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The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application

Michal Hejcman[a,b,*], Lenka Sochorová[a], Vilém Pavlů[a,b], Jan Štrobach[b], Michael Diepolder[c], Jürgen Schellberg[d]

[a] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 1176, Suchdol, Prague 6 CZ-165 21, Czech Republic
[b] Crop Research Institute, Drnovská 507, Ruzyně, Prague 6 CZ-161 06, Czech Republic
[c] Institute for Organic farming, Soil and Resource Management, Bavarian State Research Centrum for Agriculture , Lange Point 12, Freising D-85354, Germany
[d] Institute of Crop Science and Resource Conservation, University of Bonn, Katzenburgweg 5, Bonn D 53115, Germany

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ABSTRACT

The Steinach Grassland Experiment (SGE) is probably the oldest still-running grassland fertilizer experiment in Continental Europe. It was established on an alluvial *Alopecurus pratensis* meadow in southeast Germany in 1933. The aim of this study was to provide detailed information on this experiment concerning the effects of decades-long nitrogen, phosphorus, potassium, and calcium application (46 treatments in total) on (1) soil chemical properties, (2) sward height, (3) plant species composition, and (4) species richness of vascular plants derived from a field survey in spring 2008.

(1) A steep gradient of soil properties was recorded, namely plant-available (Mehlich III) P (14–161 mg kg^{-1}), K (82–1018 mg kg^{-1}) and Ca (532–3336 mg kg^{-1}) concentrations, C:N ratio (6.9–10.4) and pH (H_2O) (4.7–7.0).

(2) Compressed sward height in the third week of May 2008 ranged from 11 cm in the control to 47 cm in the limed plot with high N, P, and K application.

(3) Although fertilizer application altered the plant species composition, diversification of plant communities was not as high as in the case of fertilizer application on low productive soils in other experiments. This was caused by sufficient nutrients enabling the survival of species adapted to high nutrient availability, even in the unfertilized control. The species composition in productive alluvial grasslands is substantially less affected by fertilizer application than in low productive grasslands.

(4) Long-term fertilizer application negatively affected the species richness of vascular plants directly by soil acidification and indirectly by an increase in sward height. The extent to which N application negatively affected species richness was dependent on whether N was applied alone or in combination with other nutrients and whether N application acidified the soil.

1. Introduction

In Europe, there are many still-running decades-long grassland fertilizer experiments, but results from these experiments have rarely been published in international scientific journals (see Hejcman and Schellberg (2009) for an overview of 10 experiments). To our knowledge, there are only six experiments that have been running for at least 40 years with well-published results, of which the Park grass experiment established on *Lolium perenne* grassland in 1856 (Crawley et al., 2005; Rothamsted Research, 2006; Silvertown et al., 2006) is the best known, followed by the Palace Leas Hay Meadow Plots Experiment established on *Agrostis capillaris* grassland in 1896 (Hopkins et al., 2011), both in the UK. Recently, an analysis of plant species richness pattern was published from the Wageningen Experiment established on an *Arrhenatherion* hay meadow in the Centre of the Netherlands in 1958 (Pierik et al., 2011). The next well-published experiment is the Rengen Grassland Experiment established on low productive *Nardus stricta* grassland in SW Germany in 1941 (Schellberg et al., 1999; Hejcman et al., 2010), the Černíkovice Experiment established on alluvial *Alopecurus pratensis* meadow in the Centre of the Czech Republic in 1965 (Honsová et al., 2007; Hrevušová et al., 2009) and the Vel'ká Lúka experiment established on an alluvial *A. pratensis* meadow in 1961 in the Centre of Slovakia (Vargová

* Corresponding author: Tel.: +420224382129.
E-mail address: hejcman@fzp.czu.cz (M. Hejcman).

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et al., 2012). Decades-long fertilizer experiments are an excellent source of knowledge on complex interactions between fertilizer regime, soil chemical properties, plant species composition, productivity and forage quality (Silvertown et al., 1994; Liu et al., 2010; Pavlů et al., 2012). Each experiment is unique and all experiments together cover a wide range of environmental conditions, such as climate and soil, management, including the fertilizer regime and the frequency of defoliation, as well as the resulting productivity and forage quality.

In Europe, eutrophication mainly through the application of mineral nitrogen (N) and phosphorus (P) fertilizers is considered to be mostly responsible for the loss of species-rich grasslands (Janssens et al., 1998; Wellstein et al., 2007; Klaus et al., 2011a, 2013). In studies performed on grasslands at the landscape scale, management practices combined with fertilizer application are often insufficiently known and hence, the effects of different fertilizer regimes on plant species composition can hardly be identified correctly. In addition, changes in fertilizer regimes during the last fifty years have frequently also been connected with changes in cutting date and frequency, hampering the study of the effect of fertilizer application on plant species composition (Hopkins and Wilkins, 2006). In contrast to this, long-term fertilizer experiments under controlled and constant management allow precise identification of the real effects of fertilizer application on plant species composition.

One of the oldest still-running grassland fertilizer experiments in Continental Europe is the Steinach Grassland Experiment (SGE) established on an alluvial *A. pratensis* meadow in SE Germany (Bavaria) in 1933 (Rieder, 1988, 1989; Diepolder et al., 2005). Although treatments are not well replicated as in all similar long-term experiments established before the 1940s, a unique and rewarding feature of this experiment is the large number of fertilizer treatments applied (46 altogether), which generate a wide gradient of soil chemical properties, plant species composition and herbage production. To our knowledge, a similar large number of fertilizer treatments as in the SGE is only present in the Park Grass Experiment (Silvertown et al., 2006).

In the period from 1985 to 2001, aboveground biomass production in the SGE ranged from 4.1 $t\,ha^{-1}$ in a treatment with an application of 174 $K\,ha^{-1}$ to 10.8 $t\,ha^{-1}$ in a treatment with an application of dung water (66 $m^3\,ha^{-1}$) supplemented with 44 kg $P\,ha^{-1}$ in the form of Thomas slag, or in a treatment with mineral application of 120 kg N, 44 kg P, and 174 kg $K\,ha^{-1}$ (Diepolder et al., 2005). Another peculiarity of the SGE is the combination of organic fertilizers (dung water–mixture of urine and technological water with nutrients leached from faeces) together with synthetic chemical fertilizers (Thomas slag and lime) in some treatments, which is rarely used in long-term experiments. Details of the experimental layout and a detailed analysis of the effects of long-term fertilizer treatments on the relationship between soil chemical properties, plant species composition and species richness have never been presented to the international scientific community from this unique experiment. After a field survey of all treatments performed in 2008, we concluded that it is necessary to present an inventory of the state of the experiment, explore major drivers and effects and then assess the value of its particular combinations of fertilizer treatments for further studies. We therefore suspected that the analysis of soil chemical properties and plant species composition would be most promising in this respect and that historical herbage yield data should be considered in subsequent analyses. In this initial report, we decided to focus on the following questions:

(1) What is the effect of decades-long N, P, and K application on soil chemical properties, in particular pH (H_2O), total N, organic C and concentrations of plant-available nutrients (P, K, Ca, Mg)?
(2) How do nutrients (either alone or in combination) affect productivity of the vegetation as estimated via compressed sward height?
(3) How do different fertilizer treatments affect plant species composition of the sward? We were especially interested in understanding the impact of nutrient application on the abundance of all species, not only dominant ones. As we had observed a complete replacement of species along nutrient gradients already in other long-term experiments on poorer soils (Hejcman et al., 2007, 2012; Semelová et al., 2008), we wanted to study whether N, P, and K application affects the plant species composition similarly on a naturally richer alluvial soil, which is well supplied with water.
(4) Which of the nutrients most effectively influence the species richness of vascular plants and how is species richness related to sward height and soil chemical properties? We hypothesised that species richness in well-fertilized treatments is significantly lower than in unfertilized treatments.

2. Material and methods

2.1. Study site

The long-term Steinach grassland experiment (SGE) was established in 1933 on an alluvial meadow near the village of Steinach (Straubing-Bogen, Bavaria, Germany, 48°57’N, 12°36’E). The altitude of the study site is 344 m a.s.l., the mean annual precipitation is 840 mm and the mean annual temperature is 7.3 °C (Steinach meteorological station). The soil type is a pseudogley and in 2008, the pH(H_2O) was 5.4 in the upper 10 cm layer in the control without any fertilizer application and liming. The depth of the underground water table varied between 1 and 2 m. According to phytosociological nomenclature (Botta-Dukát et al., 2005), the vegetation of the experimental meadow was classified as *Deschampsion cespitosae* (syn. *Alopecurion pratense*).

