Agriculture, Ecosystems and Environment 130 (2009) 123–130

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Long-term dynamics of biomass production, soil chemical properties and plant species composition of alluvial grassland after the cessation of fertilizer application in the Czech Republic

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ARTICLE INFO

Article history:
Received 27 June 2008
Received in revised form 11 December 2008
Accepted 15 December 2008
Available online 20 January 2009

Keywords:
Holcus lanatus
Phosphorus
Residual soil fertility
Resilience and restoration
Species richness and diversity

ABSTRACT

The resilience, the ability of an ecosystem to recover after termination of perturbation, of highly productive *Alopecurus* grassland was investigated after the cessation of the following long-term fertilizer treatments applied under a two- or three-cut management regime: unfertilized control, PK, N_{100}PK, N_{200}PK, N_{300}PK and N_{400}PK. Annual application rates of pure nutrients per hectare were 0–400 kg for N, 40 kg for P and 100 kg for K. The dynamics of biomass production were measured for 16 years and the effect of former fertilizer treatments on soil chemical properties, biomass chemical properties, plant species composition and species richness were investigated 16 years after the last application of fertilizers. It was concluded that 16 years was not long enough to achieve resilience in plant-available soil P and K concentrations, N/P ratios in the plant biomass or plant species composition, but that it was long enough to achieve resilience in species richness. In the case of biomass production the effect of former fertilizer treatments was apparent in 10 of the 16 seasons investigated, indicating that resilience in biomass production must be evaluated using data from more than 1 vegetation season. The results of the study stress the necessity for long-term research because of high year-to-year variability in biomass production as well as long-term after-effects of fertilizer treatments in alluvial grassland.

1. Introduction

What will happen to biomass production and species diversity after cessation of fertilizer application? This question has frequently been asked by farmers as well as by scientists but it has not yet been satisfactorily answered. This is because of the high diversity of grassland types, soil properties, weather conditions, rates of fertilizers used and insufficient number of really long-term and well-documented studies. Such knowledge is extremely important for restoration of species-rich plant communities alongside the demands of farmers for satisfactory production of forage (Walker et al., 2004; Andrieu et al., 2007; Wellstein et al., 2007; Peter et al., 2008).

The ability of ecosystems to recover after termination of perturbation is called resilience (Lepš et al., 1982). The resilience of grassland ecosystems after termination of fertilizer application may be particularly low especially in the case of Ca and P application in environments with harsh climatic conditions (Hegg et al., 1992; Spiegelberger et al., 2006; Hejcman et al., 2007a). Further, resilience is dependent on the longevity of fertilizer application. Semelová et al. (2008) concluded that changes generated by long-term application of fertilizers in sub-alpine conditions may even be irreversible.

Resilience is generally substantially shorter in lowland grasslands. Olff and Bakker (1991) recorded a large decrease in dry matter biomass production from 8 to 3 t ha^{-1} and a marked increase in species richness on peaty soil 14 years after cessation of fertilizer application. Willems and van Nieuwstadt (1996) revealed a decrease in biomass production and an increase in species richness 20 years after cessation of fertilizer application on calcareous grassland. The speed of changes in productivity and species richness were negatively related to former application

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doi:10.1016/j.agee.2008.12.008

rates of P, but were independent of rates of N. Similar results were reported by Niinemets and Kull (2005), with the effect of P application on biomass production occasionally visible even 14 years after termination of P treatment. Chiarucci and Maccherini (2007) reported a residual effect of P fertilizer application on plant species composition in serpentine grassland that lasted more than 10 years. Malhi et al. (2003) recorded increased P concentration in the top soil layer even though P application was terminated 20 years prior to soil sampling. The time necessary for moor grassland resilience under continuous cutting management was estimated by Mountford et al. (1996) as 3–9 years depending on former N application rates.

The resilience of highly productive alluvial grassland after cessation of long-term fertilizer application has not yet been described. In this study, the resilience of alluvial grassland was investigated after cessation of 26 years of application of N, P and K fertilizers. For the following 16 years, the dynamics of biomass production were investigated to detect the longevity of the residual effects of the fertilizer treatments on grassland productivity. Whether the after-effects of fertilizer treatments on soil chemical properties, biomass chemical properties and plant species composition could be detected even after 16 years was also investigated.

