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The Rengen Grassland Experiment: Plant species composition after 64 years of fertilizer application

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Abstract

A long-term fertilizer experiment (the Rengen Grassland Experiment, RGE) was established in 1941 in the Eifel Mountains of Germany on low productive grassland naturally dominated by *Calluna vulgaris* and *Nardus stricta*. Six treatments combinations of Ca, N, P, and K fertilizer were applied annually: an unfertilized control, Ca, CaN, CaNP, CaNP–KCl, and CaNP–K_2SO_4. In mid-June 2005, plant cover was visually estimated and sward height was measured aiming to detect changes in floristic composition caused by long-term fertilization.

Calculated by redundancy analysis (RDA), the effect of treatment was found to be a significant predictor of sward structure in the experimental area and explained 62% of cover data variability. The largest difference in vegetation structure and composition was between the treatments without and with P application. *Briza media* was the dominant short grass in the control, Ca, and CaN treatments. *Lathyrus linifolius* was the dominant legume in the control and *Carex panicea* was dominant in the CaN treatment. Among treatments with P application, plant species composition was similar with tall grasses such as *Alopecurus pratensis*, *Arrhenatherum elatius,* and *Trisetum flavescens* dominating in the sward. Sward heights were lowest in treatments without P addition in contrast to tall canopies in the P fertilized treatments. Cover of the moss layer was continuously developed in treatments without P application only and was negatively correlated with sward height as was species richness of vascular plants.

Long-term fertilization caused significant diversification of plant communities. Species indicative of low productivity grasslands (short grasses, orchids, and sedges) survived in the CaN treatment but not in plots with P or K fertilizer. It was often thought that N enrichment is detrimental to vascular plant richness. However, the RGE shows that this is not necessarily so if N application is not accompanied by another limiting nutrient like P.

Keywords: Species richness; Long-term fertilization; Reseeding; Functional groups; Sward height

1. Introduction

The first scientific experiment aimed at assessing the effect of mineral and organic fertilizers was started by Lawes and Gilbert at Rothamsted Experimental Station (England) in 1856 (Park Grass Experiment, PGE), and this experiment is still in progress (Tilman, 1982; Silvertown et al., 2006). Lawes et al. (1882) recorded a significant diversification of floristic composition among the fertilizer regimes. In the same experiment, Silvertown (1980) showed that the portion of grasses, legumes and other species remained in dynamic equilibrium with annual fluctuations of floristic composition. Silvertown (1980) also demonstrated a remarkably consistent relationship between species diversity and biomass over a period of more than 80 years (from 1862 to 1948/9).

A prominent example of fertilizer effects on a poor substrate in a *Nardus stricta* dominated grassland is that in the Swiss mountains established in 1930 (Hegg et al., 1992; Spiegelberger et al., 2006). Liming, N and P fertilization and a combination of both were applied from 1930 to 1936 and

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from 1946 to 1950. Surprisingly, differences in floristic composition among treatments were still visible even in the abandoned experiment, although plots had not been fertilized for several decades. A similar set-aside fertilizer experiment established on *Nardus* sub-alpine grassland was reported by Hejcman et al. (2007) in the Giant Mts. (Czech Republic). The effect of fertilizer P on floristic composition especially, was still visible 37 years after the final fertilizer application.

Many fertilizer experiments were established on different types of grasslands in the 1960s in Central and Eastern Europe (Velich and Mrkvička, 1993; Královec and Prach, 1997; Galka et al., 2005; Sammul et al., 2003; Niinemets and Kull, 2005). In Austria, a series of long-term fertilizer experiments is still under observation (Trnka et al., 2006). In all these experiments, the increase of annual N, P, and K supply ultimately changed the grassland swards towards (i) lower species richness, (ii) a decreased proportion of species adapted to low soil fertility and (iii) higher dry matter yield and forage value of the grassland. According to Pennings et al. (2005), the response of individual species to nutrient supply may often be predictable. However, in most cases the response is strongly affected by biotic and abiotic conditions.

The Rengen Grassland Experiment (RGE) in Germany is one of the oldest continuously managed well-designed experiments worldwide. It consists of five fertilizer treatments and an unfertilized control on a mountainous hay meadow cut twice annually (Schellberg et al., 1999).