2.2. Experimental design

In 1933, five fertilizer treatments plus a control (group I to V, see Appendix A) were established without any replication, on plots 300 m^2 in size (Rieder, 1989). Subsequently, the experimental plots were divided and thus the number of fertilizer treatments increased. The first division was performed in 1954 (10 fertilizer treatments), the second in 1965 (20 treatments), and the last in 1986 (46 treatments). Some modifications to the fertilizer regime, particularly an increase in N application rates, were performed in several treatments in 1971. The history of the fertilizer regimes in each plot is given in Appendix A.

As a result of the reorganization of treatments, the SGE today represents three neighbouring strips (Fig. 1), which are regularly cut three times a year. Each strip is divided into 25 plots (75 monitoring plots in total). Standard individual plot area is now 18.75 m^2 (3.75 × 5 m) in 60 plots. The area of 15 additional plots is 12.5 m^2 (2.5 × 5 m).

Six control plots without any fertilizer and lime input (orange in Fig. 1) now exist, plus 69 fertilized plots. In these fertilized plots, 25 were limed in 1986 (blue) with 8 $t\,ha^{-1}$ of dolomite lime (Diepolder et al., 2005), whereas 44 plots still remain without any direct liming (green). Treatments include limed plots with and without dung water, a combination of mineral P and dung water, liming only, $(NH_4)_2SO_4$, a combination of $Ca(NO_3)_2$, $(NH_4)_2SO_4$ and NH_4NO_3 (amount of applied N was 60, 50, and 50 kg $N\,ha^{-1}$) and a combination of mineral N, P, and K fertilizers. The fertilized plots without any direct liming include plots with dung water only, a combination of P and dung water and a combination of mineral N, P, and

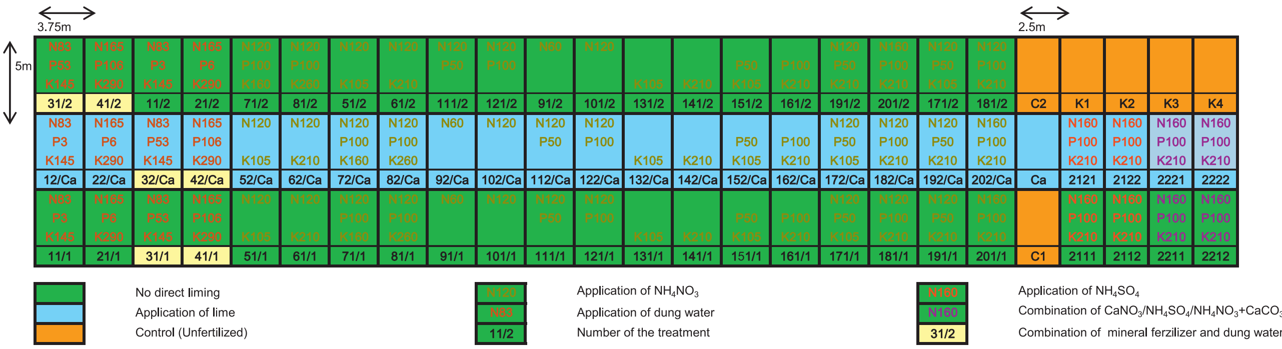

Fig. 1. Spatial arrangement of treatments in the Steinach Grassland Experiment. Numbers indicate the amount of annually applied N, P, and K (kg ha^{-1}). Black numbers and letters in the bottom of each plot are original marks of monitoring plots. Green indicates unlimed plots with application of N, P, and K fertilizers. Blue indicates plots directly limed in 1986 and with application of N, P, and K fertilizers. Orange indicates control plots without any fertilizer input. Brown letters and numbers indicate dung water application, green indicates mineral N, P, and K fertilizer application, red indicates the application of mineral N in the form of $(NH_4)_2SO_4$ and violet indicates the application of N as a combination of three different mineral fertilizers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

K fertilizers. Some plots received $(NH_4)_2SO_4$, or a combination of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, and NH_4NO_3.

Annual application rates of N were 60, 120, and 160 kg ha^{-1} in mineral fertilizer treatments and 116 and 232 kg N ha^{-1} in organic fertilizer treatments. Annual application rates of P were 22 and 44 kg ha^{-1} in mineral fertilizer treatments applied as Thomas slag and 4 and 8 kg ha^{-1} in organic fertilizer treatments. Thomas slag also supplied the soil with Ca at an annual rate of 107 and 214 kg Ca ha^{-1} in treatments with an annual application of 22 and 44 kg P ha^{-1}, respectively. Annual application rates of K were 87, 133, 174, and 216 kg ha^{-1} in mineral fertilizer treatments applied as a mixture of KCl and $MgSO_4$ and 232 and 465 kg ha^{-1} in organic fertilizer treatments. Mineral P and K fertilizers were applied in the autumn and all N fertilizers during the vegetation season. The amount of Ca and Mg applied in the form of dolomite lime (53% CaO and 25% MgO) in 1986 was 3032 and 1206 kg ha^{-1}, respectively, in limed treatments (Diepolder et al., 2005).

2.3. Soil chemical properties

In mid-April 2008, 10 separate soil samples per individual experimental plot (see Fig. 1 and Appendix A for list of all plots) were taken from a 0 to 10 cm layer and thoroughly mixed to obtain one representative soil sample per plot. The soil samples were air-dried, ground in a mortar, and sieved to 2 mm after removal of living roots. All analyses were performed at the Eko-Lab, Žamberk (http://www.ekolab.zamberk.cz), an accredited Czech national laboratory, using the Mehlich III extraction to predict plant-available Ca, K, Mg and P (Mehlich, 1984). The determination of organic C (C_{org}) was performed spectrophotometrically after oxidation in a mixture of $K_2Cr_2O_7$ solution with H_2SO_4, at 135 °C. The determination of total N (STN) was performed using a TruSpec f. Leco instrument, where the soil sample was combusted at 950 °C. Soil pH(H_2O) was measured in a suspension of 10 g dry soil mixed with 50 mL distilled H_2O.

2.4. Compressed sward height

Compressed sward height (CSH) measurements were performed with a rising plate meter (Castle, 1976) in the third week of May 2008. The mean CSH was calculated from 10 single measurements within the central part of each plot.

2.5. Plant species composition

In the third week of May 2008, the percentage cover of all vascular plant species was visually estimated in each plot. Based on description of vascular plants in the local flora (Rothmaler et al., 2000), all plant species within the study area were *a priori* classified according to their main traits. We recognized five functional groups: legumes, tall graminoids (average high ≥0.5 m), short graminoids (bellow 0.5 m), and similarly tall forbs and short forbs (see Appendix B). To obtain total cover of functional groups, the cover of individual species belonging to a particular group were totaled. To eliminate edge effects, relevés were taken only in the centre of each plot in an area of 14.6 m^2 (60 plots) and 9 m^2 (15 plots). As species richness can be affected by sampling area (Crawley et al., 2005; Hejcman et al., 2010), smaller plots (six control and nine fertilized plots) were omitted from the analyses. Species richness was determined by counting the number of species in each relevé.

2.6. Data analysis

We used simple linear regressions in STATISTICA 8.0 software (Statsoft, Tulsa, AZ, USA) to analyse the relationship between the amount of applied nutrients, soil chemical properties, CSH, species richness and cover of functional groups. We used this simple analytical approach because of the absence of proper replications of individual treatments in the SGE. However, such a large number of different fertilizer treatments created a considerably high gradient of soil chemical properties and sward heights. Thus, we analysed these gradients and applied a regression approach. Detrended Correspondence Analysis (DCA) and Redundancy Analysis (RDA) in the CANOCO 4.5 program (ter Braak and Šmilauer, 2002) were used to evaluate the plant species composition data based on the cover estimates described above. In addition, a Monte Carlo permutation test with 999 permutations was used to reveal whether the tested explanatory (environmental) variables (amount of applied nutrients, soil chemical properties and CSH) significantly affected the plant species composition data. The results of DCA and RDA analyses were visualised in the form of ordination diagrams constructed by the CanoDraw program. The percentage of plant species data variability explained by the environmental variables was used as a measure of their explanatory power.