2. Materials and methods

2.1. Study site, experiment and data collection

In 1966, a fertilizer experiment was set up at 363 m a.s.l. in a flat alluvial meadow near the village of Černíkovice, 35 km south of Prague (49°47′N, 14°45′E, Fig. 1; Velich, 1986). At the study site, mean annual precipitation and temperature were 600 mm and 8.1 °C, respectively (Benešov meteorological station, 1961–2005). The soil type was a fluvisol/gleysol with a loamy texture. The depth of the underground water table varied between 0.1 and 0.7 m during the vegetation season and flooding occurred on several occasions during the study period. According to phytosociological nomenclature (Botta-Dukát et al., 2005) the vegetation of the experimental grassland was classified as *Deschampsion cespitosae*. The experimental meadow was mown two or three times per year from 1966 to 2007 depending on climatic conditions in individual years. The experiment was arranged in four randomized blocks each with the following treatments: unfertilized control, PK, N_{100}PK, N_{200}PK, N_{300}PK and N_{400}PK. Annual application rates of P and K were 40 and 100 kg ha^{-1}, respectively. Annual application rates of N are indicated by lower case numbers in the abbreviations of treatments. The intensive N_{300}PK and N_{400}PK treatments were added in 1975 (see Honsová et al., 2007 for details). The area of individual plots was 5 m × 6 m. In 1991, each 5 m × 6 m plot was divided into two 5 m × 3 m subplots. The dosages of N were reduced by half in the first subplot and fertilizer application was terminated in the second subplot. In this paper, only data from subplots where fertilizer application was terminated are presented.

Soil samples were taken in June 2007. In each plot, four separate samples were taken from 0 to 10 cm depth after removing plant residues, and combined to form one representative sample. The soil samples were air-dried, ground in a mortar, and sieved to 2 mm after removal of living roots. All analyses were performed in an accredited national laboratory – plant-available Ca, K, Mg and P were extracted by the Mehlich III method (Mehlich, 1984) and then determined by ICP-OES. Total N was analyzed by the Kjeldahl method and organic C by means of colorimetry (AOAC, 1984).

Dry matter biomass yield from the first, second and third cut (third in favorable years only) was measured. In each plot, the