Applying ordination techniques to the analysis of plant species composition data, we studied the ‘‘explanatory power’’ of long-term treatments in the RGE. Further, the use of functional groups including legumes, grasses, and herbs, enabled us to answer the following questions: is plant height an explanatory trait of long-term fertilizer effects? Has the reseeding of productive plant species any long-term effect on plant species composition? Further, the actual and potential sward height of selected species was studied to determine the effect of fertilizer treatments on growth parameters of plants. Finally, the paper reveals the relation between species richness of vascular plants, cover of mosses and actual sward height (ASH).

2. Materials and methods

In 1941, a fertilization experiment was set up on Rengen Grassland Experimental Station of the University of Bonn in the Eifel mountains (Germany) about 60 km west of the Rhine river (50°13′N, 6°51′E) at 475 m a.s.l. At the study site, the mean annual precipitation was 811 mm and the mean annual temperature was 6.9 °C (Rengen meteorological station). The soil was a pseudogley (Arens et al., 1958) indicated by temporarily very wet conditions in the turf layer after rainfall in winter and spring and slow vertical rise of capillary water in summer. Before the initial fertilizer application, the extensively grazed area was dominated by *Calluna vulgaris*, *N. stricta,* and *Danthonia decumbens.* Baseline data about the floristic composition of the plots before the first fertilization were not available, but the original sward structure was estimated according to the data available from 1942 to 1944 (Table 1). In 1941 the soil of the study site was cultivated using a grubber in order to create a seed bed and was then reseeded with a mixture of productive grasses and herbs (Table 2). Since then, fertilizer was

Table 1
Dry-matter (DM) contribution of plant species in the biomass samples collected from 1942 to 1944 at the RGE (* values < 1%)

Plant species	Minimum value (%)	Maximum value (%)
Grasses		
Agrostis capillaris	*	7
Avenella flexuosa	2	5
Festuca ovina agg.	*	5
Molinia caerulea	1	6
Nardus stricta	8	18
Danthonia decumbens		5
Carex panicea		1
Carex pilulifera		*
Luzula multiflora		*
Herbs		
Galium saxatile		*
Hieracium spp.		*
Hypericum pulchrum		1
Platanthera bifolia		*
Polygala vulgaris		*
Potentilla erecta	*	3
Dactylorhiza maculata		*
Succisa pratensis		*
Viola canina		*
Shrubs, scrubs		
Genista pillosa	*	2
Calluna vulgaris	52	80
Frangula alnus		*
Quercus robur		*

Table 2
Percentage composition of seed mixture used for reseeding the experimental plots in 1941

Plant species	Percent by weight
Grasses	
Agrostis gigantea	6.25
Bromus inermis	10.0
Cynosurus cristatus	6.25
Dactylis glomerata	12.5
Festuca pratensis	5.0
Festuca rubra agg.	7.5
Phleum pratense	5.0
Poa pratensis agg.	7.5
Trisetum flavescens	7.5
Legumes	
Lotus corniculatus	12.5
Medicago sativa	12.5
Medicago lupulina	2.5
Trifolium pratense	5.0

applied in a single annual dressing every year except for N (two dressings). The field experiment was mown once a year in late summer from 1942 to 1944 and from 1950 to 1961 but not grazed. From 1962 onwards plots were cut twice a year, in late June to early July and in mid-October. As a post-war consequence, the plots were not fertilized, cut, or managed during 1945–1950.

The experiment was arranged in completely randomized blocks with five replications. Each block consisted of five fertilization treatments (Table 3). An unfertilized control plot was added in 1998 on a strip adjacent to the existing plots. This strip has not been fertilized or grazed since 1941 but cut when experimental plots were harvested and sampled. Individual plot size was 3 m × 5 m.

The percentage soil cover of all vascular plant species as well as the cover of the moss layer was visually estimated in each plot. To eliminate edge effects, relevés were taken in the centre of each 3 m × 5 m plot only on an area of 1.8 m × 3.2 m in mid-June 2005. Based on the descriptions of vascular plants in the regional flora (Rothmahler et al., 2000), all species within the study area were *a priori* categorised according to their main traits. We recognized the following functional groups: legumes, herbs, grasses, *Cyperaceae*, short graminoids, short herbs, tall graminoids, and tall herbs. Species reseeded to increase yield and forage quality in 1941 and other species were recognized according to the historical data of the experiment (Schellberg et al., 1999). The nomenclature of the vascular plants followed Kubát et al. (2002).

