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Year-to-year variation in plant competition in a mountain grassland

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Summary

1 We used a series of removal experiments to examine how species response to competition and climatic differences varied in three different years. We tested the interaction between removal of the dominant grass species, *Festuca rubra*, and year-to-year environmental variation in a mown mountain grassland.
2 In each year, we quantified shoot frequency and above-ground biomass of all remaining plant species. Above-ground responses were tested both by analysis of covariance and by redundancy analysis with randomization tests of changes in total species composition.
3 Analysis of above-ground biomass data showed that other species compensated for the removal of *F. rubra* biomass within 2 years and that the response in total biomass of the community did not differ among years in which the experiment was started.
4 Multivariate tests showed that species composition changed as a result of the removal; grass biomass and frequency increased more than that of dicotyledons. However, response of species composition to removal of *F. rubra* was significantly different between onset years. Specific conditions in individual years thus affect the competitive ability of individual species in a non-additive way.
5 Our results indicate that the year-to-year variation at the site has the potential to affect species coexistence and richness. As a consequence, year-to-year variation of climatic parameters may be an important driving factor in community dynamics and should be taken into account in studies of ecosystem response to climate.

Key-words: competition, environment–competition interaction, *Festuca rubra*, growth forms, randomization tests, redundancy analysis, year-to-year variation, removal experiment, temporal heterogeneity

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Introduction

A common observation in ecology is that the outcome of interactions between species depends on the environment (Sharitz & McCormick 1973; Grace 1989; Keddy *et al*. 2002). Whereas many studies have explored how spatial heterogeneity alters species interactions, fewer authors have also considered temporal variation (Likens 1989). At any site, environmental conditions vary over time and species co-occurring in one habitat should therefore experience fluctuations in competitive interactions. For example, long-term data on community composition often show dramatic fluctuations in the abundance of individual species (Likens 1989; Dodd *et al*. 1995), with peaks in abundance sometimes related to peaks in climatic variables such as temperature or precipitation (Silvertown *et al*. 1994; Fitter *et al*. 1995; Herben *et al*. 1995; Dunnett *et al*. 1998). This suggests a strong link between climatic variation and community composition, but the mechanisms underlying this link cannot be assessed using observational data.

Environmental variation is widely thought to promote coexistence of species in competitive situations (Hutchinson 1961; Huston 1979; Fowler 1990). In order for variation in environmental conditions and interspecific interactions to promote coexistence among species, the response of an individual species to competition cannot be independent of its response to environmental variation (Chesson & Huntly 1989, 1997; Chesson 1994; see Muko & Iwasa 2000 for a different result in spatially heterogeneous environments). Coexistence is promoted if there is a non-zero interaction

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(negative covariation) between species responses to abiotic and biotic factors. This typically arises from differential sensitivity of juveniles and mature individuals to environmental variation; hence the whole phenomenon has been termed the 'storage effect' (Chesson & Warner 1981).

There are surprisingly few data sets available that are appropriate for testing either the assumptions or the predictions of these theoretical findings. Long-term data on community composition change (e.g. Likens 1989; Silvertown *et al*. 1994; Dodd *et al*. 1995; Dunnett *et al*. 1998) cannot be used to infer whether absence of particular climatic factors leads to lowered species richness or altered species composition; correlations with climate are always plagued with statistical problems. These data cannot be used to demonstrate the role of climate in changing species' competitive abilities; rather, density of a species has to be manipulated experimentally over several years.

Two kinds of manipulative experiment can be used to assess how climate alters species competitive abilities through time. First, experiments could be designed to test predictions of the theory, i.e. to test the effect of temporal environmental variation on community composition by establishing treatments with constant and varying environments and comparing species composition and richness over these treatments. Such experiments have been performed in the context of global warming where whole (micro)ecosystems or communities are subjected to climate manipulation (e.g. Hillier *et al*. 1994; Chapin & Shaver 1996; Jonasson *et al*. 1999; Sternberg *et al.* 1999; Weltzin *et al*. 2000; De Valpine & Harte 2001; Graglia *et al*. 2001). Most of these experiments, however, are designed to assess the effect of the change of *averages* of specific climatic variables; this may predict change of species composition under different climate scenarios, but does not assess the role of climatic variation *itself* for species coexistence. Recent analyses indicate that climatic variation may also be subject to change (Easterling *et al*. 2000). Because these experiments generally manipulate whole communities, they cannot separate direct effects on the individual species from the indirect effects through altered interspecific interactions (Werkman *et al*. 1996). On the other hand, by treating the community as a whole, the role of species richness itself in the response to climate manipulation can be assessed (discussions in Grime 1999; Lepš *et al*. 2001).

