

# Scale- and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment

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## Summary

1. Multiple land-use change drivers affect, in most cases negatively, the biodiversity in species-rich meadows. Empirical data that can help to disentangle the effects of individual drivers and quantify the time required for a biodiversity response are seldom available. Management decisions are often based on short-term experiments or observational data.

2. A 15-year field experiment, comprising a factorial combination of fertilization, mowing and removal of the dominant species *Molinia caerulea*, was established in an oligotrophic wet meadow in Czech Republic. Each of the eight factorial combinations was replicated three times. Percentage cover for all species was monitored annually in 1-m<sup>2</sup> plots and species' presence recorded in each cell of a continuous square grid of 25 cells (0.1 × 0.1 m each). These data enabled various scale-dependent estimates of species richness.

3. The species composition of individual treatment combinations diverged over time, particularly at the start of the experiment, and by the latter stages resembled various typical grassland communities from the surrounding landscape. Fertilization had the most pronounced effect, leading to a sharp decrease in species richness, most rapidly at the smallest spatial scale. Mowing had on average a positive effect on species richness and led in most cases to spatially homogeneous species composition. The removal of *Molinia* had a positive effect on species richness, especially in unmown unfertilized plots.

4. The effects of each factor were dependent on the combination of the other two factors, and also on time, with some effects continuously increasing throughout and some diminishing by the end of the experiment. The process of competitive exclusion with fertilization and cessation of mowing was, in some treatment combinations, rather slow.

5. *Synthesis and applications.* Land-use change drivers act in combination, and their effects on the structure of species-rich wet meadows are dependent on both the temporal and spatial scales considered. Short-term experiments might underestimate the response of vegetation and thus provide erroneous conservation recommendations. Mowing was only effective in preventing species richness decline caused by fertilization in the short term. The presence of a single dominant species can modify the effectiveness of conservation measures.

**Key-words:** biodiversity decline, competitive exclusion, *Molinia caerulea*, principal response curves, response time, semi-natural grassland, species richness, species–area relationship

## Introduction

Traditionally managed oligo- and mesotrophic meadows are among the plant communities most endangered by current land-use changes throughout Europe (Isselstein,

Jeangros & Pavlů 2005). They are either used more intensively, which includes increased fertilization, or they are abandoned. Both these land-use changes lead to a substantial decline in species richness. It is unlikely, however, that either an increase in available nutrients (fertilization) or a lack of disturbance (mowing) would drive certain species to become locally extinct. Rather,

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these factors lead to changes in competition equilibria in a way that leads to the subsequent competitive exclusion of some species, which is a mechanism limiting community species richness (Palmer 1994; Wilson 2011). Although competitive exclusion has a central role in community theory, empirical data quantifying the time-scales necessary for such exclusion to occur are seldom available. The competitive exclusion of perennial plants can be a slow process (Adler, Ellner & Levine 2010) and so it can be delayed or even completely avoided by environmental variability, which results in fluctuations in the equilibria rather than a one-directional path to exclusion (Palmer 1994; Adler, Ellner & Levine 2010).

A reduction in species richness at a particular spatial scale does not necessarily signify the extinction of the species from the wider community. The species in decline can be missing from large proportions of the habitat, but still persist in a few patches. Typically, declines in species richness proceed faster at smaller spatial scales (Galvánek & Lepš 2008) – and consequently, the shape of the species–area relationship (SAR, often characterized as  $S = cA^z$ , where  $S$  is the number of species and  $A$  is the size of the area, and  $c$  and  $z$  are parameters) changes accordingly. The parameter  $z$  represents the rate of increase in the number of species as the sampling area expands, and is often considered a characteristic of beta diversity. Changes in SARs have been demonstrated for different types of disturbance regimes, such as mowing (Horník *et al.* 2012) and grazing (de Bello, Lepš & Sebastià 2007), or for successional changes (Lepš & Štursa 1989; Rejmánek & Rosén 1992).

Although the aforementioned processes have been known for a long time, they are often inferred from ‘snapshots’ of observational data (e.g. comparisons of meadows under various management regimes) or from short-term manipulative experiments. The reliance on observational data is potentially misleading in that it introduces the possibility that the management decisions were influenced by the local environmental conditions, which can also result in mutual dependence of mowing and fertilization. Additionally, the role of the dominant species in the system’s development is not clear, nor it is known how their presence affects the effectiveness of individual factors. Competitive exclusion, in fact, is often the result of just a few fiercely competitive dominants. This occurs in both abandoned and nutritionally enhanced meadows as some species, which in traditionally managed meadows are constrained by mowing and/or limited nutrients, become dominants. Despite their problems, removal experiments are the most reliable way to demonstrate the effects of competition (Aarssen & Epp 1990; Díaz *et al.* 2003).

Consequently, I have used data from a long-term (15-year) controlled factorial experiment, which includes dominant removal as one factor, and where the data are available at various spatial scales. My aim was to demonstrate (i) the combined effects and interaction of mowing and fertilization, (ii) the role of dominant species in the community response to treatments, (iii) the temporal dynamics

and spatial scale dependence of the above effects. The temporal dynamics should reveal the difference between the results of short- and long-term experiments that proceed to influence management recommendations.