ASH (*syn.* compressed sward height) was calculated as a mean of 10 measurements performed with a rising plate meter (Sanderson et al., 1999; Correll et al., 2003) within each 1.8 m × 3.2 m plot centre. To reveal the expected relationship between the average height of a particular species and its response to fertilizer treatments, the mean height of each species obtained from the local documented flora (Rothmahler et al., 2000) was weighted according to the total species cover in a particular relevé. This value is called the "potential sward height" (PSH) and has been established by Pavlů et al. (2003). Differences between actual and potential sward height were used as a measure of the so-called "environmental deficiency" in the particular treatments.

Table 3
Amounts of nutrients (kg ha^{-1}) supplied annually in the treatments since 1941 at the RGE

Treatment abbreviation	Applied nutrients (kg ha^{-1})	Nutrient
A	Unfertilized control	
B	CaO = 1000; Mg = 67	Ca
C	CaO = 1052; N = 100; Mg = 67	Ca/N
D	CaO = 1309; N = 100; P_2O_5 = 80; Mg = 75	Ca/N/P
E	CaO = 1309; N = 100; P_2O_5 = 80; K_2O = 160; Mg = 90	Ca/N/P/KCl
F	CaO = 1309; N = 100; P_2O_5 = 80; K_2O = 160; Mg = 75	Ca/N/P/K_2SO_4

2.1. Data analysis

A redundancy analysis (RDA) in the CANOCO 4.5 program (ter Braak and Šmilauer, 2002) was applied to evaluate multivariate plant species data. The RDA was used because data sets were sufficiently homogeneous and environmental variables and co-variables, *e.g.* treatments and blocks, were in the form of categorical predictors (Lepš and Šmilauer, 2003). Further, a Monte Carlo permutation test with 999 permutations was used to reveal if the tested explanatory variables (environmental variables in the CANOCO terminology) had a significant effect on the plant species composition. Results of the multivariate analysis were visualized in the form of a bi-plot ordination diagram created with CanoDraw© software. The percentage of the explained plant species data variability induced by fertilizer treatments was used as a measure of explanatory power.

All uni-variate analyses were performed using STATISTICA 5.0 software (StatSoft©, 1995). One-way ANOVA followed by post hoc comparison using Tukey's test was applied to identify significant differences between treatments for (i) cover of functional groups, (ii) cover of moss layer, (iii) vascular plant species richness, (iv) actual and potential sward height, and (v) differences between sward heights. A regression was used to evaluate the relationship between plant species richness or cover of moss layer and ASH. A correlation analysis was used to evaluate the relationship between actual and potential sward heights.

3. Results

In June 2005, 76 vascular plant species were identified within the study area: 22 grasses, 37 dicotyledonous plants, 7 legumes, 4 sedges, 3 orchids, 2 woody species and 1 woodrush species. The fertilizer treatments explained 62% variability of plant cover data (RDA, F-value = 8.1, P = 0.001). The ordination diagram (Fig. 1) clearly shows the divergence in species composition between the treatments without P fertilizer (A, B, C) and those with P (D, E, F). Treatments fertilized with P all had a similar species composition—triangles marking these treatments are closely located in the diagram. Among those treatments without P, there was also an effect of long-term lime application (treatment A versus treatment B) and an effect of N (treatments A and B versus treatment C). The length and direction of the vectors relating to the individual species in the diagram indicate the associativity of species to the respective treatments.

Alopecurus pratensis, *Arrhenatherum elatius* and *Trisetum flavescens*, all grasses with high yield and forage quality, dominated in treatments D, E, and F. *Lathyrus pratensis*, *Poa trivialis*, *P. pratensis*, *Bromus inermis*, *Trifolium hybridum*, *Anthriscus sylvestris*, *Festuca pratensis*, and *Phleum pratense* also occurred frequently in treatments D, E, and F. Remarkably, these species were not observed in the