In the second kind of experiment, the effect of temporal variation on species composition can be deduced by testing assumptions of the theory (see Hairston *et al*. 1996). In order to test the assumption that environmental variation leads to species coexistence, it is necessary to quantify the interaction between sensitivity to competition and sensitivity to temporal variation in the environment. The design of the experiment should therefore allow for testing this interaction. Few previous authors have, however, specifically addressed temporal variation in competition (Goldberg & Barton 1992). Although it is possible to argue that the space-for-time substitution (Pickett 1989) may be used in this context, justification for this argument depends on the range of environmental conditions encountered in space and time. If these vary in quantity or quality, the space-for-time substitution cannot be used, particularly if the response of a species to the environment is nonlinear.

Dunnett & Grime (1999) have identified an interaction between environmental conditions (spring heating) and competitive interactions between grassland species, but used artificial microcosms that were not similar to field conditions. This represents a rare test of non-additivity of the effect of year-to-year variation and competition, and suggests that competitive interactions can amplify climatic (between-season) effects on the performance of individual species (see also Cáceres 1997; Cerda *et al*. 1997; Lima *et al*. 1999).

We performed a long-term field experiment designed to test for changes in the competitive effects of a dominant species over several years in a mountain grassland. We used density manipulation treatments (removal of the dominant species) to detect changes in competitive response on the community-wide basis (Bender *et al*. 1984; Aarssen & Epp 1990; Laska & Wootton 1999). Although such experiments cannot be interpreted at the level of *individual* species' response to density manipulation of the dominant (Wootton 1993; Laska & Wootton 1999), they provide a good measure of the community-wide response to this manipulation. Multidimensional tests can then be used to identify which species show opposite responses in competition as environmental conditions change.

More specifically, we tested the interaction between removal of the dominant grass species (*Festuca rubra*) and year-to-year variation in the effects of removal treatments on shoot frequency and above-ground biomass of all species in the community. We used a fully factorial design with two factors: removal of the dominant and year of the removal. Removal treatments were repeated over 3 years (removal beginning in 1994, 1995, 1996). The interaction between the two main factors (removal and year) was of primary interest; significance of this interaction was taken as a demonstration of year-to-year variation in competitive response of the community.

Methods

STUDY SITE

The study site is located in the Krkonoše Mountains, North Bohemia, Czech Republic (3.75 km ESE of the centre of Pec pod Sněžkou, 50°41′28″ N, 15°47′35″ E, 880 m a.s.l.). The growing season is from mid-April after snow melt until November The grassland is *c*. 300–400 years old, is maintained by mowing in summer and is occasionally grazed late in autumn. This management regime at the site was established before

the experiment began, and has resulted in a reasonably stable species composition (see also Krahulec 1990).

The vegetation is rather short, with most (> 80%) of the above-ground biomass less than 20 cm in height. The maximum above-ground biomass is approx. 170–190 g/m^2 (dry weight). The grassland is dominated by perennials; annuals are rare (except for *Euphrasia rostkoviana*). Species richness is about 32–36 spp./m^2 (3.6 spp. in a 3.3 × 3.3 cm cell). *Festuca rubra* (the species that was removed in the experiment) is very common at the site, comprising about 33% (SD = 0.12) of the total above-ground biomass. It forms small patches several centimetres wide; spatial autocorrelation of its density (Moran's *I*) is significant over a distance of 3.3 cm, but not significant over a distance of 6.7 cm; see Appendix 2. These patches are, in most cases, mixed with other species; mean number of species per 3.3 × 3.3 cm plots is 2.68 (*Festuca* excluded) for cells with *Festuca*, and 3.28 for cells without *Festuca*.

EXPERIMENTAL DESIGN

In 1993, 18 0.5 × 0.5 m plots were established in the grassland (the initial species composition of the plots is given in Appendix 1) and divided into three groups of six plots. Experimental manipulations were applied to the first group from 1993, and from 1994 and 1995 for the second and third groups, respectively. The entire experiment was maintained until 1999. In June of the first year of treatment, the vegetation in the central part of each plot (0.25 × 0.25 m; the size was chosen for compatibility with earlier studies) was recorded (see below) and all above-ground plant parts were clipped at 3 cm over the entire 0.5 × 0.5 m plot. In early May of the following year, the dominant species, *Festuca rubra*, was removed from three plots while the other three plots were left intact. *Festuca* was removed, using forceps, from the whole 0.5 × 0.5 m in order to provide a buffer zone for the central part of plots. All above-ground parts were removed and easily accessible rhizomes were pulled from the soil, but care was taken not to disturb the soil and the root/rhizome systems of other species. Since removal was not complete, the plots had to be revisited several times in the year in which removal began and also in the following years, although biomass and numbers of *Festuca rubra* decreased rapidly (to *c*. 5% of the initial value by the end of the first year). In June of each year, the vegetation in the central part of the plots was recorded and the whole plots were once again clipped at 3 cm. Clipping simulates mowing and was performed in all treatments to ensure that there was no successional trend associated with the experiment. Recording and clipping were carried out at approximately the same time as the annual mowing.