3. Results

3.1. Soil chemical properties

Soil pH(H_2O) ranged from 4.7 in plot number 2112 ($N_{160}P_{44}K_{174}$–numbers indicate the amount of applied N, P, and K in kg ha^{-1}), with the application of $(NH_4)_2SO_4$ and in plot number 61/2 ($N_{120}P_0K_{174}$), to a value of pH 7 in the limed plot

Table 1
Soil chemical properties in a selection of the most contrasting plots. For plot abbreviations see Fig. 1. Plot C—mean values from all control plots without fertilizer input. The abbreviation "Ca" in plot numbers indicates lime application in 1986. Plots with applications of mineral P were indirectly limed by Thomas slag.

	Experimental plot									
	C	141/1	162/Ca	31/2	52/Ca	61/2	192/Ca	21/1	2112	2222/Ca
N ($kg\,ha^{-1}$)	0	0		116	100	120	120	232	160	160
P ($kg\,ha^{-1}$)	0	0	44	26			22	8	44	44
K ($kg\,ha^{-1}$)	0	174	174	232	87	174	174	485	174	174
SN_{tot} ($g\,kg^{-1}$)	3.6	3.6	2.6	2.9	3.1	3.3	2.5	3.9	5.0	3.2
SC_{org} ($g\,kg^{-1}$)	29	34	19	20	26	26	19	33	44	23
C/N	8.2	9.6	7.4	6.9	8.2	7.9	7.4	8.5	8.7	7.2
pH(H_2O)	5.1	5.2	6.7	5.9	5.5	4.7	6.4	5.9	4.7	6.5
SK ($mg\,kg^{-1}$)	135	869	214	173	135	287	170	1018	238	180
SP ($mg\,kg^{-1}$)	26	24	161	36	14	18	53	35	103	73
SMg ($mg\,kg^{-1}$)	201	218	386	164	321	164	360	222	190	388
SCa ($mg\,kg^{-1}$)	1506	565	2150	1689	1377	532	2007	1155	1153	2474

N, P, and K–amount of annually applied N, P, and K. SN_{tot}—total nitrogen content in the soil; SP, SK, SCa, and SMg—plant-available concentrations of P, K, Ca, and Mg in the soil; SC_{org}—content of organic carbon in the soil.

number 122/Ca ($N_{120}P_{44}$) with the application of NH_4NO_3 (see Table 1 for the pH in other selected plots). The mean pH value was 5.7 in plots without direct liming in 1986 and 6.1 in plots with direct liming in 1986, with significant differences between them (one-way ANOVA; DF = 1; F = 10.223; P = 0.002). As expected, there was a significant positive effect of direct liming performed in 1986 on soil pH and on the concentration of plant-available Ca in the soil (Table 2, analyses 29 and 31).

The content of organic C ranged from 18.8 $g\,kg^{-1}$ in the limed plot number 192/Ca ($N_{120}P_{22}K_{174}$) to 44.5 $g\,kg^{-1}$ in plot number 2111 ($N_{160}P_{22}K_{174}$) receiving $(NH_4)_2SO_4$. There was a significant negative relationship between the pH value and the content of organic C in the soil (Table 2, analysis 40). From the whole data set, we did not identify any effect of N and P application on organic C in the soil (Table 2, analyses 2 and 11).

The content of total N in the soil ranged from 2.5 in plot number 192/Ca to 5.0 $g\,kg^{-1}$ in plot number 2112 (Table 1). The amount of applied N, either organic or chemical-synthetic, had no effect on the content of total N in the soil (Table 2, analysis 1).

The C:N ratio of soil organic matter ranged from 6.9 in plot number 31/2 ($N_{116}P_{26}K_{232}$) with a combination of mineral P and dung water application, to 10.4 in the control plot K2 ($N_0P_0K_0$). The plant-available P concentration in the soil ranged from 14 $mg\,kg^{-1}$ in limed plot number 52/Ca ($N_{120}K_{87}$) to 161 $mg\,kg^{-1}$ in limed plot number 162/Ca ($P_{44}K_{174}$). There was a strong and significant positive effect of P application on the P concentration in the soil (Table 2, analysis 10).

The plant-available K concentration in the soil ranged from 82 $mg\,kg^{-1}$ in limed plot number 122/Ca ($N_{120}P_{44}$) to 1018 $mg\,kg^{-1}$ in plot number 21/1 ($N_{232}P_8K_{465}$) with dung water application. There was a strong and significant positive effect of K application on K concentration in the soil (Table 2, analysis 19).

The plant-available Ca concentration in the soil ranged from 532 $mg\,kg^{-1}$ in plot number 61/2 ($N_{120}K_{174}$) to 3336 $mg\,kg^{-1}$ in plot number 121/1 ($N_{120}P_{44}$).

3.2. *Compressed sward height*

The CSH ranged from 11 cm in the control plots ($N_0K_0P_0$) and in plot number 141/1 (K_{174}) to 47 cm in limed plots number 2222 and 2221 ($N_{160}P_{44}K_{174}$) with a combination of all synthetic chemical N fertilizers (Appendix B). The CSH was significantly and positively affected by N, P, and K application as well as by plant-available concentrations of P, K, and Ca in the soil (Table 2, analyses 3, 12, 21 and 13, 23, 33). In contrast, there was no effect of Ca application by liming in 1986 or of total N content in the soil on CSH (Table 2, analyses 32 and 4).

Table 2
Results of linear regression analyses of nutrient application on the content of nutrient and pH in the upper 10 cm soil layer and on compressed sward height.

Analysis	Ind. var.	Dep. var.	Equation	R	P value
1	N	SNtot	$y = 3232 - 0.3248x$	−0.04	0.750
2	N	SC_{org}	$y = 2.66 + 0.0003x$	0.03	0.811
3	N	CSH	$y = 19.0 + 0.0797x$	0.49	<0.001
4	SNtot	CSH	$y = 37.7 - 0.0033x$	−0.16	0.193
5	SNtot	TG	$y = 63.9 - 0.006x$	−0.11	0.365
6	SNtot	SG	$y = -14.2 + 0.0136x$	0.36	0.003
7	SNtot	L	$y = 25.2 - 0.0036x$	−0.12	0.354
8	SNtot	TF	$y = 9.8 + 0.0042x$	0.13	0.295
9	SNtot	SF	$y = 28.9 - 0.0014x$	−0.05	0.706
10	P	SP	$y = 19.0 + 0.7201x$	0.76	<0.001
11	P	SC_{org}	$y = 2.80 - 0.0017x$	−0.14	0.222
12	P	CSH	$y = 15.9 + 0.1716x$	0.79	<0.001
13	SP	CSH	$y = 20.0 + 0.1147x$	0.55	<0.001
14	SP	TG	$y = 29.9 + 0.2442x$	0.45	<0.001
15	SP	SG	$y = 39.5 - 0.1655x$	−0.43	<0.001
16	SP	L	$y = 15.4 - 0.0305x$	−0.09	0.447
17	SP	TF	$y = 29.9 + 0.2442x$	−0.53	<0.001
18	SP	SF	$y = 39.5 - 0.1655x$	−0.00	0.999
19	K	SK	$y = 146.5 + 0.8713x$	0.39	<0.001
20	K	SC_{org}	$y = 2.9 - 0.0016x$	−0.27	0.020
21	K	CSH	$y = 19.9 + 0.0502x$	0.45	<0.001
22	K	SR	$y = 29.2 - 0.0081x$	−0.22	0.056
23	SK	CSH	$y = 43.5 + 0.0051x$	0.11	0.377
24	SK	TG	$y = 33.3 - 0.015x$	0.04	0.723
25	SK	SG	$y = 8.3 + 0.202x$	−0.18	0.147
26	SK	L	$y = 15.4 - 0.0305x$	0.29	0.017
27	SK	TF	$y = 22.7 + 0.0021x$	0.03	0.807
28	SK	SF	$y = 29.5 - 0.0197x$	−0.30	0.012
29	Ca	SCa	$y = 1670 + 363x$	0.23	0.040
30	Ca	SC_{org}	$y = 2.8 - 0.2018x$	−0.18	0.127
31	Ca	pH	$y = 5.72 + 0.4709x$	0.35	0.002
32	Ca	CSH	$y = 23.4 + 4.4116x$	0.19	0.097
33	SCa	CSH	$y = 20.5 + 0.0036x$	0.27	0.026
34	SCa	TG	$y = 30.4 + 0.0077x$	0.22	0.072
35	SCa	SG	$y = 35.6 - 0.0033x$	−0.13	0.289
36	SCa	L	$y = 27.5 - 0.0075x$	−0.36	0.003
37	SCa	TF	$y = 37.0 - 0.0074x$	−0.35	0.004
38	SCa	SF	$y = 7.27 + 0.0092x$	0.47	<0.001
39	SCa	SR	$y = 31.2 - 0.0017x$	−0.25	0.029
40	pH	SC_{org}	$y = 5.52 - 0.4808x$	−0.57	<0.001
41	TG	SR	$y = 31.3 - 30.07x$	−0.39	<0.001

Ind. var.–independent variable; Dep. var.–dependent variable; Equation–regression equation; r–coefficient of determination; P value–corresponding probability value; N, P, and K–applied nutrients; Ca–amount of Ca applied by liming in 1986; SNtot–total nitrogen content in the soil; SP, SK, and SCa–plant-available concentrations of P, K and Ca in the soil; CSH–compressed sward height; SC_{org}–content of organic carbon in the soil; TG–tall graminoids; SG–short graminoids; L–legumes; TF–tall forbs; SF–short forbs; SR–species richness.