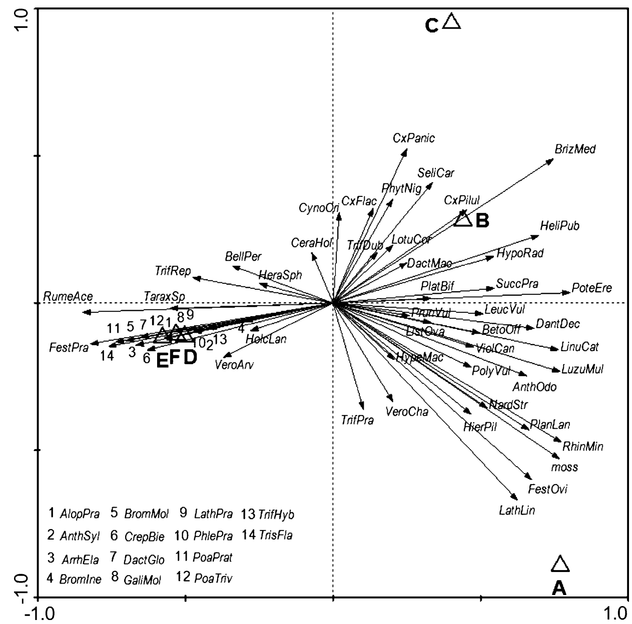

Fig. 1. Ordination diagram showing the result of RDA analysis of plant species composition data. Treatments abbreviations (A–F) are given in Table 3. Species abbreviations: *AlopPra*, *Alopecurus pratensis*; *AnthOdo*, *Anthoxanthum odoratum*; *AnthSyl*, *Anthriscus sylvestris*; *ArrhEla*, *Arrhenatherum elatius*; *BetoOff*, *Betonica officinalis*; *BrizMed*, *Briza media*; *BromIne*, *Bromus inermis*; *BromMol*, *Bromus hordaceus*; *CeraHol*, *Cerastium holosteoides*; *CrepBie*, *Crepis biennis*; *CxFlac*, *Carex flacca*; *CxPanic*, *Carex panacea*; *CxPilul*, *Carex pilulifera*; *CynoCri*, *Cynosurus cristatus*; *DactGlo*, *Dactylis glomerata*; *DactMac*, *Dactylorhiza maculate*; *DantDec*, *Danthonia decumbens*; *FestOvi*, *Festuca ovina*; *FestPra*, *Festuca pratensis*; *GaliMol*, *Galium mollugo*; *HeliPub*, *Helictotrichon pubescens*; *HierPil*, *Hieracium pilosella*; *HolcLan*, *Holcus lanatus*; *HypeMac*, *Hypericum maculatum*; *HypoRad*, *Hypochaeris radicata*; *LathLin*, *Lathyrus linifolius*; *LathPra*, *Lathyrus pratensis*; *LeucVul*, *Leucanthemum vulgare*; *LinuCat*, *Linum catharticum*; *ListOva*, *Listera ovata*; *LotuCor*, *Lotus corniculatus*; *LuzuMul*, *Luzula multiflora*; *NardStr*, *Nardus stricta*; *PhlePra*, *Phleum pratense*; *PhytNig*, *Phyteuma nigrum*; *PlanLan*, *Plantago lanceolata*; *PlatBif*, *Platanthera bifolia*; *PoaPrat*, *Poa pratensis*; *PoaTriv*, *Poa trivialis*; *PolyVul*, *Polygala vulgaris*; *PoteEre*, *Potentilla erecta*; *PrunVul*, *Prunella vulgaris*; *RhinMin*, *Rhinanthus minor*; *RumeAce*, *Rumex acetosa*; *SeliCar*, *Selinum carvifolia*; *SuccPra*, *Succisa pratensis*; *TaraxSp*, *Taraxacum* spp.; *TrifDub*, *Trifolium dubium*; *TrifHyb*, *Trifolium hybridum*; *TrifPra*, *Trifolium pratense*; *TrifRep*, *Trifolium repens*; *TrisFla*, *Trisetum flavescens*; *VeroArv*, *Veronica arvensis*; *VeroCha*, *Veronica chamaedrys*; *ViolCan*, *Viola canina*.

unfertilized control. Plant species present in all treatments but much more abundant in treatments D, E, and F were *Cardamine pratensis*, *Crepis biennis*, *Dactylis glomerata*, *Rumex acetosa*, *Taraxacum* sp., *Trifolium repens*, and *Holcus lanatus*. *Heracleum sphondylium* was present in treatments C, E, and F, whereas *Bellis perennis* and *Galium mollugo* were found only in treatments C, D, E, and F. *Bromus hordaceus* was present with highest cover in treatments D, E, and F and sporadically occurred in treatment B. *Cerastium holosteiodes*, *Cynosurus cristatus*, *Trifolium dubium*, *T. pratense*, and *Veronica chamaedrys* were present in all plots regardless of fertilizer treatment. *Lotus corniculatus* was present in all plots but most abundant in treatment B. *Hypericum maculatum* had highest cover in A and *Selinum carvifolia* in B and C treatments; both species were completely missing in treatment F. *Veronica arvensis* was recorded in A, D, E, and F treatments.