The three groups in the 'onset of experiment' treatment are referred to hereafter as '1994', '1995' and '1996', indicating the year when removal began. Within each group, the time elapsed since manipulation started is denoted as Year 1 (first clipping), Year 2 (removal started), etc. For example, calendar year 1996 was Year 2 for the '1996' treatment, while it was Year 4 for the '1994' treatment (Table 1).

The vegetation in the plots was recorded by means of a grid with 3.3 × 3.3 cm cells positioned exactly at the same position in the plots every year. The cell size was selected for compatibility with earlier studies at this and other grasslands. During the recording, the number of tillers (for grasses and graminoids), the number of rosettes (for small dicotyledons) and the number of leaves (for larger rosette dicotyledons, i.e. *Alchemilla* spp., *Polygonum bistorta*, *Geranium sylvaticum*, *Hieracium* subg. *Pilosella*, *Leontodon hispidus*, *Plantago lanceolata*, *Ranunculus acris*, *Trifolium* spp., *Taraxacum officinale*; names follow Tutin *et al*. 1964–80) rooting in each cell were counted (hereafter 'numbers of shoots'). Because the plots were clipped after the recording, the clippings were collected, sorted into species and used to determine above-ground biomass of each species.

DATA ANALYSIS

The data set was analysed in order to obtain four different kinds of information: (i) the overall response of community biomass at the plot level to *F. rubra* removal, year of onset of experimental treatments

Table 1 Overview of the experiment. Beginning of treatment in each group ('onset of the experiment') is indicated by boldface; italics indicate groups that were not part of the experiment in the particular year

	Treatment (onset of the experiment)		
	1994	1995	1996
Before 1993	*Mown*	*Mown*	*Mown*
1993 summer	Recorded/clipped	*Mown*	*Mown*
1994 spring	**Removal in treatment plots**		
1994 summer	Recorded/clipped	Recorded/clipped	*Mown*
1995 spring		**Removal in treatment plots**	
1995 summer	Recorded/clipped	Recorded/clipped	Recorded/clipped
1996 spring			**Removal in treatment plots**
1996 summer	Recorded/clipped	Recorded/clipped	Recorded/clipped
1997 summer	Recorded/clipped	Recorded/clipped	Recorded/clipped
1998 summer		Recorded/clipped	Recorded/clipped
1999 summer			Recorded/clipped

Table 2 Design of multivariate randomizations tests at the plot and cell levels. Plot ID, code of the experimental plot; Cell ID, code of the cell nested within the plot (only when tests at the cell level were carried out); each cell therefore had a unique identification variable; 'Initial *Festuca*' refers to the number of shoots of *Festuca* in each cell at the beginning of the experiment. Time was always randomized by cyclic shifts within each plot; recordings of cells were always kept together within one plot. Within-plot randomization of cells (if applicable) was carried out by grid rotation, reflexion and toroidal shifts

Effect to be tested	Levels of analysis	Variable(s) tested	Covariables	Plots randomized within blocks defined by	Randomization of cells within plots
Removal	Plots, cells	Removal × Time	Time, Plot ID/Cell ID	Onset	No
Onset	Plots, cells	Onset × Time	Time, Plot ID/Cell ID	Removal	No
Onset × Removal	Plots, cells	Onset × Removal × Time	Time, Plot ID/Cell ID, Onset × Time, Removal × Time	No constraintNo	No
Removal × Initial *Festuca* density	Cells only	Removal × Time × Initial *Festuca* density	Time, Cell ID	Onset	Yes
Onset × Removal × Initial *Festuca* density	Cells only	Removal × Onset × Time × Initial *Festuca* density	Time, Cell ID, Removal × Time, Onset × Time	No constraint	Yes

and their interaction; (ii) the response of community species composition at the plot level to removal, onset and their interaction; (iii) the fine-scale response of the community species composition at the cell level to removal, onset and their interaction; and (iv) correlation of the fine-scale community response to initial density of the removed dominant, *F. rubra*. The first analysis was carried out by means of a repeated measurements analysis of covariance, whereas multivariate tests were used for the remaining analyses. Multivariate tests enable us to assess the compositional variability in the whole community response and minimize likelihood of inflated Type I errors due to multiple testing carried out on the same data.

Analysis of changes in total above-ground biomass included onset and removal as main factors and above-ground biomass prior to the treatment as covariate. *Festuca rubra* biomass (in untreated variants) was also included as a covariate. Since Mauchly's test indicated a significant violation of the assumption of sphericity (approx. chi-squared = 11.51, d.f. = 5, $P = 0.043$), significance levels for within-subject effects were calculated using the Greenhouse–Geisser correction of the number of degrees of freedom. All the univariate calculations were carried out using SPSS ver. 8 (Anonymous 2000).