## Materials and methods

### STUDY SITE

The experiment was established in a wet oligotrophic meadow close to village of Ohrazení, in South Bohemia, Czech Republic, in 1994. The experiment is centred on the coordinates 48°57′11.3″N 14°35′34.0″E, at 510 m a.s.l. The mean annual temperature at the nearby České Budějovice meteorological station (400 m a.s.l.) is 7.8 °C, and the mean annual precipitation is 620 mm. Soil nutrient levels are low (total nitrogen 6–8 g kg<sup>−1</sup> dry soil weight, total phosphorus 400–500 mg kg<sup>−1</sup> dry soil weight, C/N ratio 16–20, all in 1994). The first 4 years of the experiment were reported by Lepš (1999), together with a detailed description of the locality, and some of the results were used to test various ecological hypotheses (e.g. Titus & Lepš 2000; Lepš 2004; Lepš *et al.* 2011; Mason *et al.* 2011). This is the first description of the biodiversity dynamics during the first 15 years of the experiment.

The meadow was traditionally mown once or twice a year until the late 1980s. The plant community at the start of the experiment could be characterized as Molinion with some elements of Violion caninae. The dominant grasses were *Molinia caerulea* (L.) Moench., *Nardus stricta* L., *Festuca rubra* L., *Holcus lanatus* L. The meadow was species rich, hosting over ten species of sedges (e.g. *Carex panicea* L., *C. hartmanii* Cajander, *C. nigra* (L.) Reichard, *C. palescens* L., *C. pilulifera* L., *C. pulicaris* L.) and many forb species. In the 15 years the project has been running, I recorded a total of 91 species of vascular plants and six bryophytes in the 24 1-m<sup>2</sup> quadrats.

### EXPERIMENTAL SETTINGS

The experiment combines mowing, fertilization and dominant removal in a factorial design, yielding eight possible combinations, each with three replications. Each replication is a 4-m<sup>2</sup> quadrat, located in a 4 × 6 quadrat lattice (Fig. S1, Supporting Information). Originally, fertilization consisted of 65 g m<sup>−2</sup> of commercial NPK fertilizer: 12% N (nitrate and ammonium), 19% P (as P<sub>2</sub>O<sub>5</sub>) and 19% K (as K<sub>2</sub>O). The first part of the dose consisting of 50 g m<sup>−2</sup> was applied in autumn and remaining 15 g m<sup>−2</sup> in spring. From 1997 onwards, the full dosage was applied only in spring. Since 2003, the total dosage was reduced to 50 g m<sup>−2</sup> and the commercial fertilizer changed to Cererit (8% N, 13% P, 11% K), in order to prevent the accumulation of nutrients, particularly phosphorus, in the soil (as there is no nutrient export in the form of biomass from the unmown plots). Mowing was carried out annually in the second half of June, by clipping the vegetation with scissors [for biomass measurements, only in the mown plots, as reported in Lepš (2004)] for the central 0.25 m<sup>2</sup> at c. 1–2 cm above ground, and using a sickle for the rest of the quadrat, cutting approximately at the same height. *Molinia caerulea* individual tillers were manually removed with a screwdriver in April 1995 with a minimum of soil disturbance and subsequent individuals were removed annually, if necessary.

## SAMPLING

Plots were sampled in the growing season prior to mowing (usually in early June) each year, starting in 1994, that is, before the first experimental manipulations started – the baseline year, 1994, is referred to as ‘year 0’. Percentage cover of all vascular plant species, the moss layer and litter was visually estimated in the central 1 m<sup>2</sup> of each 2 × 2 m plot. It was followed by detailed analysis of the central 0.5 × 0.5 m using a continuous grid of 25 0.1 × 0.1 m subplots and included the recording of rooting presence of all vascular plant species in each individual 0.1 × 0.1 m cell.

## DATA ANALYSIS

The data from the 1-m<sup>2</sup> plots were used for the analysis of temporal changes in vascular plant and bryophyte species composition by various multivariate methods (Šmilauer & Lepš 2014), using log ( $x + 1$ )-transformed values for percentage cover. Overall changes were visualized using detrended correspondence analysis (DCA). In order to visualize the dynamics of the process, the position of each treatment combination in each year is displayed (technically, these are the centroids of the interaction of year as a dummy variable with all the treatment interactions). Changes in total species richness could be inferred from the species richness isolines overlaid on the ordination diagram. The isolines were plotted by fitting the richness values to the two DCA axes using generalized additive models, with stepwise selection of optimal complexity using the AIC criterion. The effects of the treatments were analysed using principal response curve (PRC, Van den Brink & ter Braak 1999). The basic difference between the two approaches is that DCA is an unconstrained ordination and displays both the dynamics of all the treatment combinations and their differentiation, whereas PRC is based on constrained ordinations (and consequently, it also enables formal tests of the treatment effects). In PRC, time is used as a covariate, and consequently, we can see the differentiation of each treatment combination against the designated control at each time point, but we do not see the possible dynamics of the control itself, or any dynamics common to all the treatment combinations, for example adverse weather conditions (Šmilauer & Lepš 2014; chap. 10.1). I designated the unfertilized, unmown, non-removal plots as ‘controls’. Note that the selection of ‘control’ does not affect the interpretation of the results of PRC. If we selected another combination as the control, the only difference would be in the graphical output – the differences between the treatment combinations would remain the same, but the newly selected control would correspond to the x-axis, and the others would be shifted so that their positions, relative to each other, would remain the same. I used neither standardization by samples, nor by species – consequently, this PRC is more affected by dominants and less by rare species than the DCA, where both standardizations are implicit. *Molinia caerulea* was omitted from all analyses (because it is the manipulated species, see Šmilauer & Lepš 2014; chap. 16), so that the results represent the response of the rest of the community. All multivariate analyses were carried out using Canoco 5 (ter Braak & Šmilauer 2012).