Briza media was the dominant short grass in A, B, and C treatments. Further species present only in treatments without P fertilizer were *Betonica officinalis*, *Carex palescens*, *C. panicea*, *Dactylorhiza maculata*, *D. decumbens*, *Festuca ovina*, *Helictotrichon pubescens*, *Hypochoeris radicata*, *Linum catharticum*, *Listera ovata*, *Luzula multiflora*, *N. stricta*, *Polygala vulgaris*, *Potentilla erecta*, *Platanthera bifolia,* and *Succisa pratensis*. *Lathyrus linifolius*, occurring only in A and B treatments, was the dominant legume in A and the sedges *C. panicea* and *C. flacca* were the dominant species in the C treatment. *Anthoxanthum odoratum*, *Leucanthemum vulgare*, *Plantago lanceolata*, *Rhinanthus minor*, and *Viola canina* were species with highest cover in treatments without P application and only sporadically occurring in those with P addition. *Prunella vulgaris* occurred in A, B, C, and D treatments.

The ANOVA analyses of functional groups data are given in Table 4. A significant treatment effect on the total cover of legumes was detected, but no differences among treatments were revealed by *post hoc* comparisons (Fig. 2). The functional group of herbs achieved the highest percentage cover in treatments A and B, with treatment A significantly differing from treatments C, D, and E. Differences among treatments B and C and E on the other hand were significant. The functional group of grasses achieved the highest percentage cover with NPK-fertilization.

Cyperaceae were dominant only in treatment C, short graminoids prevailed in treatment C followed by treatments B and A (Fig. 2). In contrast, the cover of short graminoids was negligible in treatments D, E, and F. Short herbs obtained highest total cover in A and B treatments and significantly differed from the other treatments. By contrast, tall graminoids prevailed in treatments D, E, F, and

Table 4
Results of the ANOVA analyses of several sward structure variables in the RGE

Tested variable	*F*-value	*P*-value
Legumes	3.9	0.009
Herbs	7.5	<0.001
Grasses	5.4	0.001
Cyperaceae	12.4	<0.001
Short graminoids	74.4	<0.001
Short herbs	42.2	<0.001
Tall graminoids	51.6	<0.001
Tall herbs	6.0	<0.001
Other species	4.9	0.003
Sown species	9.9	<0.001
Cover of moss layer	32.1	<0.001
Number of species	3.8	0.012
Actual sward height (ASH)	97.1	<0.001
Potential sward height (PSH)	36.8	<0.001
Difference between ASH and PSH	10.1	<0.001

Degrees of freedom were five in all analyses.

