensis (Poa pra)
Ranunculus acris (Ranu acr)
Rosa spp. (Rosa spp.)
Rubus idaeus (Rubu ida)
Rumex acetosella (Rume aca)
Sedum acre (Sedu acr)
Tanacetum vulgare (Tana vul)
Taraxacum sect. *Ruderalia* (Tara rud)
Thlaspi caerulescens (Thla cae)
Trifolium medium (Trif med)
Trifolium pratense (Trif pra)
Trifolium repens (Trif rep)
Ulmus glabra (Ulmu gla)
Verbascum nigrum (Verb nig)
Vicia cracca (Vici cra)

The following 47 species had a presence ≤ 3%:
Alchemilla vulgaris, Allium oleraceum, Alnus glutinosa, Alnus incana, Angelica sylvestris, Anthyllis vulneraria ssp. carpatica, Artemisia vulgaris, Botrychium lunaria, Bromus sp., Carex pallescens, Carum carvi, Cerastium fontanum, Deschampsia cespitosa, Draba incana, Epilobium montanum, Erigeron acer, Galeopsis sp., Galium aparine, Geranium sylvaticum, Geum urbanum, Heracleum sibiricum, Hieracium spp., Leontodon autumnalis, Lotus corniculatus, Luzula multiflora, Luzula pilosa, Melica nutans, Myosotis arvensis, Pinus sylvestris, Poa annua, Poa glauca, Potentilla argentea, Potentilla crantzii, Rhinanthus minor, Rubus saxatilis, Rumex acetosa, Salix caprea, Senecio viscosus, Silene vulgaris, Sorbus aucuparia, Stellaria graminea, Succisa pratensis, Veronica chamaedrys, Veronica officinalis, Vicia sepium, Viola canina, Viola tricolor.

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