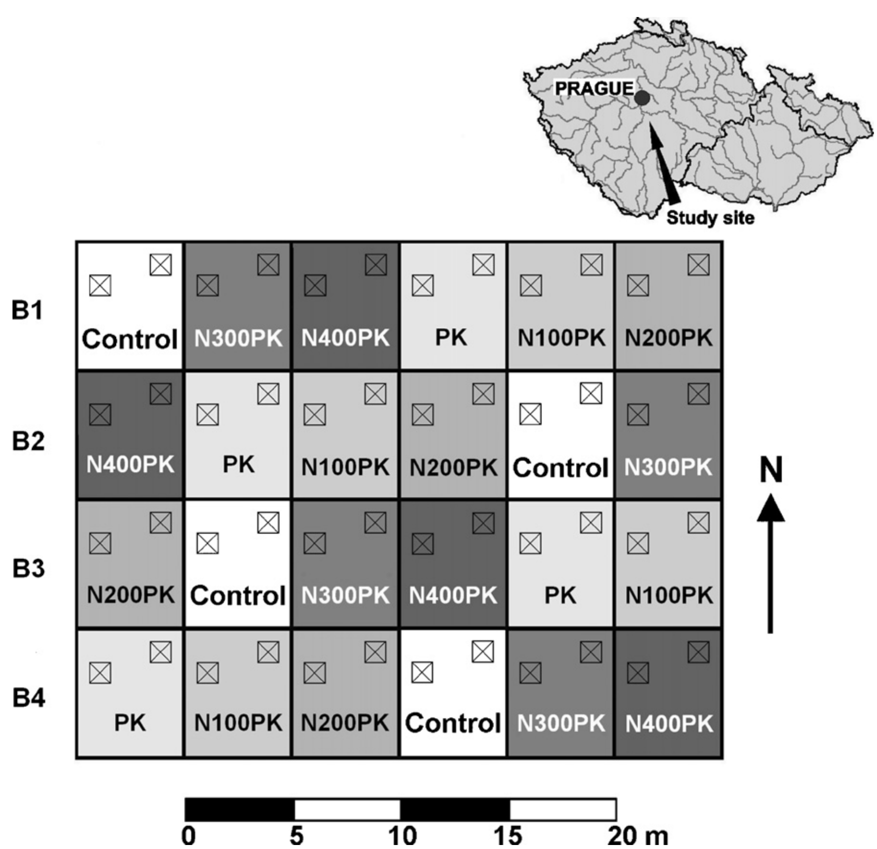

Fig. 1. Location of the study site in the Czech Republic and a design of the experiment—the spatial arrangement of randomized blocks of five fertilizer treatments and the control. Positions of 1 m^2 plots used for monitoring the vegetation are indicated by squares with diagonals. B1–B4 indicate complete randomized blocks. Treatment abbreviations reflects nutrients applied up to 1991—P (40 kg ha^{-1}), K (100 kg ha^{-1}) and N (0–400 kg ha^{-1}).

Table 1
Results of one-way ANOVA analyses—effect of former fertilizer treatments on soil chemical properties, biomass chemical properties, amount of removed nutrients and vegetation characteristics analyzed 16 years after the last fertilizer application under continual two- or three-cut management.

Tested variable	*F*-value	*P*-value
Soil chemical properties		
Carbon (organic)	0.597	0.702
$pH/CaCl_2$	0.85	0.532
pH/H_2O	1.04	0.424
Calcium	1.278	0.316
Magnesium	1.924	0.14
Nitrogen (total)	0.862	0.525
Phosphorus	4.631	**0.007**
Potassium	4.838	**0.006**
Biomass chemical properties		
Calcium	0.567	0.724
Magnesium	0.545	0.74
Nitrogen	1.273	0.318
Phosphorus	1.892	0.146
Potassium	0.243	0.938
N/P ratio	3.2	**0.035**
Amount of removed nutrients		
Calcium	0.201	0.958
Magnesium	0.353	0.873
Nitrogen	0.951	0.543
Phosphorus	1.145	0.373
Potassium	0.555	0.733
Vegetation characteristics		
Species richness	1.908	0.113
Short grasses (cover)	0.387	0.855
Tall grasses (cover)	3.240	0.015
Total grasses (cover)	1.304	0.281
Total legumes (cover)	1.769	0.140
Short herbs (cover)	0.572	0.721
Tall herbs (cover)	1.063	0.394
Total herbs (cover)	1.118	0.366
Holcus lanatus (cover)	3.411	**0.011**
Ranunculus acris (cover)	5.47	<**0.001**

Degrees of freedom were five in all analyses. Significant results are faced in bold.

sward was mown by machine leaving a stubble height of approximately 5 cm. The harvested biomass was immediately weighed and the percentage dry matter determined in the laboratory after 48 h of drying at 85 °C. Biomass production per plot was then recalculated as dry matter yield (t ha^{-1}).

N, Ca, Mg, P and K concentrations in the biomass were determined in the same way as in soil samples (ICP-OES). The amount of nutrients removed in the harvested biomass was calculated from biomass production data and concentrations of elements in the biomass.

The cover of all vascular plant species was visually estimated directly in percentages in each plot and cover estimates ranged from 0.5% to 100%. This was done before the first harvest in mid-May 2007. In each plot, two 1 m × 1 m relevés were recorded (eight relevés per treatment, Fig. 1). Based on the descriptions of the vascular plants in the national flora (Kubát et al., 2002), all plant species within the study area were categorized into five functional groups (short grasses, tall grasses, short herbs, tall herbs and legumes). The cover of each functional group was calculated for each 1 m^2 plot. Tall grasses were *Alopecurus pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata*, *D. cespitosa*, *Elytrigia repens*, *Festuca pratensis*, *Holcus lanatus*, *Poa pratensis*, *P. trivialis* and *Trisetum flavescens*.