Scale-dependent changes in species richness were based on the analysis of the central continuous grid of 25 0.1 × 0.1 m cells (only for vascular plants). The term ‘scale’ is used to denote sampling unit size, and so, scale dependence means the effects of

sampling unit size on the (dynamics of) patterns of richness. Although I have data available for species in the 1-m<sup>2</sup> plots, they were not used because there is a higher chance that a species would be overlooked in these plots than in the detailed analysis of 0.1 × 0.1 m cells. The number of species was estimated at the following scales: 0.1 × 0.1 m, 0.2 × 0.2 m, 0.3 × 0.3 m, 0.4 × 0.4 m and 0.5 × 0.5 m. The number of species was obtained as the average species number in all possible quadrat positions of a given size that fit into the 0.5 × 0.5 m frame. The species richness data were analysed using a repeated-measures (split-plot) ANOVA model with three between-subject (whole plot) factors – Mowing, Fertilization and Removal – and two within-subject (repeated-measures) factors – Year and Scale (i.e. plot size as a factor). As the number of species was log-transformed, the null hypothesis of additivity of effects in the tests of interaction using the log-transformed data translates to multiplicativity at the original scale. For example, the interaction between Scale and Time applied to the log-transformed number of species tests the null hypothesis that the log-transformed value decreases or increases by the same amount at all spatial scales, which means that the same proportion of species is lost or gained at each scale. Finally, the SARs were fitted using the power function  $S = cA^z$  for each individual 0.25-m<sup>2</sup> plot in each year;  $z$  and  $c$  are parameters fitted by the linear regression after log transformation of both  $S$  and  $A$ . The  $z$ -values were analysed with a repeated-measures ANOVA. In the repeated-measures ANOVA, several error levels are used. Consequently, for the corresponding figures, we present standard errors of the differences between means for corresponding comparisons, calculated as  $SE = \sqrt{\frac{MS_e}{2} \left( \frac{1}{n_A} + \frac{1}{n_B} \right)}$ , where  $MS_e$

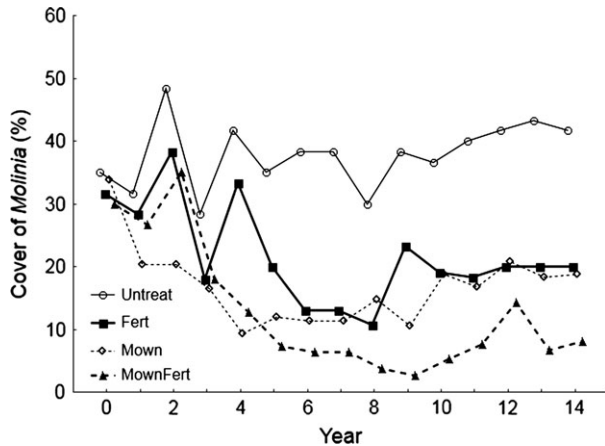
is the corresponding error mean square and  $n_A$  and  $n_B$  are the numbers of replications in the compared groups. Univariate analyses were carried out using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA).

## Results

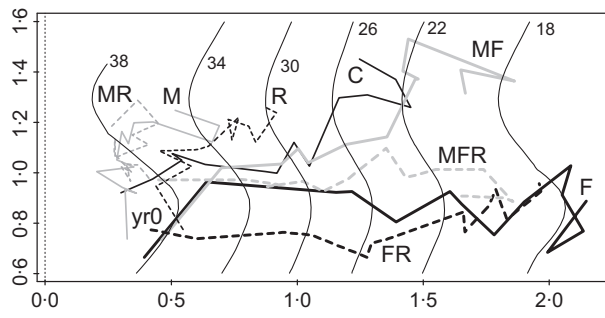
## CHANGES IN SPECIES COMPOSITION

Species composition changed over time and diverged depending on the factorial combination of fertilization, mowing and dominant removal (Fig. S2, Supporting Information). In evaluating the effects of dominant removal, it is important to note that in the non-removal plots, the cover of *Molinia* itself was affected by all fertilization and mowing combinations. Specifically, in the long term, *Molinia* is negatively affected by both mowing and fertilization (Fig. 1, full ANOVA in Table S1, Supporting Information). In comparison with the experimental plots at year zero, *Molinia* increased overall only in unmown unfertilized plots, which would correspond with the total abandonment of the meadow. Initially, there was a brief proliferation in fertilized plots followed by a sharp decrease in mown fertilized plots.

The DCA shows that species composition among plots was highly similar before the treatments were imposed, but then quickly diverged depending on treatment (Fig. 2, Fig. S3, Supporting Information). Fertilization caused fast and pronounced changes, which were accompanied by



**Fig. 1.** Changes in mean cover of *Molinia caerulea* as a response to mowing and fertilization over 15 years. Dashed line – mown, solid line – unmown, full symbols and thick line – fertilized, open symbols and thin line – unfertilized. Repeated-measures ANOVA revealed the negative effects of both mowing and fertilization (significant as both main effects and interactions with year). The full ANOVA in Table S1 (Supporting Information), SE for between-subject comparison = 14.35, SE for within-subject comparison = 4.08.