Otherwise, we used redundancy analysis (RDA, a multivariate generalization of linear regression) on the correlation matrix (data standardized by species). This is a canonical form of principal components analysis that identifies major gradients within the set of dependent variables (Jongman *et al*. 1987). In addition, it maximizes the correlation of these gradients with another set of independent variables, assuming linear relationships between the two sets of variables. To account for the complex structure of independent variables and several error strata, we used the partial form of RDA that identifies the correlation of one set of independent variables with residuals of dependent variables after the effect of another set of independent variables (covariates) has been removed. Covariates were used to remove differences between plots and cells from the analysis (Table 2). Redundancy analysis was used to analyse three data matrices: (i) shoot counts of species per plot (obtained by pooling cell counts of each species within plots, 18 plots × 5 recordings × 41 species); (ii) species biomass per plot (measured directly, 18 plots × 5 recordings × 41 species); and (iii) shoot counts of species per cells [measured directly, 1152 cells (nested within 18 plots) × 5 recordings × 41 species]. These variables were taken as dependent variables; quantity (shoot number or above-ground biomass) of the removed species, *Festuca rubra*, was always excluded from the dependent variables (species).

Different independent variables were used depending on the test performed (Table 2). Significance testing of the effect of these independent variables used permutation procedures that maintained plot identity (i.e. all recordings of one plot were permuted together) and, in the case of the cell counts, also the spatial structure within grids. To test a specific effect, a partial test was made by treating all other relevant independent variables as covariates; in this case, the permutation procedure randomized only the variable in question with respect to the residuals after the effect of covariates had been removed (ter Braak & Šmilauer 1998). In all cases, significance levels reported are for all canonical axes together.

Multivariate significance tests at the plot level were performed by (i) complete randomization of whole plots with respect to onset or treatment, while keeping the other variable not randomized, together with (ii) cyclic shifts of time using the same onset for all the plots (Table 2, first three analyses). 'Dummy' covariates identifying plots were used to factor out differences between plots. Multivariate tests of the cell-based data (Table 2, last two analyses) either used the same randomization procedure as above (for tests of plot-level factors such as onset and removal) or a randomization within grids was used in addition to the randomization among plots and recording times (for tests of initial *Festuca* density and its interactions). All randomizations

of cell-based data had to take into account the fact that the data structure is a double split-plot design (repeated recordings within cells and cells within plots). To test treatment effects, plots were always randomized as a whole; any other type of randomization would lead to pseudoreplication. The within-plot randomization used grid rotation and/or reflexion, and toroidal shifts within grids by a random onset. Again, 'dummy' covariates identifying cells were used to factor out differences between cells.

Rare species, i.e. those that were recorded in less than 10 cells (out of 1152) were not included in the multivariate analyses (13 species). The removed dominant, *Festuca rubra*, was also not included and the remaining data set thus contained 41 species. (Density of *Festuca rubra* at the beginning of the experiment was used as an independent variable in the analysis of the data at the cell level.)

When assessing the response of individual species to independent variables (removal, onset, onset × removal interaction), species scores on the canonical axes were used. When there was only one canonical axis (i.e. there were only two levels of one independent variable), the scores themselves were taken as the approximate measure of the amplitude and direction of the species' response to the independent variable. If there were two or more canonical axes, the square root of the sum of squared scores of all canonical axes (Euclidean distance to the origin) was used as a summary measure of the amplitude of the response to the independent variables. In order for the axes be comparable, scoring was based on the weighted sum of species scores not adjusted by species variance (option-2 in CANOCO ver. 4 was used) (see ter Braak & Šmilauer 1998; Šmilauer pers. comm.).

All multivariate analyses were carried out using the program CANOCO ver. 4 (ter Braak & Šmilauer 1998); since the data on shoot counts within cells are a double split-plot design, which cannot be handled by CANOCO, a special randomization program was written for this type of analysis (for details of the randomizations, see Table 2 and Appendix 3).

Species were classified into simple functional types using data from Grime *et al.* (1988). Grasses were compared with other species and grasses and graminoids with non-rosette dicotyledon species, rosette dicotyledon species and annuals.

CLIMATE VARIATION DURING THE EXPERIMENT

Information on weather during the study period was taken from a nearby climatic station (Pec pod Sněžkou), which records temperature and precipitation, and the presence and thickness of snow cover daily. Several months having extreme weather (with z-score greater than 1.5 compared with the 15-year average) occurred during the experimental period: high precipitation in August 1994, May 1996, September 1998 and especially July 1997; low precipitation in June 1994, September 1997 and August 1999; high temperature in July 1994, July 1995, October 1995, September 1999; low temperature in September 1996.