2.2. Data analysis

One-way ANOVA followed by post hoc comparison using the Tukey HSD test was applied to identify differences among treatments for soil and biomass chemical properties, vegetation properties and annual yields. To analyze dynamics in biomass production, repeated measures ANOVA was used to evaluate the effect of treatments, year and interaction of treatments with year. Blocks were treated as a random factor in all ANOVA analyses.

Redundancy analysis (RDA) was used to evaluate multivariate plant cover data. This method was used because the data set was relatively homogeneous, and environmental variables and covariables were categorical. A Monte Carlo permutation test with 999 permutations restricted into blocks was used to reveal the significance of the treatment effects on plant species composition. The percentage of the explained plant species data variability induced by fertilizer treatments was used as a measure of explanatory power. ANOVA analyses were performed using the STATISTICA 5.0 program (StatSoft, 1995) and RDA analysis using the CANOCO 4.5 program (ter Braak and Šmilauer, 2002).

3. Results

3.1. Soil chemical properties

There was no significant effect of former fertilizer treatments on organic C, Ca, Mg and total N concentrations in the soil (Tables 1 and 2). Similarly the effect of former fertilizer treatments on soil pH was not significant, although both pH/H_2O and $pH/CaCl_2$ values were lowest in the $N_{400}PK$ treatment. The effect of treatment on plant-available P and K concentrations was significant even though fertilizer application had been terminated 16 years prior to soil sampling. The most interesting result was the increased P concentration in the PK treatment in contrast with the never-fertilized control. Further, although $N_{100}PK$–$N_{400}PK$ treatments received the same amount of P as the PK treatment during the period of fertilization, the concentration of P was not significantly different from the control.

3.2. Biomass chemical properties

The effect of treatments on the N/P ratio in the biomass was significant (Tables 1 and 3). In the control treatment, the mean N/P ratio was 8.7 and significantly differed from 7.0 in the PK treatment. Although the effect of treatment on concentrations of all analyzed elements was not significant, the highest concentra-

Table 2
Effect of former fertilizer treatments on soil chemical properties analyzed 16 years after the last fertilizer application under continual two- or three-cut management.

Treatment	N-tot (mg kg^{-1})	C-org (%)	$pH/CaCl_2$	pH/H_2O	K (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})
Control	$4718^{a} \pm 217$	$3.75^{a} \pm 0.09$	$5.41^{a} \pm 0.16$	$5.72^{a} \pm 0.08$	$173^{ab} \pm 6.1$	$13^{a} \pm 0.83$	$520^{a} \pm 21$	$3919^{a} \pm 118$
PK	$4478^{a} \pm 282$	$3.53^{a} \pm 0.12$	$5.25^{a} \pm 0.26$	$5.52^{a} \pm 0.25$	$186^{a} \pm 13.3$	$24^{b} \pm 8.58$	$454^{a} \pm 41$	$3697^{a} \pm 472$
$N_{100}PK$	$4829^{a} \pm 268$	$3.67^{a} \pm 0.24$	$5.37^{a} \pm 0.18$	$5.67^{a} \pm 0.16$	$167^{ab} \pm 12.0$	$19^{ab} \pm 2.49$	$462^{a} \pm 26$	$4067^{a} \pm 124$
$N_{200}PK$	$4893^{a} \pm 305$	$3.63^{a} \pm 0.36$	$5.37^{a} \pm 0.19$	$5.64^{a} \pm 0.12$	$152^{b} \pm 13.9$	$17^{ab} \pm 1.30$	$477^{a} \pm 13$	$4299^{a} \pm 389$
$N_{300}PK$	$4757^{a} \pm 272$	$3.58^{a} \pm 0.26$	$5.23^{a} \pm 0.18$	$5.54^{a} \pm 0.09$	$149^{b} \pm 9.3$	$15^{ab} \pm 0.87$	$457^{a} \pm 40$	$3896^{a} \pm 131$
$N_{400}PK$	$4781^{a} \pm 250$	$3.47^{a} \pm 0.17$	$5.10^{a} \pm 0.31$	$5.50^{a} \pm 0.17$	$159^{ab} \pm 9.6$	$17^{ab} \pm 2.49$	$441^{a} \pm 53$	$3769^{a} \pm 492$

Using Tukey post hoc test, treatments with the same letter were not significantly different. ± values indicate S.D.