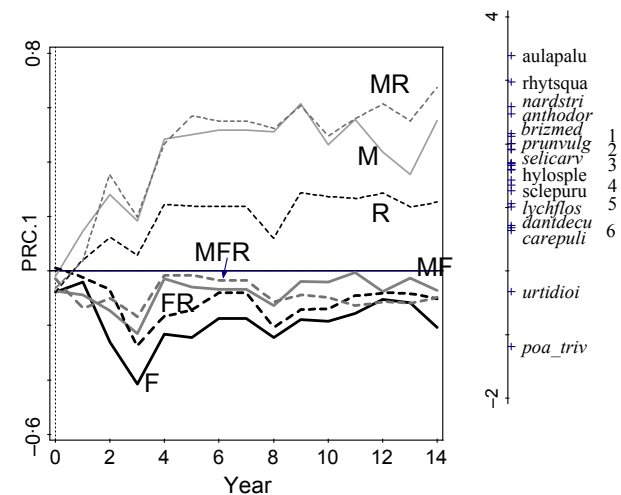


**Fig. 2.** Results of detrended correspondence analysis (DCA) including all samples across all years (first two axes shown). The DCA1 (horizontal) axis explains 11.24% and the DCA2 (vertical) explains 5.96% of the total variation. Percentage cover of individual species was  $\log(x+1)$ -transformed, and *Molinia* was excluded from the ordination. Line trajectories of centroids of treatment combinations signify the changes in species composition over the years, with all treatment combinations starting from the lower left corner (yr0). The different starting points show the natural variability of that baseline data. Fertilized and unfertilized plots are shown by thick and thin lines, unmown and mown plots by black and grey, and removal and non-removal plots by dashed and solid lines, respectively. Letters represent different combinations of treatments (Mowing, Fertilization, Removal), C for control, that is, unmown, unfertilized, non-removal. The curves are isolines of species richness per quadrat (including both vascular plants and mosses), overlaid over the ordination space by fitting the species richness by the two ordination axes using generalized additive models. The corresponding ordination of species is in Fig. S2 (Supporting Information).

species loss. This differentiation between fertilized and unfertilized plots (regardless of other factors) determined the first DCA axis. Unmown plots also suffered the loss of a similar set of species, whereas in the mown fertilized

plots (both with and without removal) grasses began to dominate (e.g. *Agrostis capillaris* L., *A. canina* L., *F. rubra*). The unmown fertilized plots were progressively dominated by *Scirpus sylvaticus* (mostly those with dominant removal), and they were also invaded by *Urtica dioica* L. The smallest changes were observed in unfertilized mown plots which ‘moved’ slightly in the direction of the second DCA axis, without any species loss. The removal plots were very similar to the corresponding combinations of non-removal plots, except in unmown unfertilized plots where a greater differentiation was observed. All the moss species responded in similar way, practically disappearing from all fertilized and unmown non-removal plots, and were most common in unfertilized mown plots.

In the PRC, the individual treatment combinations are compared year-by-year against the designated ‘control’, which, by definition, is represented by the horizontal axis (Fig. 3). The PRC confirmed the homogeneity of the plots at year zero. The major divergence, also shown by the DCA, between fertilized and non-fertilized plots (which in



**Fig. 3.** Principal response curves for the treatment combinations. The first axis is highly significant ( $P < 0.001$ ). Fertilized and unfertilized plots are shown by thick and thin lines, unmown and mown plots by black and grey, and removal and non-removal plots by dashed and solid lines, respectively. The species on the right-hand side show 26 species best fitted by the first principal axis. The unfertilized, unmown, non-removal combination was designated as a ‘control’, so it corresponds to the x-axis. Species labels (mosses labels are not in italics): *anthodor* – *Anthoxanthum odoratum* L., *aulapalu* – *Aulacomnium palustre* (Hedw.) Schwägr., *brizmed* – *Briza media* L., *carepuli* – *Carex pulicaris* L., *dantidecu* – *Danthonia decumbens* (L.) DC., *hylosple* – *Hylocomium splendens* (Hedw.) Schimp., *lychflos* – *Lychnis flos-cuculi* L., *nardstri* – *Narus stricta* L., *poa\_triv* – *Poa trivialis* L., *prunvulg* – *Prunella vulgaris* L., *rhytsqua* – *Rhytidiadelphus squarrosus* (Hedw.) Warnst. – *selicary* – *Selinum carvifolia* (L.) L., *sclepuru* – *Scleropodium purum* (Hedw.) Limpr., *urtidioi* – *Urtica dioica* L.; numerals are for (multiple) labels that do not fit into the graph: 1 – *Carex pilulifera* L., *Luzula multiflora* (Ehrh.) Lej.; 2 – *Carex palescens* L., *Potentilla erecta* (L.) Rauesch., *Climacium dendroides* (Hedw.) F. Weber et D. Mohr; 3 – *Carex panicea* L., *Festuca ovina* L., *Succisa pratensis* Moench; 4 – *Plantago lanceolata* L., *Ranunculus acris* L., 5 – *Scorzonera humilis* L.; 6 – *Ranunculus nemorosus* DC.



fact determined the first PRC axis, the y axis in the figure) occurred within the first 4 years. In contrast, differentiation between mown and unmown plots, particularly in the unfertilized plots, continued to increase for the first 10 years. The effect of mowing, in this sense, was opposite to that of fertilization. Whereas the effect of *Molinia* removal in mown unfertilized plots was small, the differentiation between removal and non-removal unmown unfertilized plots was pronounced (which is particularly visible in the DCA). In the case of the latter, *Molinia* was not replaced there by other grasses, but mostly by dicots.