Results

RESPONSE TO REMOVAL AT THE PLOT LEVEL

Removal of the dominant species, *F. rubra*, had a major effect on the total above-ground biomass of the community (Table 3). In the first year after the removal, the total biomass decreased by *c.* 40%, although it then increased to compensate for the loss of the dominant during the second year after removal (Fig. 1, the difference is significant in Year 2 (two-way ANCOVA with onsets as blocks and the above-ground biomass in Year 1 as a covariate: $F = 6.551$; d.f. = 1,13; $P = 0.024$), but not significant in subsequent years (Year 3: $F = 0.226$, $P = 0.643$; Year 4: $F = 0.461$, $P = 0.509$; Year 5:

Table 3 Changes in total above-ground biomass in the removal experiment tested by analysis of covariance with repeated measurements. Biomass of *Festuca rubra* (from untreated variants or values at the beginning of the experiment before the removal began) is included in the analyses. Significance levels for the effects involving time are calculated using the Greenhouse–Geisser correction of the number of degrees of freedom. Effects significant at alpha = 0.05 are shown in bold

	F	d.f.	*P*
Removal	0.85	1,11	0.376
Onset	1.52	2,11	0.261
Removal × Onset	0.59	2,11	0.573
Biomass in the year preceding treatment	7.84	1,11	**0.017**
Time	1.85	3,33	0.189
Removal × Time	5.07	3,33	**0.022**
Onset × Time	5.69	6,33	**0.005**
Removal × Onset × Time	0.18	6,33	0.923
Biomass in the year preceding treatment × Time	2.13	3,33	0.152

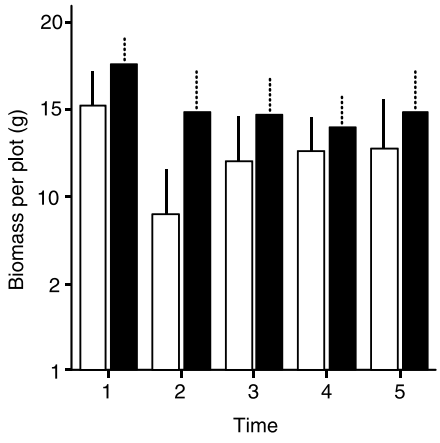

Fig. 1 Differences in the total above-ground biomass (g/plot) between plots with (filled columns) and without (open columns) dominant species removal (all onsets combined; each point represents the mean of nine plots). Removal began in Year 2 (1994 for the '1994' onset, etc). Bars indicate 95% confidence intervals.

Table 4 Multivariate tests (redundancy analysis on the correlation matrix) of effects of *Festuca* removal, onset of the experiment and their interactions at the plot and cell levels. Species data are not transformed. For the exact structure of the tests and analyses, see Table 2. *F*, pseudo-*F* statistic (see ter Braak & Šmilauer 1998); *P*, significance level; –, not applicable. Numbers in parentheses indicate number of canonical axes in the analysis (number of independent variables – 1, including dummy variables used to code categorical variables, see Table 2). Effects significant at alpha = 0.05 are shown in bold. In the text, the analyses are referred to by a combination of rows (numbers) and columns (letters)

	Plot level shoot counts A	Plot level biomass B	Cell level shoot counts C
Plots/cells	18	18	1152 (nested in 18 plots)
Recordings of each plot/cell	5	5	5
Number of dependent variables (species)	41	41	41
(1) Removal (1)			
F	1.57	1.51	5.14
P	**0.040**	0.055	0.100
(2) Onset (2)			
F	2.50	2.52	7.88
P	**0.005**	**0.005**	**0.005**
(3) Onset × Removal (2)			
F	1.56	1.29	5.66
P	**0.040**	0.125	**0.005**
(4) Removal × Initial *Festuca* density (1)			
F	–	–	1.492
P	–	–	0.145
(5) Onset × Removal × Initial *Festuca* density (3)			
F	–	–	2.026
P	–	–	**0.010**

Table 5 Difference between functional types in their response to removal. Values in the table are approximate chi-squared values of the Kruskal–Wallis tests (significance levels in parentheses). Effects significant at alpha = 0.05 are shown in bold

	Growth form (No rosette, rosette, annual)	Grass vs. dicotyledon
Number of levels	3	2
Parameter		
Response to removal – plot level counts	0.35 ($P = 0.840$)	3.57 ($P = 0.059$)
Response to removal – plot level biomass	1.02 ($P = 0.600$)	1.59 ($P = 0.208$)
Response to removal – cell level counts	0.57 ($P = 0.751$)	5.00 ($P = 0.025$)

$F = 0.016$, $P = 0.902$). There was a significant effect of above-ground biomass prior to the treatment which was taken as a covariate (Table 3).

Removal had a significant effect on the total species composition of the plots, expressed both as total shoot counts or above-ground biomass sorted into species (marginally significant in case of biomass, $P = 0.055$; Table 4, analysis 1). There was a very close relation between the response of individual species in the analysis based on biomass and on shoot counts. The species scores on the first canonical axis in analysis of biomass and that of shoot counts are approximately linearly related; there is one outlier species (*Prunella vulgaris*) whose responses by number of shoots and biomass are both strong, i.e. it has high scores in both analyses, but they are of opposite signs. If this species is removed, the relationship has $R^2 = 0.545$ ($n = 41$, $P < 0.001$); the intercept of the relationship is statistically indistinguishable from zero ($P = 0.262$ using the *t*-test).