Table 3
Effect of former fertilizer treatments on biomass chemical properties of the first cut analyzed 16 years after the last fertilizer application under continual two- or three-cut management.

Treatment	K (%)	Ca (%)	Mg (%)	P (%)	N-tot (%)	N/P ratio
Control	$2.37^{a} \pm 0.09$	$0.91^{a} \pm 0.08$	$0.24^{a} \pm 0.02$	$0.28^{a} \pm 0.04$	$2.37^{a} \pm 0.09$	$8.7^{a} \pm 0.89$
PK	$2.38^{a} \pm 0.12$	$0.89^{a} \pm 0.12$	$0.25^{a} \pm 0.03$	$0.33^{a} \pm 0.02$	$2.31^{a} \pm 0.22$	$7.0^{b} \pm 0.63$
N_{100}PK	$2.28^{a} \pm 0.08$	$0.86^{a} \pm 0.10$	$0.23^{a} \pm 0.02$	$0.30^{a} \pm 0.01$	$2.12^{a} \pm 0.03$	$7.2^{ab} \pm 0.26$
N_{200}PK	$2.33^{a} \pm 0.20$	$0.96^{a} \pm 0.24$	$0.24^{a} \pm 0.04$	$0.30^{a} \pm 0.02$	$2.35^{a} \pm 0.28$	$7.7^{ab} \pm 0.62$
N_{300}PK	$2.36^{a} \pm 0.16$	$0.81^{a} \pm 0.22$	$0.23^{a} \pm 0.03$	$0.30^{a} \pm 0.02$	$2.10^{a} \pm 0.20$	$7.2^{ab} \pm 0.97$
N_{400}PK	$2.35^{a} \pm 0.05$	$0.76^{a} \pm 0.17$	$0.21^{a} \pm 0.03$	$0.30^{a} \pm 0.02$	$2.18^{a} \pm 0.18$	$7.4^{ab} \pm 0.95$

Using Tukey post hoc test, treatments with the same letter were not significantly different. ± values indicate S.D.

tion of P was recorded in the PK treatment and the lowest in the control. There was no obvious effect of treatment on concentrations of other analyzed elements.

3.3. Biomass production

A repeated measures ANOVA revealed a significant effect of treatments ($F = 7.8$, $P < 0.001$), year ($F = 57.0$, $P < 0.001$) and treatment × year interaction ($F = 2.4$, $P < 0.001$) on biomass production (Fig. 2). The large effect of treatments on total aboveground biomass production was apparent in the last year of fertilizer application in 1991. In this year, dry matter biomass production gradually increased with the amount of fertilizers used from control to N_{400}PK treatment. There was no clear pattern of biomass production after cessation of fertilizer application. A significant residual effect of treatment on biomass production was recorded in 1992–1994, 1997–2001, 2004 and 2006. Biomass production decreased substantially in the first year without fertilizer application in 1992. Surprisingly biomass production was lowest in the N_{100}PK treatment followed by N_{200}PK and control treatments, and highest in the N_{400}PK treatment in this year. In 1993, biomass production was paradoxically highest in the