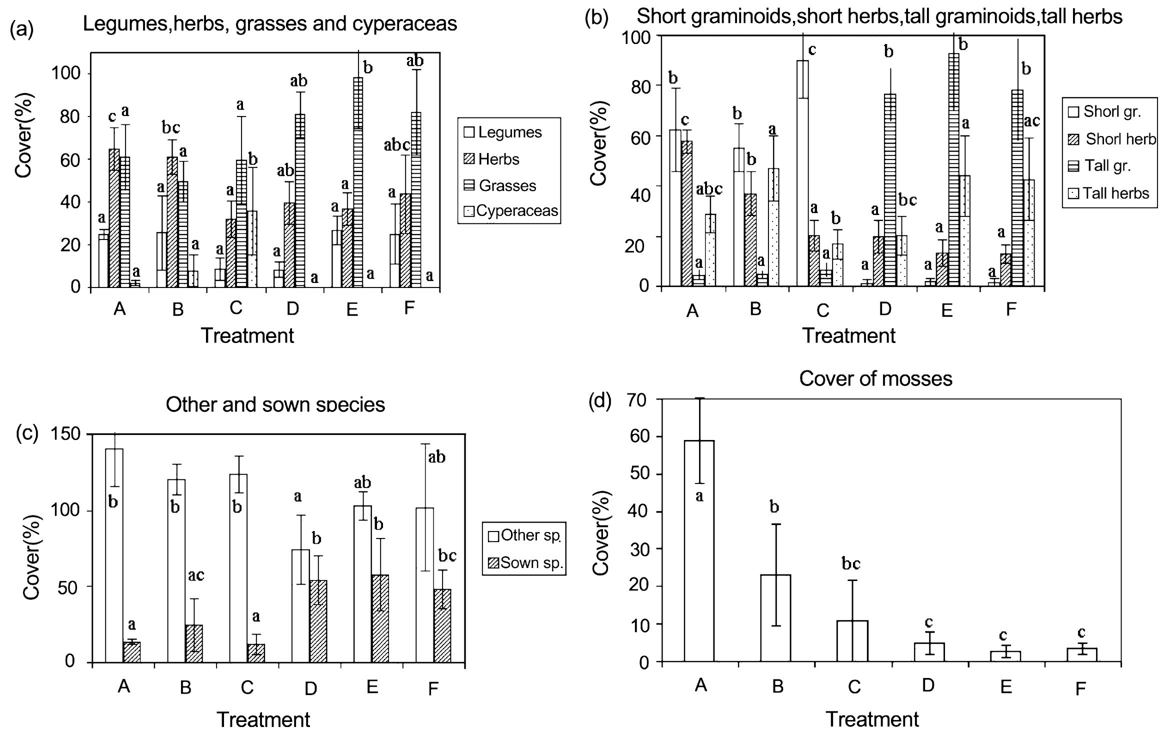

Fig. 2. Percentage cover of different functional groups in the six treatments investigated in the RGE. Treatment abbreviations are in accordance with Table 3. Vertical lines indicate S.D.

significantly differed from treatments A, B, and C. Although total cover of tall herbs varied among treatments, no response to a particular treatment or group of treatments was found. Other species not sown in 1941 had higher total cover in treatments A, B, and C in comparison with treatments D, E, and F; the opposite was true for sown species.

The moss layer increased in the unfertilized control plots where almost 60% cover was recorded, whereas in treatments D, E, and F the moss layer occurred sporadically. The cover of mosses significantly decreased with an increase in ASH with the threshold value of ASH at approximately 20 cm ($R = -0.62$; $F = 17.2$; $P < 0.001$; Fig. 4).

The overall species richness of vascular plants ranged from 24 to 39 per 1.8 m × 3.2 m plot size depending on fertilizer application. Species richness was highest in treatment B, which differed from treatments D and F (Fig. 3). There was a clear trend towards a decrease of species richness with increased ASH but the relationship was not linear ($R = -0.44$; $F = 6.7$; $P = 0.015$; Fig. 4).

Large differences in ASH occurred among fertilizer treatments. In the unfertilized control plot, the mean ASH was 10 cm, whereas ASH was more than 40 cm in treatments E and F. Treatments A, B, and C did not differ in ASH, but differed from treatments D, E, and F (Fig. 3). Similarly to ASH, the potential sward height (PSH) was lowest in treatments A, B, and C, which differed from treatments D, E, and F. ASH and PSH were positively correlated ($R = 0.88$; $F = 22.3$; $P < 0.001$), indicating that an increase of ASH was not due to an increase in individual height of particular plant species, but predominately due to a change in plant species composition in plots with more complex fertilization. Differences between ASH and PSH were strictly dependant on the type of fertilization (Table 4, Fig. 3). Generally, ASH was lower than PSH in treatments A, B, and C (Fig. 3). The results indicate that plant species in treatments A, B, and C exhibited generally lower actual heights as compared to the mean height given in the local flora.

4. Discussion

The observed strong explanatory power of fertilizer treatments (62%) recorded in RGE contrasts with results obtained from the Černíkovice experiment established on a highly productive alluvial meadow near Prague (Czech Republic) in 1966 (Velich and Mrkvička, 1993). A similar experiment as RGE has been established in the Beskid Sadecki Mts. (Poland) on oligotrophic *N. stricta* and *F. rubra* dominated grassland in 1968; although the explanatory power of treatments has not been observed, a strong effect of fertilizer application on plant species composition has been published (Galka et al., 2005).

No obvious trend occurred for legumes in relation to P application in RGE. This was surprising and it differs from observations in several other experiments (Jančovič et al., 1999; Velich and Mrkvička, 1993). The first explanation could be a strong variation in the presence of legume species in the respective treatments: *L. pratensis* and *T. hybridum* were abundant in plots where P was applied, whereas *L.*