There was no difference in the response of different plant life forms to the removal treatment, either at the level of cells or plots (Table 5). There was a significant difference between the response of grasses relative to dicotyledons at the cell level ($P = 0.059$ at the plot level; Table 5). Grasses generally increased their shoot counts after removal relative to dicotyledons. Only two grasses had negative scores (i.e. indicating relative decrease after removal), viz. *Alopecurus pratensis* and *Deschampsia caespitosa*; neither species is common in the plots. There was no significant difference between grasses and dicotyledons in their above-ground biomass response to removal treatment (Table 5).

YEAR-TO-YEAR VARIATION IN COMPETITIVE RESPONSE AT THE PLOT LEVEL

The rate of change of total above-ground biomass after *F. rubra* removal differed between different onsets, but there was no significant onset × removal interaction (Table 3); this indicates that biomass compensation after removal was similar among years.

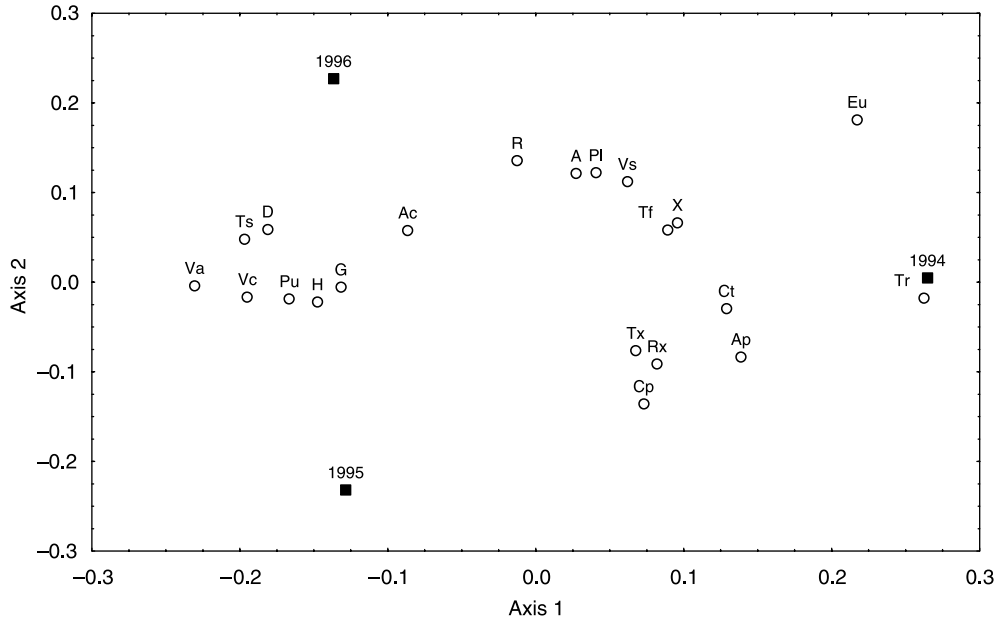

Fig. 2 Redundancy analysis biplot (Table 2, analysis 3, shoot counts) of the year-to-year difference in the individual species' response to the removal of the dominant species. '1994', '1995' and '1996' are scores of the environmental variables (given year × dominant removal × time; cf. Table 2). Circles are species; only species with distance to the origin greater than 0.7 are displayed. Species abbreviations: A, *Agrostis capillaris*; Ac, *Acer pseudoplatanus*; Ap, *Alopecurus pratensis*; Cp, *Campanula patula*; Ct, *Cerastium holosteoides*; D, *Deschampsia flexuosa*; Eu, *Euphrasia rostkoviana*; G, *Galium pumilum*; H, *Cardaminopsis halleri*; R, *Ranunculus acris*; Rx, *Rumex acetosa*; Ts, *Trisetum flavescens*; Tx, *Taraxacum officinale*; Va, *Vicia cracca*; Vc, *Veronica chamaedrys*; Vs, *Vicia sepium*; X, *Anthoxanthum odoratum*; Pu, *Prunella vulgaris*; Pl, *Plantago lanceolata*; Tr, *Trifolium repens*; Tf, *Trifolium pratense*.

Multivariate tests (Table 4, analysis 2) showed that species composition of the plots differed among onsets of the experiment. Again, the effect was significant no matter whether the response was expressed as total shoot counts or above-ground species-specific biomass. In addition, there was a significant interaction between the onset and removal treatments for the shoot counts (Table 4, analysis 3); but the interaction for the above-ground biomass was not significant ($P = 0.125$).

The removal of the dominant favoured different species in the 1994, 1995 and 1996 treatments (Fig. 2; species with points lying in the same direction from the origin as the point of a given year responded positively to the removal of the dominant in that year). In 1994 and 1995 rosette species were more successful both at the cell and at the plot levels using shoot counts (difference among growth forms in the scores on the two canonical axes is significant using MANOVA, plot level shoot counts: $F = 2.59$, d.f. = 4,74, $P = 0.044$; cell level shoot counts: $F = 3.73$, d.f. = 4,74, $P = 0.008$). Most of the non-rosette species were favoured by the removal in 1996. No such difference was recorded for biomass data (MANOVA, $F = 0.28$, d.f. = 4,74, $P = 0.887$).