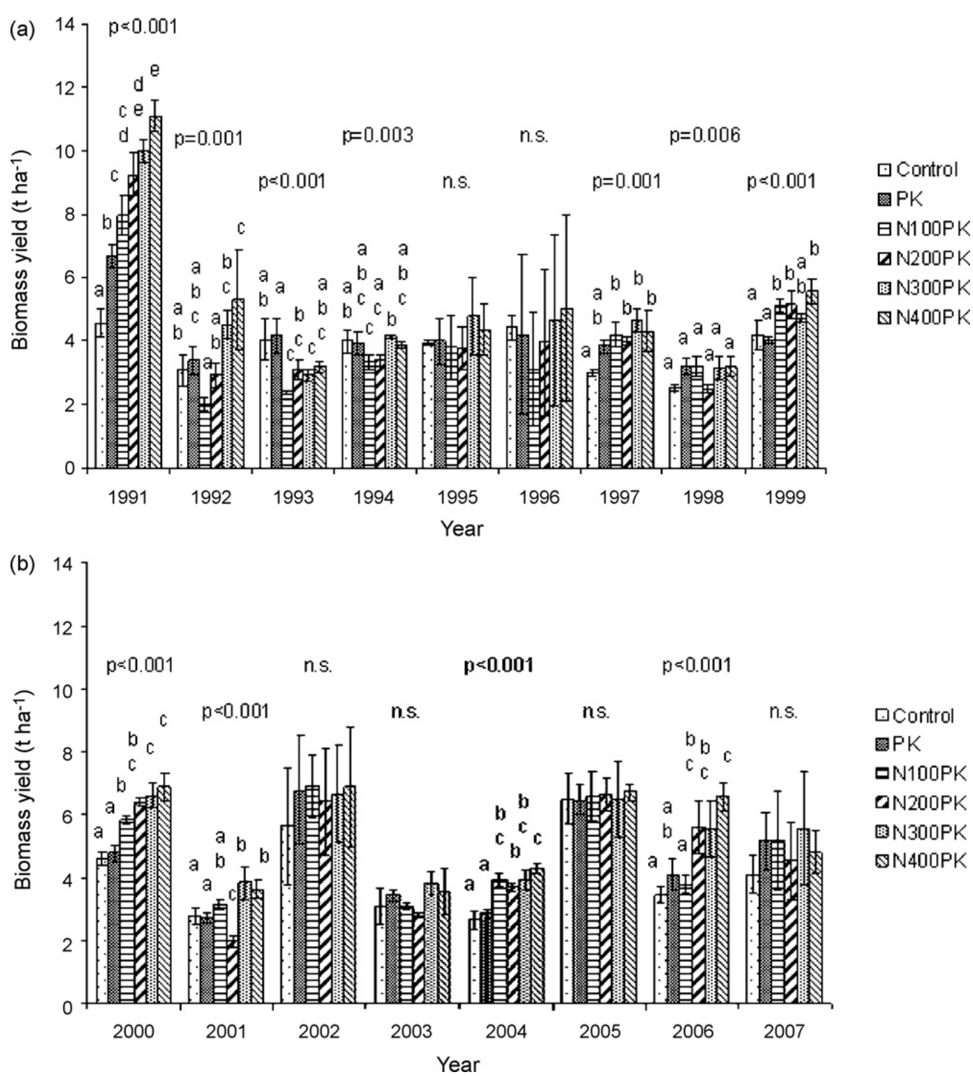

Fig. 2. Dynamic of total aboveground biomass production during 16 years following the last fertilizer application in 1991. Data from year 1991 to 1999 (a) and from year 2000 to 2007 (b). *Abbreviations*: *P*—probability value obtained from one-way ANOVA analyses. Using Tukey post hoc test, treatments with the same letter were not significantly different. n.s.—results of ANOVA analyses were not significant. Error bars indicate S.D.