#### CHANGES IN SPECIES RICHNESS

The repeated-measures ANOVA (Table 1) provided a long list of significant effects (the trivial ones, as increase of species richness with scale, are not interpreted). The most pronounced effect was the decrease in species richness

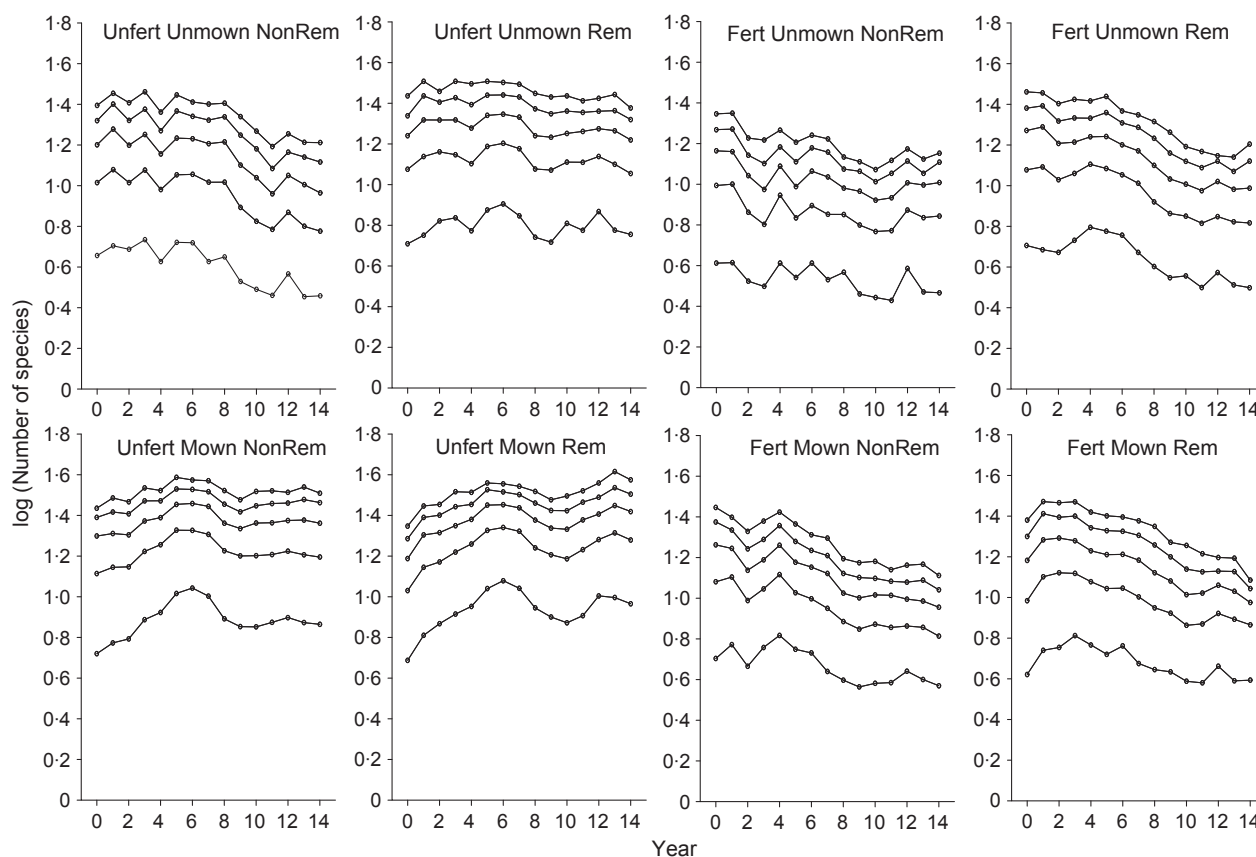
in fertilized plots, followed by a positive main effect of mowing (i.e. a positive effect when averaged across all the other factors), and a minor, but still significant, positive main effect due to removal. The many significant interactions (particularly the highly significant Year\*Scale\*Mowing\*Fertilization, and interactions with Removal) suggest that each treatment combination affected species richness in its own specific way (Fig. 4). These significant interactions also suggest that the effects of individual factors changed with time and scale, and they also depend on the factor levels with which they were combined. This can be best appreciated from pairwise comparisons, by comparing the trajectories of the two treatment levels of one factor, while the other factors remain constant (Fig. S4, Supporting Information). Specifically, the effect of fertilization was negative and developed rapidly at all spatial scales. It is most pronounced in mown plots (both removal and non-removal), where the differentiation

**Table 1.** Results of the repeated-measures ANOVA of log-transformed species richness. The between-subject factors are Mowing (MOW), Fertilization (FER) and Removal (REM), and the within-subject factors are YEAR and SCALE