RESPONSES AT THE CELL LEVEL

Overall results at the cell level are very similar to those at the plot level. The effect of removal *per se* at the cell level was weak ($P = 0.10$; Table 4, analysis 1). There was a tight relationship between the species' score on the canonical axis at the plot level and on the canonical axis at the cell level ($r^2 = 0.876$); the intercept of this relationship is statistically indistinguishable from zero ($P = 0.597$ using the *t*-test). However, as at the plot level, the effects of onset and onset × removal were significant (Table 4, analyses 2 and 3). There was strong correlation between scores on the canonical axes between plot and cell levels (shoot counts: 1st axis plot level vs. 1st axis cell level: $r = 0.879$, $n = 41$, $P < 0.0001$; 2nd axis plot level vs. 2nd axis cell level: $r = 0.861$, $n = 41$, $P < 0.0001$).

The overall test of removal × initial *Festuca* density was not significant (Table 4, analysis 4: $P = 0.145$), suggesting that the compositional change at the level of cells was independent of the initial density of *Festuca* in these cells. On the other hand, the interaction of onset × removal × initial *Festuca* density was significant (Table 4, analysis 5), indicating that there was a consistent difference among years in the compositional change that followed *Festuca* removal in cells differing in initial *Festuca* density. The responses in 1994 and 1996 were similar; the response in 1995 was quite different with many dicotyledon seedlings recruiting in cells which originally had a high *Festuca* density.

Discussion

IMPLICATIONS FOR THE COMMUNITY DYNAMICS AND SPECIES COEXISTENCE

Release from competition by *Festuca rubra* affected species composition in this grassland community

differently among years (Table 4). This means that sensitivities of the component species to year-to-year variation and to competition are not independent. More importantly, this suggests that the response of individual species to competition cannot be predicted using information on the year-to-year variation in the performance of that species and the *average* value of the competitive effect of *Festuca*. This effect is significant in both tests with shoot counts; since this test is based on only three full replicates and so has relatively lower statistical power, deviation from the null hypothesis is likely to be large.

This experiment cannot reveal anything about the mechanisms underlying the differential response of species to removal in individual years, but one explanation could be that this response is due to climatic variation among years. Even if the effects are due to climate, the response time of the community is unknown; the specific response in one particular year is due to signals integrated over the previous year or years. Nevertheless, in comparison, spring was considerably shorter in 1995 and 1996 than in 1994. This may account for differential recruitment of annuals and non-clonal species, since shorter springs may favour clonal plants that can mobilize resources faster. There was also a marked increase of legumes (*Trifolium repens*, *Trifolium pratense* and *Vicia sepium*) in 1994, whereas non-leguminous clonal forbs (*Veronica chamaedrys*, *Prunella vulgaris*, *Cardaminopsis halleri*) increased in 1995 and 1996. If initial *Festuca* density at the cell-level is taken into account, only the three-way interaction of removal × *Festuca* density × onset is significant. This means that a specific group of species seems to invade *Festuca*-rich cells in individual years; this is likely to be due to differential seedling recruitment of dicotyledons at different onsets.

Most of the research on climate-driven variation in community composition has been done in communities where year-to-year fluctuations of environmental conditions is a major determinant of the community composition, such as in arid habitats e.g. Pake & Venable 1996, and references therein) or in ephemeral ponds (e.g. Bonis *et al*. 1995). Such communities regenerate each year from a persistent diaspore bank and different climatic conditions among years are likely to favour different subsets of species. In order for the storage effect to be of importance, there should be differential sensitivity of juveniles and mature individuals to environmental variation. In communities of long-lived plants, such as grasslands, there are always strong year-to-year biological legacies, for example, due to the presence of underground storage organs that form new shoots every year. Again, regeneration of new above-ground shoots from the underground 'bud bank' and of seedlings from the seed bank are the plant developmental stages that are most sensitive to environmental conditions.

If the climatic variation that occurs as the regular part of the ecological regime of the site produces our observed interaction between experimental onset and competition, then there is a good reason to suspect that this variation has an important role in control of species richness at the site. The results of our experiment indicate that there is no dominant competitive ranking of species in this community (Keddy *et al*. 2002). Such hierarchies are often rather consistent when different ecological conditions are compared (Keddy *et al*. 1994; Keddy *et al*. 2000; Keddy *et al*. 2002) and therefore they should also remain consistent over the climatic variation observed during our study. However, competitive hierarchies are typically found for plants that vary in size over several orders of magnitude; in contrast, there is only limited variation in plant size in the experimental grassland species present there. If plants are of similar sizes, competition becomes more symmetric (Hara 1993; Kikuzawa & Umeki 1996; Keddy *et al*. 1997) and a strong competitive hierarchy is less likely to develop (see also, e.g. Aarssen 1988; Hara & Wyszomirski 1994).