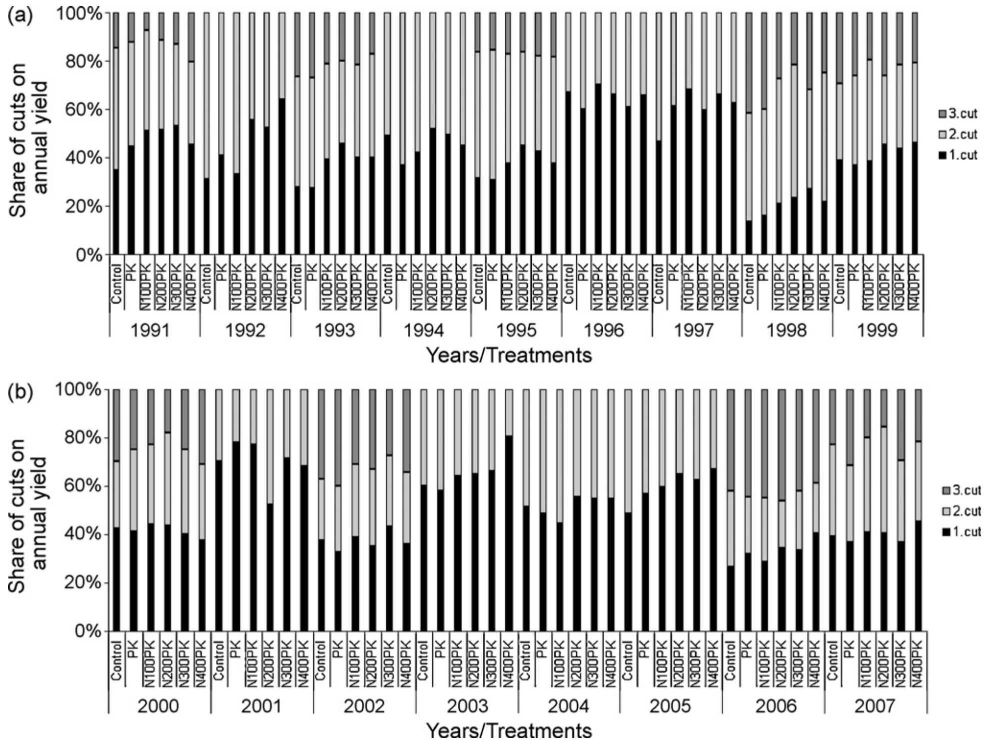

Fig. 3. Percentage proportion of the first, second and third cut on total biomass yield. Third cut was performed in favorable vegetation seasons only. Data from year 1991 to 1999 (a) and from year 2000 to 2007 (b).

PK and control treatments. Biomass production in the increasing order from control to N_{400}PK treatment was recorded in 1997, 1999, 2000, 2004 and 2006. Total biomass production was highly affected by year-to-year variability. In the control for example, dry matter biomass production ranged from 2.6 to 6.5 t ha^{-1} in 1998 and 2005, respectively. Biomass production and the proportion of individual cuts out of total biomass yield differed substantially because of year-to-year variability (Fig. 3). The proportion of total biomass production of individual cuts was little affected by treatment effect. The mean share of the first and second cuts over 17 years was 47% and 39%, respectively. The three-cut management regime was only applied in favorable vegetation seasons in 1991, 1993, 1995, 1998, 1999, 2000, 2002, 2006 and 2007. The third cut accounted for 26% of total biomass production in seasons with three-cut management.

In 2007, the effect of treatment on the amount of nutrients removed in the harvested biomass was not significant (Tables 1 and 4) although the lowest amount of all analyzed elements was removed in the control.

3.4. Plant species composition

According to RDA, the effect of treatment on plant species composition was significant 16 years after the last fertilizer application in 2007 ($F = 1.5$, $P = 0.038$). Treatment effect (all constrained axes together) explained 15.2% of the variability of the plant species composition data (the ordination diagram is given in Fig. 4). N_{400}PK treatment differed substantially from all other treatments as the distance between triangles for this and other treatments was the highest in the ordination diagram. Species associated with N_{400}PK treatment were the tall grasses *H. lanatus*, *P. pratensis* and *T. flavescens* and the tall forb *Anthriscus sylvestris*. On the other hand, species associated with the control were particularly dicotyledonous: *Alchemilla* sp., *Ranunculus auricomus* and *R. acris*.

The effect of treatment on the cover of functional groups was significant only for tall grasses (Table 1, Fig. 5b). The cover of tall grasses reflected former application rates of N as the cover of this functional group was lowest in the control and highest in the N_{400}PK treatment. *H. lanatus* most positively reflected former application rates of fertilizers (Table 1, Fig. 5c). *R. acris*, a short herb species, most negatively reflected the former application rates of fertilizers (Table 1, Fig. 5d). The effect of treatment on vascular plant species richness per 1 m^2 was not significant, but a decreasing number of species from control to N_{400}PK treatment was apparent 16 years after termination of long-term fertilizer application (Table 1, Fig. 5a).