	d.f.	MS	<i>F</i>	<i>P</i>
MOW	<b>1</b>	<b>5.242</b>	<b>26.15</b>	<b>0.000104</b>
FER	<b>1</b>	<b>15.512</b>	<b>77.37</b>	<b>0.000000</b>
REM	<b>1</b>	<b>2.688</b>	<b>13.41</b>	<b>0.002105</b>
MOW*FER	<b>1</b>	<b>1.246</b>	<b>6.21</b>	<b>0.024022</b>
MOW*REM	<b>1</b>	<b>1.199</b>	<b>5.98</b>	<b>0.026405</b>
FER*REM	1	0.002	0.01	0.928131
MOW*FER*REM	1	0.129	0.64	0.435081
Error	16	0.200		
YEAR	<b>14</b>	<b>0.443</b>	<b>28.11</b>	<b>0.000000</b>
YEAR*MOW	<b>14</b>	<b>0.053</b>	<b>3.35</b>	<b>0.000067</b>
YEAR*FER	<b>14</b>	<b>0.197</b>	<b>12.51</b>	<b>0.000000</b>
YEAR*REM	14	0.022	1.41	0.150534
YEAR*MOW*FER	<b>14</b>	<b>0.082</b>	<b>5.20</b>	<b>0.000000</b>
YEAR*MOW*REM	14	0.015	0.98	0.478951
YEAR*FER*REM	<b>14</b>	<b>0.063</b>	<b>4.01</b>	<b>0.000004</b>
YEAR*MOW*FER*REM	14	0.026	1.65	0.066893
Error	224	0.016		
SCALE	<b>4</b>	<b>24.254</b>	<b>4365.49</b>	<b>0.000000</b>
SCALE*MOW	<b>4</b>	<b>0.069</b>	<b>12.49</b>	<b>0.000000</b>
SCALE*FER	4	0.004	0.70	0.597317
SCALE*REM	4	0.006	1.02	0.402189
SCALE*MOW*FER	<b>4</b>	<b>0.016</b>	<b>2.82</b>	<b>0.032050</b>
SCALE*MOW*REM	4	0.004	0.76	0.552968
SCALE*FER*REM	<b>4</b>	<b>0.037</b>	<b>6.66</b>	<b>0.000152</b>
SCALE*MOW*FER*REM	4	0.001	0.27	0.897611
Error	64	0.006		
YEAR*SCALE	<b>56</b>	<b>0.006</b>	<b>9.66</b>	<b>0.000000</b>
YEAR*SCALE*MOW	<b>56</b>	<b>0.001</b>	<b>1.44</b>	<b>0.021420</b>
YEAR*SCALE*FER	<b>56</b>	<b>0.002</b>	<b>2.83</b>	<b>0.000000</b>
YEAR*SCALE*REM	56	0.001	1.21	0.139860
YEAR*SCALE*MOW*FER	<b>56</b>	<b>0.002</b>	<b>2.68</b>	<b>0.000000</b>
YEAR*SCALE*MOW*REM	56	0.000	0.68	0.967328
YEAR*SCALE*FER*REM	56	0.001	0.87	0.741054
YEAR*SCALE*MOW*FER*REM	56	0.001	1.00	0.485832
Error	896	0.001		

MS, mean square.

The significant effects ( $P < 0.05$ ) are highlighted in boldface.



**Fig. 4.** Changes in species richness during the 15-year experiment at various spatial scales. The five lines in each panel correspond to the average of log species richness in quadrat size from top down  $0.5 \times 0.5$  m,  $0.4 \times 0.4$  m,  $0.3 \times 0.3$  m,  $0.2 \times 0.2$  m and  $0.1 \times 0.1$  m. For the repeated-measures ANOVA results, See Table 1. There are four error levels, one for between-subject comparisons ( $SE = 0.258$ ) and three for within-subject comparisons: for year ( $SE = 0.073$ ), for scale ( $SE = 0.043$ ) and for year \* scale interaction ( $SE = 0.015$ ).

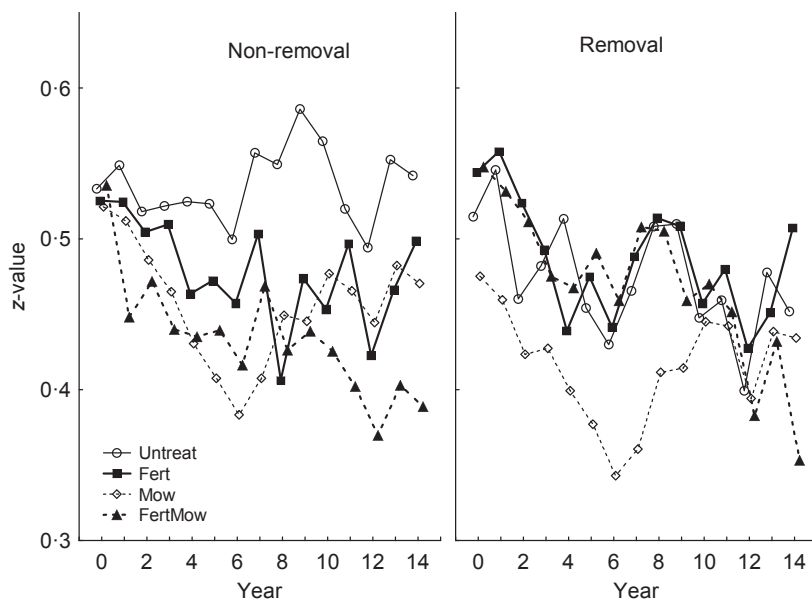
between fertilized and unfertilized plots continuously increased throughout the entire experiment. The effect was less pronounced in the unmown plots, and the differentiation disappeared completely in the unmown non-removal plots in the final years. Here, the differences were most pronounced at larger spatial scales in the early years, and they disappeared faster at the smallest scale. Absence of mowing had the most pronounced negative effect in unfertilized non-removal plots, mainly due to competitive suppression by *Molinia*, and the effect was more pronounced at smaller spatial scales. The effect of mowing was smaller in unfertilized removal plots. In fertilized non-removal plots, the positive effect of mowing disappeared in the last years, and in the removal fertilized plots, the differences between mown and unmown plots never developed. The effect of removal was positive in unmown unfertilized plots, most obvious at the smallest spatial scale, and the effect size generally increased with time. In unmown fertilized plots, there was a general divergence between removal treatments in the first half of the experiment, but after 7 years the effect diminished until non-existent. The effect of removal in mown plots was negligible.