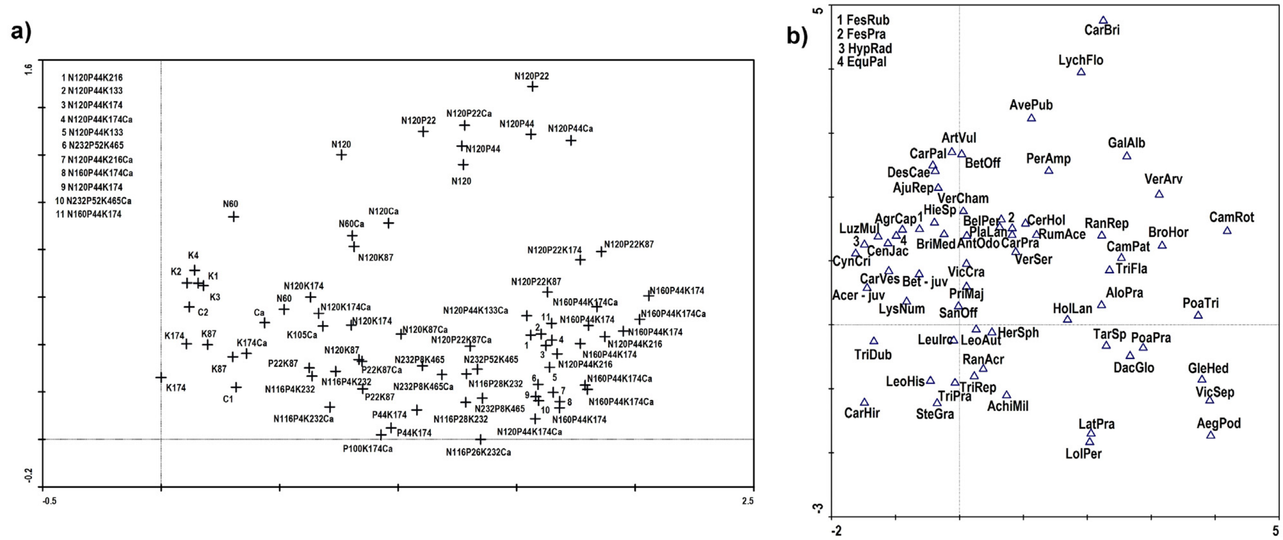

Fig. 2. Ordination diagrams showing the results of DCA analysis of plant species composition data: (a) the scatter plot of individual experimental plots and (b) the scatter plot of plant species. Letters and numbers in plot names indicate the amount of applied N, P, and K in kg ha^{-1} according to Appendix 1. Ca indicates lime application in 1986. Abbreviations of control plots without any N, P and K application are the same as in Fig. 1: Ca–control limed in 1986, K1–K4, C1 and C2. Plant species abbreviations: Acer–juv–*Acer* seedlings, AchiMil – *A. millefolium*, AegPod—*A. podagraria*, AgrCap—*A. capillaris*, AjuRep—*A. reptans*, AloPra—*A. pratensis*, AntOdo—*A. odoratum*, AvePub—*A. pubescent*, BelPer—*B. perennis*, BetOff—*B. officinalis*, Bet–juv–*Betula* seedlings, BroHor—*B. hordaceus*, CamPat—*C. patula*, CarBri—*C. brizoides*, CarHir—*C. hirta*, CarPal—*C. pallescens*, CarVes—*C. vesicaria*, CarPra—*C. pratensis*, CerHol—*C. holosteoides*, CynCri—*Cynosurus cristatus*, DacGlo—*D. glomerata*, DesCae—*D. caespitosa*, EquPal—*E. palustre*, FesPra—*F. pratensis*, FesRub—*F. rubra*, GalAlb—*G. album*, GleHed—*G. hederacea*, HerSph—*H. sphondylium*, HolLan—*Holcus lanatus*, HypRad—*H. radicata*, LatPra—*L. pratensis*, LeoHis—*L. hispidus*, LeuIrc—*L. ircutianum*, LolPer—*L. perenne*, LuzMul—*L. multiflora*, LychFlo—*L. flos-cuculi*, LysNum—*L. nummularia*, PerAmp—*P. amphibia*, PimMaj—*P. major*, PlaLan—*P. lanceolata*, PoaPra—*Poa pratensis*, PoaTri—*P. trivialis*, RanAcr—*R. acris*, RanRep—*R. repens*, RumAce—*R. acetosa*, SanOff—*Sanguisorba officinalis*, SteGra—*S. graminea*, TarSp—*T.* sp., TriDub—*Trifolium dubium*, TriPra—*Trifolium pretense*, TriRep—*T. repens*, TriFla—*T. flavescens*, UrtDio—*U. dioica*, VerArv—*V. arvensis*, VerCham—*V. chamaedrys*.

3.3. Plant species composition

Across all plots, 68 vascular plant species were recorded, i.e. 21 graminoids, 6 legumes, 38 other dicotyledonous and 3 seedlings of woody species (Appendix B). Calculated by DCA, the first axis and the first four canonical axes explained 29 and 50% of the variability in the plant species composition data, respectively. The length of the environmental gradient in DCA was 2.0. In the ordination diagram derived from this analysis, the plots without any N application together with their characteristic species grouped on the left side, whereas plots receiving high rates of N with their characteristic species were located together on the right side of the diagram (Fig. 2).

Calculated by RDA, the amount of applied nutrients, soil chemical properties, and CSH together explained 49.5% of the variability in the plant species composition data (Table 3, analysis 1). This analysis is visualised in an ordination diagram in Fig. 3, which displays the response of individual species to nutrient application, soil chemical properties and CSH.

The amount of applied N, P, K, and Ca together explained 43% of the variability in the plant species composition data (Table 3, analysis 2). The highest amount of variability from applied nutrients was explained by N (Table 3, analysis 3), followed by P (Table 3, analysis 4), and K (Table 3, analysis 5). Therefore, long-term N application most effectively diversified plant species composition in the SGE, either applied alone or in combination with other nutrients. The amount of applied Ca by direct liming in 1986 alone explained only a non-significant part of the variability in plant species composition (Table 3, analysis 6).

All soil chemical properties together explained 28.2% of the variability in plant species composition data (Table 3, analysis 7) and this is much less than the amount of fertilizer applied. The

Table 3
Results of RDA analyses of plant species composition data.

Analysis	Expl. var.	Covariables	% ax 1 (all)	F 1 (all)	P1 (all)
1	N, P, K, Ca, SNtot, SP, SK, SCa, CSH	–	33.5% (49.5%)	32.8 (8.1)	0.001 (0.001)
2	N, P, K, Ca	–	31.0% (43.0%)	32.0 (14.0)	0.001 (0.001)
3	N	P, K, Ca	19.6%	17.0	0.001
4	P	N, K, Ca	13.4%	10.8	0.001
5	K	N, P, Ca	12.5%	10.0	0.001
6	Ca	N, P, K	2.1%	1.5	0.091
7	SNtot, SP, SK, SCa	–	19.4% (28.2%)	16.8 (6.8)	0.001 (0.001)
8	SNtot	SP, SK, SCa	3.0%	2.1	0.023
9	SP	SNtot, SK, SCa	10.0%	7.8	0.001
10	SK	SNtot, SP, SCa	2.8%	2.0	0.035
11	SCa	SNtot, SP, SK	7.8%	5.90	0.001
12	CSH	–	26.7%	26.6	0.001
13	CSH	N, P, K, Ca	6.7%	4.9	0.001
14	CSH	SNtot, SP, SK, SCa	17.6%	17.7	0.001

Expl. var.—explanatory (environmental in Canoco terminology) variables; % ax 1 (all)—species variability explained by canonical axis 1 or by all constrained axes (measure of explanatory power of the environmental variables); F1 (all)–statistics for the test of particular analyses; P 1 (all)—corresponding probability value obtained by the Monte Carlo permutation test; N, P,K, and Ca—amount of applied nutrient; SNtot—total nitrogen content in the soil; SP, SK, and SCa–plant-available concentrations of P, K and Ca in the soil; CSH—compressed sward height.