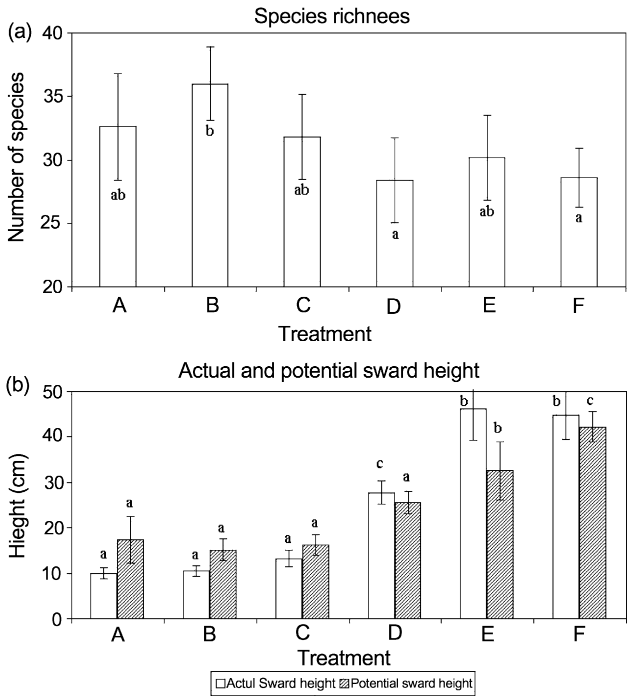

Fig. 3. Species richness (a) and actual and potential sward height (b). Treatment abbreviations are in accordance with Table 3. Vertical lines indicate S.D.

linifolius was prevalent in the unfertilized control. Secondly, P was applied together with N in this experiment but not as a sole treatment like in many other long-term studies where a positive effect of P on legumes was detected. *L. pratensis* is known to grow in tall canopies dominated by grasses as well (Grime et al., 1988; Hejcman et al., 2005a), but was probably less competitive in a P limited environment in A, B, and C treatments.

The dominance of short graminoids and herbs in the A, B, C treatments and of tall graminoids in D, E, and F treatments supports the conclusion of Lepš (1999), that plant height is the best predictor of species response to fertilization. The significance of mean species height in response to fertilization was obvious from PSH as well, but only partly from ASH as this parameter was affected by environmental conditions in the investigated treatments.

One novel finding of the present study was that species not reseeded in 1941 were dominant in the A, B, and C treatments whereas sown species were found mainly in fully fertilized treatments. It remains unclear whether the sown species originated from the seed mixture applied to the sward in 1941. Many individuals of these species were present in the neighbouring semi-natural grasslands, but a genetic analysis has not been performed yet. The only exception was *B. inermis* as it was absent in the surrounding grasslands. Thus, it was hypothesized that reseeding of low productive grassland by species of high forage yield and quality has long-term effects on plant species composition only with continuous application of adequate fertilizer nutrients.

The moss layer increased in treatments without P application and was negatively affected by sward height. The main effect of fertilization on the moss cover was therefore probably an indirect one through competition of vascular plants causing a decrease in light availability as demonstrated in the PGE by Virtanen et al. (2000) as well as in montane ecosystems where sedge and grass cover increased with N enrichment (van der Wal et al., 2005). In contrast to Virtanen et al. (2000), the decrease of moss cover with increasing grassland productivity was not linear, but curvi-linear in the present study. A rapid decline in cover of mosses with increasing height at low ASH was recorded, but above a threshold (ASH approximately 20 cm), there was no extra effect of ASH on cover of mosses. Above ASH 20 cm, light availability was relatively constant and so was the moss cover.

The highest species richness found on limed plots in the RGE was probably caused by an improvement of originally acid soils increasing the environmental suitability for a wider range of plant species. This agrees with Gough et al. (2000) who recorded higher species richness on soils with a higher pH. In the RGE, the addition of P was the most