Scale dependence was also confirmed by the analysis of  $z$ -value estimates (as a measure of beta diversity) in the

SARs (Fig. 5; Table S2, Supporting Information). Here, large variation among individual years was observed. On average, mowing decreased the  $z$ -value implying a more homogenous species composition within plots. In the non-removal plots, those that were also mown nearly always exhibited a lower  $z$ -value than the corresponding unmown plots. However, the differentiation was prominent at the beginning of the experiment and diminished later. The highest  $z$ -values were always found in the unfertilized, unmown, non-removal plots.

## Discussion

Our experiment is rather exceptional in that it combined a manipulative experiment with species removal and the detailed recording of vegetation over a lengthy and continuous observational period – the removal experiments are usually followed for much shorter time (Jonasson 1992; Hulme & Bremner 2005). The data were gathered over the entire 15 years solely by the author, thus ensuring a consistent quality of observation across the whole time period. Nevertheless, notes on some potential problems and practical considerations regarding measurements and experimental settings are presented in Appendix S1 (Supporting Information). Our experiment demonstrated



**Fig. 5.** Changes in the average  $z$ -value in species–area relationship for the eight treatment combinations over 15 years. For repeated-measures ANOVA, see Table S2 (Supporting Information). Dashed line – mown, solid line – unmown, full symbols and thick line – fertilized, open symbols and thin line – unfertilized. The SE for between-subject comparison = 0.072, SE for within-subject comparison = 0.021.

that drivers of land-use change act in combination, and their effects on the community structure are dependent on both the temporal and spatial scales considered and can be modified by the presence of a single dominant species.

As the experiment progressed, the species composition of the communities developed in different ways that mirrored typical vegetation types in the surrounding landscape (Fig S2, Supporting Information), confirming that the results of the experiment could be pertinent to the conservation of these endangered species-rich grasslands. The mown unfertilized plots corresponded with species-rich semi-natural meadows, mown fertilized plots roughly resembled intensively managed meadows, and unmown fertilized plots resembled meadows affected by nutrient run-off from surrounding fields. In all three combinations, the removal of *Molinia* modified the community composition only slightly. The unmown unfertilized non-removal plots were similar to abandoned meadows, which are commonly dominated by *Molinia*. The removal unmown unfertilized plots had no natural counterpart in the landscape.

#### INTERDEPENDENCY OF INDIVIDUAL FACTORS

Although the experiment confirms the effects of mowing and fertilization, which are already well known, it also highlights that the effect of each factor is conditioned by the combination of other factors and that the effects change considerably over time and with the spatial scale. While the positive effects of mowing and negative effects of fertilization on species richness in semi-natural meadows have been demonstrated many times (e.g. Lepš 1999; Eek & Zobel 2001; Rajaniemi 2002; Silvertown *et al.* 2006; Hejman *et al.* 2007; Galvnek & Lepš 2008; Hejman *et al.* 2007), contrasting results for fertilization have also been obtained in differing community types,

for example in Arctic tundra (Jonasson 1992). Recently, a negative effect of mowing frequency on species richness was demonstrated by Socher *et al.* (2012). However, they compared the grasslands that were mown one to four times per year, whereas our experiment compares plots mown once per year with corresponding unmown plots.

The results also demonstrate that the effectiveness of management and conservation measures might depend on the behaviour of a single dominant species. *Molinia* was positively affected by fertilization during the early years of the experiment, but thereafter its cover declined, particularly when mown. In fertilized plots, mowing was apparently able to mitigate for the negative effects of *Molinia*. However, in the latter stages, *Molinia* was out-competed in fertilized mown plots by strongly competing grasses (e.g. *F. rubra*), which are not negatively affected by mowing. These grasses suppressed species richness in mown plots as did *Molinia* in unmown plots. In unmown non-removal plots, the disparity in richness between fertilized and unfertilized plots disappeared towards the end of experiment: a single species, *Molinia*, was finally able to suppress species richness without fertilization.

#### EFFECTS OF DOMINANT REMOVAL

The removal of a dominant species and its subsequent effects on species richness has been less frequently studied, and with variable results (e.g. Jonasson 1992; Hulme & Bremner 2006); in species-poor communities, the removal resulted in changes in species abundance rather than in change in species richness (Herben *et al.* 1997). The positive effect of removal in our case, most noticeable in unmown unfertilized plots (where *Molinia* is most abundant), shows that this species is a threat to species

diversity predominantly (and perhaps only) in semi-natural communities (meadows and pastures) where traditional management has been abandoned. Unfortunately, regular mowing is not an economically viable management strategy at large scale in habitats where *Molinia* is considered a biodiversity threat – for example, in UK moorlands (Marrs *et al.* 2004). In Molinion meadows mown at least once a year, *Molinia* can coexist with many species in a species-rich community.

The results also demonstrate that the presence of a single competitively strong native species can result in the loss of community diversity, similar to frequently documented effects of non-native species (Hulme & Bremner 2005). This scenario is well known in applied nature conservation, when some native expansive dominants, for example *Calamagrostis epigeios* (L.) Roth, are able to out-compete many species from local communities (e.g. Somodi, Virágh & Podani 2008).