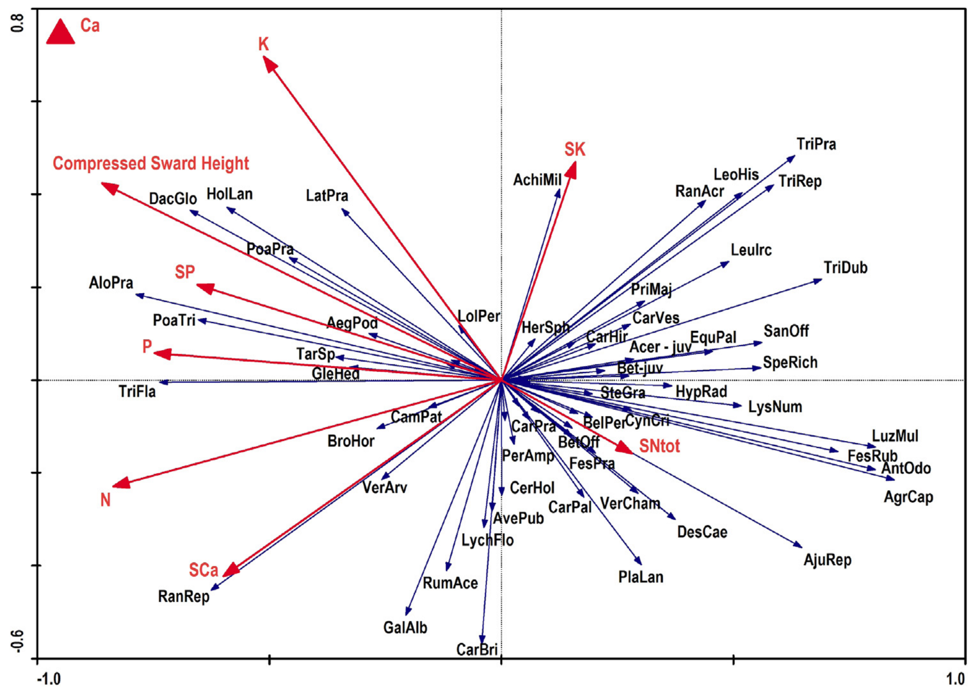

Fig. 3. Ordination diagram showing the result of RDA analysis of plant species composition data in relation to the amount of applied nutrients, soil chemical properties, and compressed sward height according to analysis a1 in Table 3. Environmental variables: N, P, K, and Ca—applied N, P, K, and Ca by fertilizers; SNtot—total nitrogen content in the soil; SP, SK, SCa—plant available concentrations of P, K and Ca in the soil. Abbreviations of plant species are given in Fig. 2.

highest amount of variability from soil chemical properties was explained by plant-available P (Table 3, analysis 9), followed by plant-available Ca (Table 3, analysis 11), total N (Table 3, analysis 8), and plant-available K in the soil (Table 3, analysis 10). Here, plant-available P rather than total N in the soil had the strongest impact on the explained variability in the data set.

Compressed sward height alone explained 26.7% of the variability in plant species composition data (Table 3, analysis 12). The explained variability by CSH strongly decreased after removal of the effect of applied nutrients (Table 3, analysis 13) and soil chemical properties (Table 3, analysis 14) from the analysis, thus indicating a relatively high effect of applied nutrients and soil chemical properties on sward height.

Calculated by RDA, soil chemical properties, the amount of applied N, P, K, and Ca, and CSH together explained 50% of the variability in plant functional group data ($F = 7.4$, $P = 0.001$, results are visualized in Fig. 4). A high cover of tall graminoids was associated with high values of CSH and high application rates of N, P, and K and high plant-available concentrations of P and K in the soil. Alternatively, a high cover of tall graminoids was connected with a low cover of legumes, short and tall forbs, short graminoids, and with a low plant species richness.

Calculated by linear regression, there was a positive significant effect of total soil N content on the cover of short graminoids, but there was no significant effect on the cover of tall graminoids, legumes, tall forbs, and short forbs (Table 2, analyses 5–9). Furthermore, we found a significant positive relationship between the concentration of plant-available P in the soil and the cover of tall graminoids (Table 2, analysis 14), but a negative correlation between P and short graminoids or tall forbs (Table 2, analyses 15 and 17). A significant effect of soil P on legumes and short forbs was not observed (Table 2, analyses 16 and 18). The response of functional groups on soil K was positive for legumes and short forbs and no effect of K was recorded on tall graminoids, short graminoids, and tall forbs (Table 2, analyses 24–28). The concentration of plant-available Ca caused a negative significant effect on legumes and tall

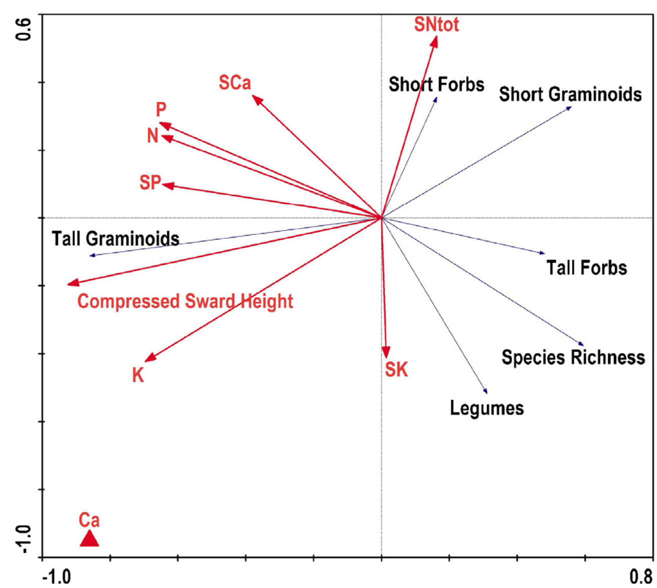

Fig. 4. Ordination diagram showing the result of RDA analysis of functional group data and species richness in relation to the amount of applied nutrients, soil chemical properties, and compressed sward height. Environmental variables: N, P, K, and Ca—applied N, P, K, and Ca by fertilizers; SNtot—total nitrogen content in the soil; SP, SK, SCa—plant-available concentrations of P, K and Ca in the soil.

forbs, a positive effect on short forbs, but no significant effect on tall and short graminoids (Table 2, analyses 34–38).

With respect to plant species composition, a highly positive response to combined N, P, and K application was found, with an increasing cover of especially tall grasses, namely *Holcus lanatus*, *A. pratensis*, and *Dactylis glomerata*. In contrast, the short grass *Anthoxanthum odoratum* and the tall grass *Festua rubra* responded negatively to N, P, and K application. We also observed that N application decreased the cover of legumes. Only the legumes