LIMITATIONS OF THE EXPERIMENT

There is a major limitation of the experiment due to unknown interactions between the effects of variation in 'target' years (1994, 1995, 1996) and in the years that followed and preceded the treatment. Climate and other environmental conditions of, for example, 1995, influenced the first-year effects of the removal treatment for the '1995'-treatment, but the second-year effects of the '1994' treatment. As a result, effect of onsets of the experiment are difficult to test independently from background variation among years. In an ideal world, onsets should differ in the ambient conditions only in the period of the treatment, while the rest of the experiment should be identical across the onsets. This is a conceptual difficulty that cannot be easily overcome. If treatments with different onsets are compared, the difference between onsets is not limited to the year(s) when the removal was done, but necessarily continues to differ throughout the whole experiment. As a result, comparison across onsets means comparing different years throughout the experiment. There is however, no method for separating variation in climate that is part of the experimental treatment from that which is the nuisance factor in the background (and should be removed by a good experimental design!). The only option is to accept the fact that the response times to different environmental signals are difficult to separate and that a conservative solution is to include the total environmental variation into the treatment factor (see, e.g. Swetnam & Betancourt 1998).

This experiment does not permit separation of the direct effects of the removed competitor (*Festuca*) on any remaining species from the indirect effects mediated via additional species (Wootton 1993) or from simply creating gaps in the community. Our data cannot therefore be used to argue that the responses of individual species are *separate* from the removal of the dominant

among years. While this is quite likely (particularly at the cell level where the higher-order effects may play less of a role), only the community-level response can safely be said to respond to the interaction between climate and competition. The statistical techniques used do allow us to test both aggregate (as determined by ANOVA) and compositional (as determined by RDA) components of the multivariate variability on the same data set (Michelli *et al*. 1999). Whereas the aggregate variability (change in total biomass) shows no removal × onset interaction, the significance of the removal × year interaction in the compositional variability indicates a changed interaction structure within the community over the three onsets of the experiment.

Finally, results from any removal experiment may be due to rapidly responding plant species that are able to occupy gaps either by fast clonal growth or seedling recruitment, traits which need not be correlated with competitive release (Glenn & Collins 1993; Wardle *et al*. 1999). In our experiment, seedling recruitment differed among years and is less likely to be important, since the spatial structure of the grassland is extremely fine-scaled (i.e. *Festuca* patches are small – only a few cm in diameter and always mixed with other species) and therefore removal itself produced no large gaps.

TYPES OF PLANT RESPONSE

Although there is a broad correlation between biomass and shoot number responses to treatments, neither response was fully predicted by the other. A plant may clearly respond to release from competition or to year-to-year variation either by producing larger modules or by producing a greater number of modules (Herben *et al*. 1995). The principal interaction of interest, removal × onset, is stronger for the number of shoots than for biomass (the same is true for the removal *per se*; Table 4), indicating that species in this community change proportions of shoot numbers more readily than biomass proportions.

Interestingly, in the test done at the plot level (both shoot counts and biomass) both onset × removal and removal *per se* effects are significant; indicating that, although the response differs among years, there is still an overall change following removal, which is captured by the main effect of year. This is mainly due to the systematic increase in shoot numbers and biomass of major grasses following *Festuca* removal, possibly because *Festuca* exerts a stronger competitive effect on species that are more ecologically similar. Alternatively, grasses may be weaker competitors or species capable of a faster response and thus respond more strongly to the lowered level of competition after removal. Although such species may be less important for community dynamics when the superior competitor is present, their density may have important cascade effects on other trophic levels and consequently on the dynamics of the whole community (Richardson *et al*. 2002; see also Grime 1999).

Conclusions

This experiment provides evidence that interspecific interactions are modified by climate (and vice versa), with a significant climate × competition interaction (cf. Dunnett & Grime 1999). This means that sensitivity of grassland species to competition (*Festuca* removal) is not independent of that to the environmental change (onset of the experiment), suggesting that climate variation has the potential to maintain species-rich communities (Chesson & Huntly 1997). This has two major implications. First, it is known that climate variation itself may change as a consequence of climate change (e.g. Easterling *et al*. 2000); the role of inter-annual variation therefore has to be taken into account in studies of ecosystem response to climate because response simply to changing means of climatic variables may not be sufficient to capture the whole effect. Second, since species richness affects many functions of the community (Lepš *et al*. 2001; Richardson *et al*. 2002), the effects of climate variation in maintaining these functions through effects on species richness may be considerable.

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Supplementary material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JEC/JEC746/JEC746sm.htm:

Appendix 1 Species composition of the plots at the beginning of the experiment.

Appendix 2 Spatial autocorrelation of *Festuca rubra* density at the cell level at the beginning of the experiment.

Appendix 3 Code listing of the BASIC program for different types of (restricted) randomisation of double split plot designs.

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