#### SCALE-DEPENDENT EFFECTS

Changes in the environmental conditions or in management strategies affect species richness differently at different spatial scales, thus altering the shape of the SAR (Lepš & Štursa 1989; Rejmánek & Rosén 1992; de Bello, Lepš & Sebastià 2007). In our data set, where the range of spatial scales is small to very small plots, but the total species richness is still high, the most pronounced effect is the initial decrease in the  $z$ -value in mown plots. This implies homogenization, where individual cells within the mown plots become more similar to each other, and is probably caused by two factors. First, the compact tussocks are more frequently formed under unmown conditions. Many species, including the dominant *Molinia* and others (typically *Carex umbrosa*, Fig. S4, Supporting Information), form more compact tussocks under unmown conditions (Š. Janeček, J. Lepš, unpublished data). Tussocks formed by competitively strong species result in low species richness in the affected cells; however, the lost species do not get excluded from non-tussock cells, and so the increase in species richness with area is faster. Secondly, in the smallest areas, the number of species is limited by the number of genets. In mown plots, the genets are usually smaller and so the number of genets that fit into a single  $0.1 \times 0.1$  m cell is considerably higher and consequently the number of species is higher. Because the number of species is far less limited by the number of genets in larger areas, the effect of the higher number of genets on species richness is considerably smaller in larger plots. Heterogeneity, represented by the  $z$ -value, decreased in most plots as a result of mowing at the beginning of the experiment, when all plots shared similar species. Later on, when species compositions diverged, different species began to dominate based on the treatment combination, and the tussock morphology of various species affects the  $z$ -value on this small scale.

#### COMPETITIVE EXCLUSION

In both unmown and fertilized plots, species richness declined, presumably as a result of competitive exclusion (Hautier, Niklaus & Hector 2009). This assumption is supported by the fact that plant height is the best predictor of species response to fertilization, with short species losing out due to the competition for light (see Lepš 1999), and similarly, tall species dominating the fertilized plots (Lepš *et al.* 2011). Tall species also prevailed in the unmown plots as opposed to mown plots, and also, the same species are on average taller in fertilized and unmown plots (Lepš *et al.* 2011). The short species (including all bryophytes) survived predominantly in the mown unfertilized plots, resulting in similar effects of fertilization and abandonment on both species composition and richness. Although convincing, this is still only indirect evidence for the increased importance of light competition, as light was not directly manipulated (as in Eek & Zobel 2001; Rajaniemi 2002 and Hautier, Niklaus & Hector 2009). However, the fact that *Molinia* modifies the effects of fertilization and mowing demonstrates that the competitive effect cannot be simply partitioned into competition for light and for nutrients. First, we have already demonstrated (Janeček & Lepš 2005) how *Molinia* affects both the established vegetation and seedling recruitment; these effects vary during a season, and their important components are an effect of slow decomposing litter (Pálková & Lepš 2008) and an effect of the basal internodes of *Molinia*. Large amounts of litter, particularly in the unmown plots, suppressed also seedling recruitment (unpublished data). The importance of this factor depends on the longevity of genets, which is potentially a very long time, as most of the species are clonal, but reliable data are lacking. From this perspective, the duration of the experiment is probably shorter than the maximum life span of the genets of most species.

The results also show that the time it takes for species exclusion to occur can be rather long; for some perennial species, competitive exclusion can take decades, which can lead to increasing extinction debt. This time is still short in comparison with the estimated 300–400 years suggested by Adler, Ellner & Levine (2010) in sagebrush steppe. Similarly, Helm, Hanski & Pärtel (2006) and Purschke *et al.* (2012) suggested that the persistence of species in a community can be rather long-lasting. On the other hand, some species (mainly the short ones) disappeared within a few years, especially from fertilized unmown plots.

Species exclusion is a scale-dependent process. The decrease in species richness in unmown unfertilized plots started 2 years before a sharp decline in the larger plots (similarly to GalvANEK & Lepš 2008). In the mown fertilized removal plots, the number of species in the smallest plots seems to have stabilized by the end of the experiment, whereas at the largest scale, the decline continued. On the other hand, in some cases, species richness



decrease was immediate and concordant at all spatial scales (e.g. in unmown fertilized plots).

## CONCLUSIONS

This study highlights the potential differences between the management recommendations based on short- and long-term experiments. For example, if a three- or even 7-year project be deemed sufficient, then our results would suggest that the species richness of mown fertilized meadows does not decline. This could lead to the misleading conclusion that this type of management can reconcile the economic interests of higher productivity with nature conservation goals of maintaining high diversity. Moreover, the scale of observation can further shift the timing in the detection of patterns of change.

Although the experiment confirmed that fertilization has a negative and mowing a positive effect on species richness, these effects change considerably in combination with each other and with the presence or absence of the most dominant species in the meadow. The response of the dominant species is an important driver of the biodiversity response to land-use changes. Mowing once a year enabled the coexistence of *Molinia* with other species in a species-rich community in unfertilized plots, whereas without mowing, the species is a serious threat to biodiversity.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Reliability of the data.

**Fig. S1.** Design of the experiment.

**Fig. S2.** State of the plots under individual treatment combinations in 2007 (photos).

**Fig. S3.** DCA Ordination of species corresponding to the Fig. 2.

**Fig. S4.** Pairwise comparisons of species richness changes.

**Fig. S5.** Compact tussock of *Carex umbrosa* in unmown unfertilized plot.

**Table S1.** Repeated-measures ANOVA of *Molinia* cover.

**Table S2.** Repeated-measures ANOVA of the z-value.