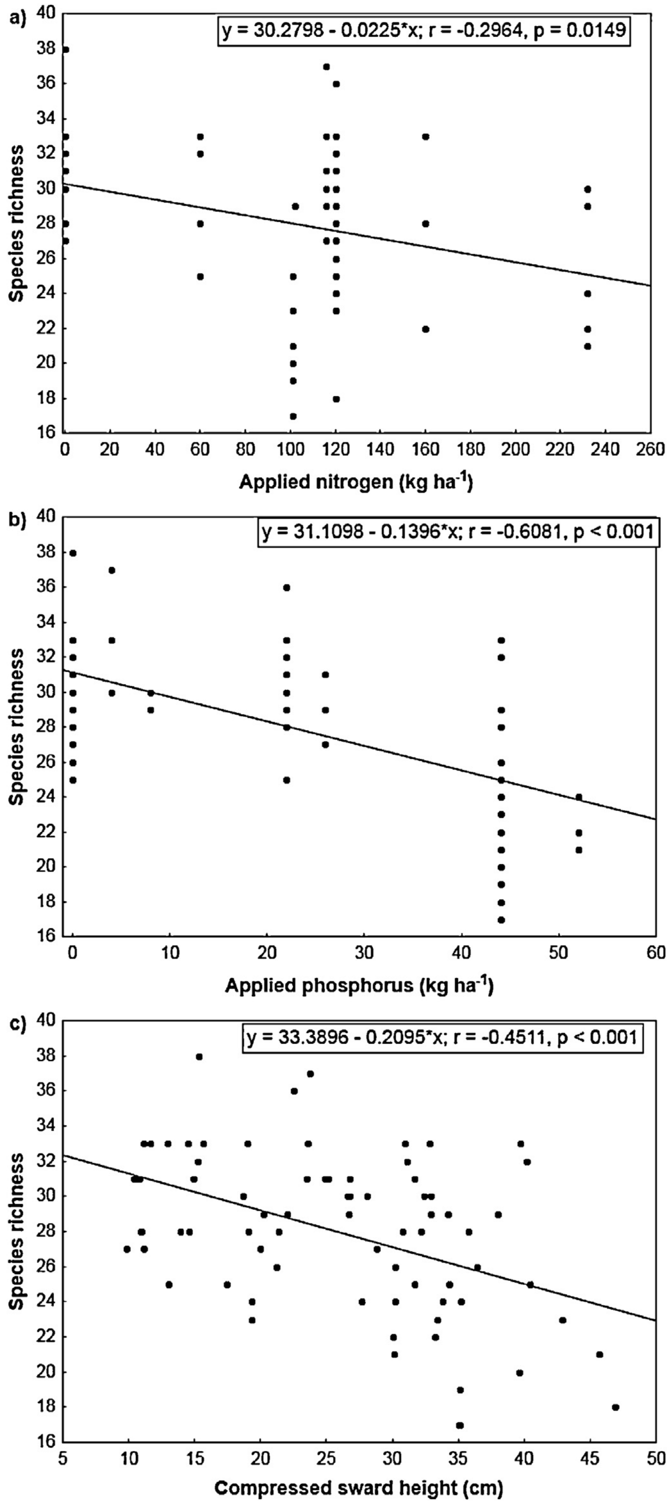

Fig. 5. Effect of (a) nitrogen and (b) phosphorus application and (c) compressed sward height on species richness (per 18.75 m^2 area) of vascular plants.

Lathyrus pratensis, *Trifolium pratensis*, and *Trifolium dubium* were able to survive moderate application of N. The highest cover of the herbs *Leontodon hispidus*, *Lysimachia nummularia*, *Plantago lanceolata*, *Ranunculus acris*, and *Sanguisorba officinalis* was recorded in non-fertilized control plots.

The species richness of vascular plants ranged from 17 to 38 species per plot (18.75 m^2) and its overall relation to applied nutrients, soil chemical properties and cover of individual species and functional groups is visible in the RDA ordination diagrams (Fig. 3 and Fig. 4). As depicted in Fig. 5, species richness was negatively affected by N and P application and by CSH. In addition, species richness decreased with the Ca concentration in the soil and with the cover of tall graminoids (Table 2, analyses 39 and 41). The total soil N content, soil pH and plant-available K in the soil did not affect species richness (data not shown).

4. Discussion

4.1. Soil chemical properties

The application of different fertilizers resulted in a wide gradient of soil pH that ranged from moderately acid to neutral. High soil acidification was already recorded in treatments with a long-term application of $(NH_4)_2SO_4$ (Silvertown et al., 2006; Clark et al., 2007; Hopkins et al., 2011). Soil acidification can substantially change the soil chemical properties and availability of many elements. Furthermore, the accumulation of soil organic matter in plots with the most acid soils has been recorded previously, indicating a negative effect of soil acidity on the mineralization of soil organic matter and on the release of mineral N. The accumulation of soil organic matter under low soil pH was also recorded in other long-term fertilizer experiments (Diepolder and Schröpel, 2003; Silvertown et al., 2006; Hejcman et al., 2010; Hopkins et al., 2011). In contrast, neutral soils in the SGE plots with Ca application and with the lowest content of organic matter indicate a positive effect of Ca application on soil microbial activity and the associated rate of N and C cycling. This conclusion is also supported by the fact that the C:N ratio in soil organic matter was lowest in neutral soils and highest in acid soils.

Although N fertilizers were applied for several decades, we did not observe any effect of N application on the total soil N content. Instead, the total N content was affected by soil pH rather than by N application. The fact that there was no effect of N application on the total soil N content supports results from other long-term grassland experiments (Silvertown et al., 2006; Hejcman et al., 2010; Hopkins et al., 2011). Probably, increased N uptake by harvested biomass was sufficient to remove the N applied by fertilizers.

In the SGE, the content of plant-available P and K in the soil was positively affected by P and K fertilizer application, as in other long-term experiments (Hejcman et al., 2009). We also found a strong relationship between plant-available P and K concentrations and N application, since these were highest in plots with P and K but without N application. This can be explained by a negative effect of N deficiency on plant growth and therefore, on herbage yield and a reduced uptake, leading to a higher accumulation of applied P and K in the soil (Hrevušová et al., 2009; Hejcman et al., 2010; Pavlů et al., 2011a). In addition, we measured a high concentration of plant-available P in all plots with an annual application of 44 kg P ha^{-1}, because P application probably exceeded P uptake by harvested biomass and thus P accumulated in the soil.

The same is true for plant-available Ca in the soil, which substantially increased with lime application and partly because of Thomas slag application (P fertilizer) because of the high amount of applied Ca exceeded the amount of Ca removed by harvested biomass and leaching.

4.2. Compressed sward height

It is well known that the height of a sward is highly correlated with the standing aboveground biomass and is thus a predictor of herbage yield (O'Donovan et al., 2002; Hakl et al., 2012; Pavlů et al., 2013). Therefore, an increase in sward height with N application even without any P and K application, indicates N limitation of biomass production at the experimental site. Nitrogen limitation of biomass production in alluvial grasslands agrees with results of other authors from different regions in Europe (Beltman et al., 2007; Hrevušová et al., 2009; Loeb et al., 2009).

4.3. Plant species composition

Although N, P and K fertilizers have been applied in the SGE for several decades, there were relatively small differences in plant species composition across the full range of fertilizer treatment, compared to long-term experiments established on naturally low productive soils (Silvertown et al., 2006; Hejcman et al., 2007; Semelová et al., 2008). One possible explanation is that the plant species composition of the alluvial grassland has adapted to relatively high nutrient concentrations even without any fertilizer input. A high tolerance of plant species composition to nutrient input is a typical feature of alluvial grasslands, because a high amount of nutrients can be deposited during floods. Furthermore, high rates of N, P and K might be released through mineralization of soil organic matter or clay minerals (Beltman et al., 2007; Klaus et al., 2011b). A clear indication of nutrient deposit and mineralization in the SGE is given by the fact that the majority of species, including those with high nutrient requirements such as *A. pratensis*, were recorded even in the control plots of the SGE, whereas in other long-term experiments on low productive soils, unfertilized control and fertilized plots rarely shared the same species.

Apart from small differences in floristic composition, we found some fertilizer effects, as depicted in Fig. 3, i.e., a strong positive effect of N application on the cover of tall graminoids, but a negative effect on species richness as well as on the cover of legumes, short graminoids and forbs. This is consistent with results from several other long-term experiments on alluvial grasslands and indicates high N requirements of tall grasses and therefore their N limitation without any N application (Honsová et al., 2007; Rothamsted Research, 2006; Vargová et al., 2012). In addition, tall graminoids were partly limited in their expansion by obviously insufficient P supply in plots with N and K application only. The concentration of plant-available P in the soil was lowest from all plots and the cover of tall graminoids was not as high as in plots with simultaneous N, P, and K application. The lowest cover of tall graminoids in plots with K application was caused by a strong N and partly P limitation. In treatments that received K only, the concentration of plant-available N was probably below the tolerance limit for *A. pratensis*, *D. glomerata*, *P. pratensis*, and *P. trivialis.* It is well documented that these grasses generally require high N supply rates throughout the growing season (Prach, 2008; Čop et al., 2009; Liu et al., 2010; Klaus et al., 2011b; Pavlů et al., 2011b; Hejcman et al., 2012).

In contrast to other tall graminoids, *H. lanatus* responded positively to the application of $(NH_4)_2SO_4$. This is generally due to its relatively high N requirements connected with its tolerance to acid soils and aluminium toxicity (Kidd and Proctor, 2001; Rothamsted Research, 2006). In addition, this grass species has been dominant in plots with dung water application, where applied N is mainly in the form of NH_4^+. A high tolerance of *H. lanatus* to high soil acidity can be thus also connected to N nutrition, i.e. a preference for NH_4^+ rather than for NO_3^-. In contrast, *Poa trivialis* negatively responded to the application of $(NH_4)_2SO_4$, probably because of its lower tolerance to acid soil reaction. *A. pratensis* responded highly positively to N application under a wide range of soil pH, indicating its high N requirements, ability to take up NH_4^+ and NO_3^- and tolerance to acid soils. *Festuca rubra* was the only tall grass that exhibited a negative response to N application and its highest cover was recorded in the control. The low N requirements of *F. rubra* in comparison to other tall forage grasses were also recorded by other authors (Berendse et al., 1992; Gastal et al., 2010; Hejcman et al., 2012; Pavlů et al., 2012).