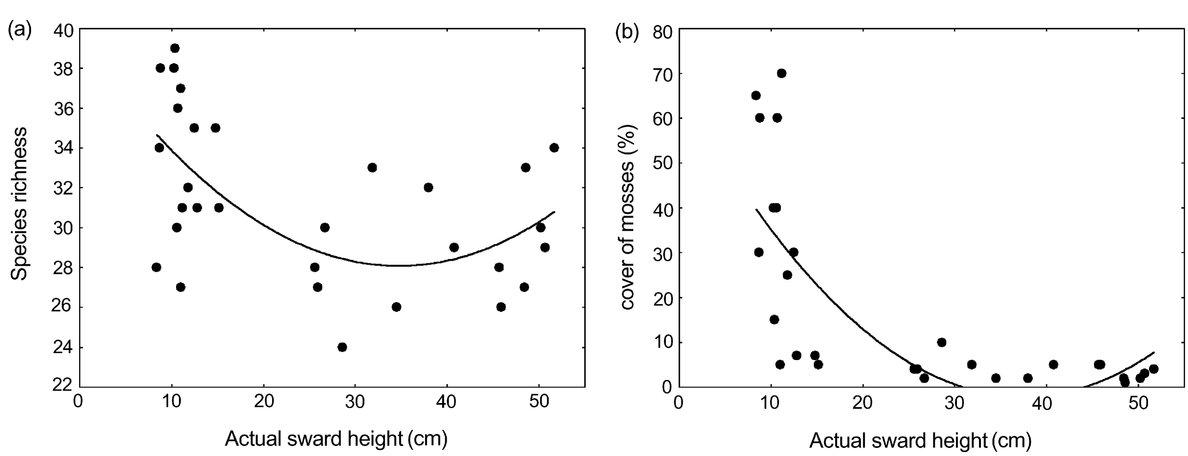

Fig. 4. Species richness (a) and cover of mosses (b) as a function of actual sward height.

responsible for a decrease in species richness. When N was not limiting, P was the nutrient limiting growth, and hence its addition caused a strong shift in floristic composition. A limitation of growth by P availability was obvious from soil analyses published by Schellberg et al. (1999). In 1993, P concentration was 1.9, 1.9, 12.3, 11.5, and 11.5 mg $\times$ 100 g in B, C, D, E, and F treatments, respectively. Further, the addition of K in treatments E or F had no effect on species richness.

The lowest species richness occurred in plots where a combination of N and P was applied. The limited effect of K agrees with results of Crawley et al. (2005) from the PGE or with conclusions of Kayser and Isselstein (2005). In the PGE, species typical of mountainous grasslands disappeared from plots receiving N, P, and K fertilizers. The strong impact of P on sward structure and plant species richness in RGE was consistent with results of Janssens et al. (1998), Wassen et al. (2005) or Bullock and Pywell (2005) who concluded that conservation management of grasslands should focus on reducing P rather than N availability.

The floristic composition in the unfertilized control and in the P treatments sharply contrasted with the remaining plots indicating that P generally limits the persistence and dominance of highly productive and tall growing grasses such as *A. pratensis*, *A. elatius,* or *T. flavescens*. In this respect, the RGE is unique due to the occurrence of three orchid species in treatments without P application. These species were not able to withstand plant competition under a two cut system in plots where sward height and therefore productivity was high. Further, the occurrence of *D. maculata* and *P. bifolia* in the treatment C fertilized with N was surprising. Both species were able to tolerate long-term application of ammonium nitrate. The reason for their absence in completely fertilized plots may be due to the intolerance of higher phosphorus availability but, most probably, due to the competitive exclusion caused by the suppression of highly productive species.

The sedges *C. panicea* and *C. flacca* dominated in the C treatment and so were obviously promoted by long-term N application which resulted in lowest P concentration in the soil (data not shown). Such a positive effect of N on the occurrence of *C. panicea* was in agreement with results of van der Hoek et al. (2004) from species-rich fen meadows in the Netherlands. According to Boeye et al. (1997), Grime et al. (1988), and Gusewell (2005), *C. panicea* is a slow growing stress-tolerant sedge well disposed to take up P, and is hence not restricted by a low P supply as are tall growing grasses.

The dominance of *B. media* and the sedges and the low plant cover of tall growing grasses in the C treatment in RGE were caused by lowest P availability. In reverse, the positive effect of N addition on tall grasses has often been shown in long-term experiments (Jančovič et al., 1999; Galka et al., 2005) but can only be expected if P is not limiting. In the present experiment, *N. stricta*, a characteristic slow growing species of acid low productivity grasslands (Krahulec, 1985; Hejcman et al., 2005b), was able to survive not only in the unfertilized control but also in treatments B and C, supporting the fact that the calcifuge *N. stricta* is able to survive long-term liming even if this is not accompanied by sward productivity and competition for light (Hejcman et al., 2007).

The RGE provides one important conclusion in view of the conservation of low productivity grassland. It was often argued that N enrichment is detrimental to vascular plant diversity, but the RGE showed that this must not necessarily be the case if N fertilizer application is not accompanied by another limiting nutrient like P. The presence of three orchid species as well as *B. media* and short sedges recorded in the N fertilized plots and the absence of tall grasses in the same environment support this conclusion.

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