As well as the effects of N, P, and K application in the SGE, the dominance of some species might also have been influenced by the permanent three-cut system in this experiment. For instance, *A. pratensis* obviously benefits from a low cutting frequency and suffers from frequent cutting, in contrast to *T. repens* (Pavlů et al., 2011a, 2012).

The highest cover of the short graminoids, especially *A. odoratum* and *A. capillaris*, in plots with N and K application but without any P application, was caused by lower P requirements than that of tall grasses (Merunková and Chytrý, 2012; Pavlů et al., 2012). Because of insufficient P supply, rapidly growing tall grasses were probably not able to suppress short grasses with lower P requirements under N and K application. *Trisetum flavescens* positively responded to the application of $(NH_4)_2SO_4$, probably because of its tolerance to acid soil reaction (Pavlů et al., 2011b).

The low cover of legumes in plots with N application agrees with results from other long-term experiments (Velich, 1986; Pavlů et al., 2011a, 2012). The increased competitive ability of tall graminoids suppressed legumes under high N supply. Furthermore, a higher sensitivity of legumes to an increased mineral N concentration in the soil is known to reduce N_2 fixation and the competitivity of legumes (Neuberg et al., 2011). Only some legumes such as *L. pratensis*, *Trifolium repens, T. pratensis* and *T. dubium* survived moderate applications of N similar to in other long-term experiments (Hejcman et al., 2007; Honsová et al., 2007; Pavlů et al., 2011a).

Herbs, predominately short ones, possess a lower competitive ability under high application rates of N, P, and K fertilizers, mainly because of shading by tall grasses. The only forbs that exhibited a high competitive ability under N, P, and K application were *Glechoma hederacea* and *Achillea millefolium* (Honsová et al., 2007). *G. hederacea* is well known as a shade-tolerant species that can survive in dense canopies of tall vegetation (Daßler et al., 2008). *Ranunculus repens* positively responded to the application of $(NH_4)_2SO_4$, probably due to its high N requirements connected with a tolerance to an acid soil reaction.

Ajuga reptans was most commonly found in the control as well as in K, and NK treatments and *Leonthodon hispidus*, in plots with K application, whereas *L. nummularia* and *Veronica chamaedris* preferred plots without any fertilizer input. All these short species were probably suppressed by shading of tall graminoids in plots with N, P, and K application. Notably, *P. lanceolata* was the most common species in all plots, irrespective of fertilizer input. A great tolerance of this species to a wider range of nutrient availability in grassland soils has also been found in the Rengen grassland experiment (Hejcman et al., 2013).

4.4. Species richness of vascular plants

Species richness was negatively affected the most by simultaneous N, P, and K application, similar to in other experiments (Crawley et al., 2005; Honsová et al., 2007; Hejcman et al., 2010; Pierik et al., 2011; Wesche et al., 2012; Dufková and Libichová, 2013). Under non-limiting N, P, and K supply, tall swards developed in the SGE, with a dominance of several tall graminoids. This partly explains the negative effect of sward height on species richness also recorded in other experiments (Clark et al., 2007; Gough et al., 2012). Most importantly, there was almost no negative effect of N application in the form of NH_4NO_3 on species richness, since almost the same number of species was recorded in the control (29 species) as in plots with only N application (27 species under 60 and 120 kg N ha^{-1}). In contrast, we found a negative effect of $(NH_4)_2SO_4$ application on species richness, similar to observations by Crawley et al. (2005). In plots which received only N in the form of $(NH_4)_2SO_4$, the number of species per plot was only 18. We suggest that this effect on species richness was predominately due to soil acidification. In Europe, acid soils are generally associated with a low species richness, because the majority of vascular plants in the regional species pool are adapted to neutral and alkaline soils. This is mainly due to evolutionary processes within the current flora that occurred on predominantly alkaline soils during the Pleistocene (Ewald, 2003; Merunková and Chytrý, 2012). This result indicates that not only the amount of applied N is important for species richness, but also the form of applied N and whether N is applied together with other limiting nutrients, in particular, P and K. A low plant species richness in acid soil conditions also directly explains the results of observational studies showing that a high atmospheric deposition of N compounds markedly decreases the species richness of European grasslands (Stevens et al., 2011). This decrease is evidently caused not only by indirect effects of N application via an increase in biomass production, competition for light and therefore by exclusion of less competitive species, but also directly via soil acidification, which creates a pH that lies outside the tolerance range of many species.

5. Conclusions

(1) Over decades of different fertilizer application, a steep gradient of soil chemical properties developed, namely in N, P, K, and Ca availability, the content of soil organic matter and pH.
(2) Soil chemical properties were responsible for the development of a steep gradient of aboveground biomass production expressed by sward height.
(3) Although long-term fertilizer application induced significant diversification of the plant species composition in the SGE on the alluvial meadow, this effect was not as high as that resulting from fertilizer application on naturally low productive soils of other long-term experiments discussed here. Clearly, a high nutrient availability enabled the survival of species that were well adapted to these conditions, even in the unfertilized control. We therefore conclude that the plant species composition in alluvial grasslands responds substantially less dramatically to N, P, and K application than in low productive grasslands. Alluvial grasslands dominated by *A. pratensis* are thus substantially less affected by fertilizer application than other low productive grasslands.
(4) Long-term fertilizer application negatively affected plant species richness directly by soil acidification ($(NH_4)_2SO_4$ particularly) and indirectly by an increase in aboveground biomass production, thus increasing competition for light, which supports the exclusion of low competitive species. The magnitude of the negative effects of N application on species richness was dependent on (i) whether N was applied alone or in combination with other nutrients, particularly P, K, and Ca, and (ii) whether the N application acidified the soil.

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Appendix A.

List of plots and fertilizer regimes applied since the start of the experiment in 1933. Numbers indicate the amount of annually applied N, P and K in kg ha^{-1}. The abbreviation "Ca" in plot numbers indicates lime application in 1986. Bold frames indicate plots exposed to the same fertilizer treatment. Since 1933, original groups of plots I to VI have been divided into subgroups and therefore, the amounts of either N, P, and K applied have been increased in most plots. Plots C_1, C_2 and C_{Ca} and K_1–K_4 have never received any fertilizer input.

Group	Plot number	Period 1	Period 2	Period 3	Period 4	Period 5
		1933–1953 (21 years)	1954–1964 (11 years)	1965–1970 (6 years)	1971–1986 (17 years)	1987–2008 (22 years)
I Dung water	11/1, 11/2 & 12/Ca	N116P4K232	N116P4K232	N116P4K232	N116P4K232	N116P4K232[1]
	21/1, 21/2 & 22/Ca	N116P4K232	N116P4K232	N232P8K465	N232P8K465	N232P8K465[1]
	31/1, 31/2 & 32/Ca	N116P4K232	N116P4K232	N116P26K232	N116P26K232	N116P26K232[1]
	41/1, 41/2 & 42/Ca	N116P4K232	N116P4K232	N232P52K465	N232P52K465	N232P52K465[1]
II	51/1, 51/2 & 52/Ca	N40K87	N40K87	N40K87	N120K87	N120K87[1]
N-	61/1, 61/2 & 62/Ca	N40K87	N40K87	N80K100	N120K174	N120K174[1]
K	71/1, 71/2 & 72/Ca	N40K87	N60P40K133	N80P44K133	N120P44K133	N120P44K133[1]
	81/1, 81/2 & 82/Ca	N40K87	N60P40K133	N80P44K216	N120P44K216	N120P44K216[1]
III	91/1, 91/2 & 92/Ca	N40	N40	N40	N60	N60[1]
N	101/1, 101/2 & 102/Ca	N40	N40	N80	N120	N120[1]
	111/1, 111/2 & 112/Ca	N40	N40P22	N40P22	N120P22	N120P22[1]
III	121/1, 121/2 & 122/Ca	N40	N40P22	N80P44	N120P44	N120P44[1]
IV	131/1, 131/2 & 132/Ca	P22K87	K87	K87	K87	K87[1]
	141/1, 141/2 & 142/Ca	P22K87	K87	K174	K174	K174
	151/1, 151/2 & 152/Ca	P22K87	P22K87	P22K87	P22K87	P22K87[1]
	161/1, 161/2 & 162/Ca	P22K87	P22K87	P44K174	P44K174	P44K174[1]
V	171/1, 171/2 & 172/Ca	N40P22K87	N40P22K87	N40P22K87	120N22P87 K	120N22P87K[1]
	181/1, 181/2 & 182/Ca	N40P22K87	N40P22K87	N80P44K174	120N44P174 K	120N44P174K[1]
V	191/1, 191/2 & 192/Ca	N40P22K87	N40P40K133	N120P22K174	N120P22K174	N120P22K174[1]
	201/1, 201/2 & 202/Ca	N40P22K87	N40P40K133	N120P44K174	N160P44K174	N160K44P 174[1]
VI	2111, 2112, 2121/Ca, 2122/Ca	–	–	–	–	N160P44K174[2]
	2211, 2212, 2221/Ca, 2222/Ca	–	–	–	–	N160P44K174[3]

[1] Application of NH_4NO_3.
[2] Application of NH_4SO_4.
[3] Combination of $CaNO_3/NH_4SO_4/NH_4NO_3$.

Appendix B.

Cover (in %) of bryophytes, functional groups of vascular species and vascular species, compressed sward height (cm), and species richness (per 9 m^2 in C, 2112, and 2222 plots and per 14.6 m^2 in all other plots) measured in May 2008. Species with a cover below 0.1% are not presented. C–mean values from all control plots without any fertilizer input. Many species were recorded only in several control plots, therefore, the mean species richness per control plot was 29.3 and the number of species recorded over all control plots was 64. The abbreviation “Ca” in plot numbers indicates lime application in 1986. Plots with an application of mineral P were indirectly limed by Thomas slag.

Species	Experimental plot									
	C	141/1	162/Ca	31/2	52/Ca	61/2	192/Ca	21/1	2112	2222/Ca
N (kg ha^{-1})	0	0		116	100	120	120	232	160	160
P (kg ha^{-1})	0	0	44	26			22	8	44	44
K (kg ha^{-1})	0	174	174	232	87	174	174	485	174	174
Bryophytes	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Compressed sward height	11.3	9.8	26.7	31.7	24.9	19	26.8	32.4	35.1	46.9
Species richness	29.3	27	29	31	31	33	31	30	19	18
Tall graminoids	15.6	5.2	49	64.2	37.2	17.2	55	75.2	96.1	52
Alopecurus pratensis	1.6	–	10	8	20	8	20	10	30	30
Avenula pubescent	0.1	–	–	0.1	–	–	–	–	–	–
Carex vesicaria	0.1	–	–	–	–	–	–	–	–	–
Deschampsia caespitosa	0.1	0.1	–	–	–	–	–	–	–	–
Dactylis glomerata	0.1	–	5	5	5	0.1	20	3	5	5
Festuca pratensis	0.8	0.1	6	0.1	4	0.1	3	12	1	–
F. rubra	10.2	3	–	–	0.1	3	–	–	–	–
Holcus lanatus	2.2	2	25	50	8	6	10	50	60	5
Poa pratensis	0.1	–	2	–	–	–	1	0.1	0.1	–
P. trivialis	0.3	–	1	1	0.1	–	1	0.1	–	12
Short Graminoids	55.8	31.2	5.2	25.2	12	64	24	20.2	18.2	40.1
Agrostis capillaris	22.5	6	–	–	2	15	–	–	–	–
Anthoxanthum odoratum	13.7	20	5	10	8	45	3	8	0.1	0.1
Briza media	0.1	–	–	–	–	–	–	–	–	–
Bromus hordaceus	0.1	–	0.1	0.1	–	–	1	0.1	0.1	–
Carex hirta	0.1	–	–	–	–	–	–	–	–	–
Carex pallescens	0.1	–	–	–	–	1	–	–	–	–
Carex brizoides	9	0.1	–	–	–	–	–	–	–	–
Cynosurus cristatus	0.6	–	–	–	–	–	–	–	–	–
Lolium perenne	0.1	–	0.1	0.1	–	–	–	0.1	–	–
Luzula multiflora	9.2	5	–	–	–	1	–	–	–	–
Trisetum flavescens	0.3	0.1	–	15	2	2	20	12	18	40
Legumes	28.5	31	35	13.2	7	1.2	2.2	11	0	2.2
Trifolium repens	12.2	10	20	0.1	4	0.1	0.1	8	–	0.1
T. pretense	4.7	1	12	3	2	1	0.1	2	–	0.1
T. dubium	10.3	20	2	0.1	–	–	–	–	–	–
Lathyrus pratensis	1.1	–	1	10	1	0.1	2	1	–	2
Vicia cracca	0.1	–	–	–	–	–	–	-	–	–
Vicia sepium	0.1	–	–	–	–	–	–	–	–	–
Tall Forbs	20.3	16.2	23.1	18	42.3	58.2	15	30.2	3.5	4
Aegopodium podagraria	0.1	–	–	–	–	–	1	–	–	–
millefolium	1.1	6	4	2	4	40	1	15	3	–
Artemisia vulgaris	0.1	–	–	–	–	–	–	–	–	–
Betonica officinalis	0.1	–	–	–	–	–	–	–	–	–
Centaurea jacea	0.1	–	–	–	–	–	–	–	–	–
Leucanthemum ircutianum	1.6	10	8	–	0.1	5	5	0.1	0.1	–
Galium album	0.1	–	–	2	4	–	–	0.1	–	1
Lychnis flos-cuculi	0.1	–	0.1	–	–	–	–	–	–	–
Pimpinella major	0.1	–	–	–	0.1	0.1	–	–	–	–
Persicaria amphibia	0.1	–	1	–	–	–	–	–	0.1	–
Ranunculus acris	7.3	0.1	7	2	0.1	0.1	1	1	0.1	–
Rumex acetosa	2.5	0.1	2	5	4	5	7	8	0.1	3
Sanquisorba officinalis	6.8	–	1	7	30	8	–	5	0.1	–
Silaum silaus	0.1	–	–	–	–	–	–	1	–	–
Urtica dioica	0.1	–	–	–	–	–	–	–	–	–
Short Forbs	25.9	17.5	8.4	9.6	20.5	16.4	34.6	13.5	6.2	43.2
Ajuga reptans	4.4	5	–	0.1	0.1	5	–	–	–	–
Alchemilla vulgaris	0.1	–	–	–	–	–	–	–	–	–
Bellis perennis	0.1	0.1	0.1	0.1	0.1	–	–	0.1	–	–
Campanula patula	0.1	–	–	–	–	–	0.1	–	–	–
Campanula rotundifolia	0.1	–	–	–	–	–	–	–	–	–
Cardamine pratensis	0.4	–	–	–	–	–	0.1	–	–	–
Cerastium holosteoides	0.6	0.1	0.1	0.1	0.1	0.1	1	0.1	0.1	0.1
Equisetum palustre	0.1	–	–	–	–	–	–	–	–	–
Glechoma hederacea	0.1	–	–	0.1	–	–	1	–	–	–
Heracleum sphondylium	0.1	–	–	–	2	4	–	0.1	–	–
Hieracium sp.	0.1	–	–	–	–	1	–	–	–	–
Hypochaeris radicata	0.1	–	–	–	–	0.1	–	–	–	–

Appendix B (*Continued*)

Species	Experimental plot									
	C	141/1	162/Ca	31/2	52/Ca	61/2	192/Ca	21/1	2112	2222/Ca
Leontodon autumnalis	0.1	–	–	–	1	0.1	–	–	–	–
L. hispidus	2	12	4	0.1	3	–	0.1	2	–	–
Lysimachia nummularia	3.8	–	–	2	0.1	0.1	0.1	0.1	–	–
P. lanceolata	2.4	0.1	1	4	10	2	2	7	0.1	1
Ranunculus repens	3.5	0.1	3	2	4	2	25	4	5	40
Stellaria graminea	0.1	–	–	0.1	–	–	–	–	–	–
Taraxacum sp.	0.1	–	0.1	1	0.1	2	4	0.1	1	2
Veronica chamaedrys	7.4	0.1	0.1	–	–	–	1	–	–	–
Veronica serpyllifolia	0.1	–	–	–	–	–	0.1	–	–	–
Veronica arvensis	0.1	–	–	–	–	–	0.1	–	–	0.1

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