Table 4
Amount of removed nutrients in harvested biomass in 2007. ± values indicate S.D.

Treatment	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	P (kg ha^{-1})	N-tot (kg ha^{-1})
Control	94 ± 14	58 ± 8	13 ± 2.0	11 ± 1.6	96 ± 14
PK	115 ± 20	66 ± 11	17 ± 3.0	17 ± 2.9	112 ± 20
N_{100}PK	114 ± 35	66 ± 20	17 ± 5.1	17 ± 5.1	116 ± 36
N_{200}PK	97 ± 26	67 ± 18	17 ± 4.6	14 ± 3.8	99 ± 27
N_{300}PK	123 ± 40	73 ± 24	17 ± 5.6	17 ± 5.6	125 ± 41
N_{400}PK	106 ± 15	65 ± 9	15 ± 2.1	15 ± 2.1	106 ± 15

No significant effect of treatment was revealed.

4. Discussion

In grasslands, the longevity of residual effects of fertilizer application is believed to be a negative function of site productivity as decades-long residual effects on plant species composition and soil chemical properties have been recorded in extreme soil and climatic conditions (Hegg et al., 1992; Spiegelberger et al., 2006; Hejcman et al., 2007a; Semelová et al., 2008). Results from this experiment show that detectable residual effects of N, P and K fertilizer application can be significant on highly productive grassland even 16 years after termination of long-term fertilizer application.

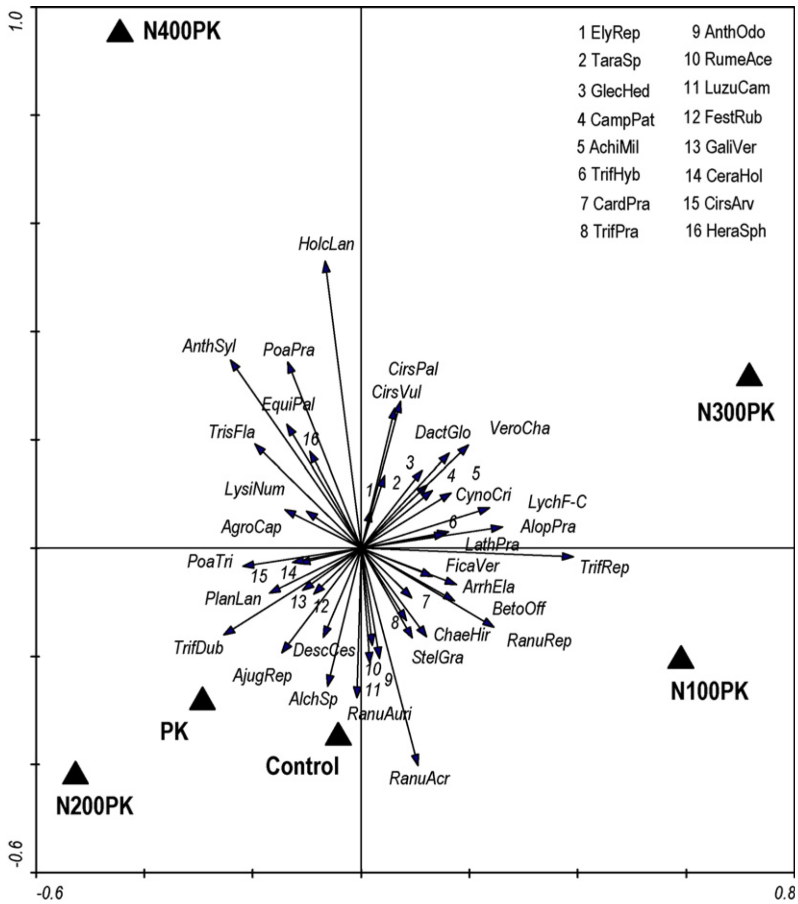

Fig. 4. Ordination diagram showing result of RDA analysis. Species abbreviations: AgroCap, *Agrostis capillaris*; AchiMil, *Achilea millefolium*; AjugRep, *Ajuga reptans*; AlchSp, *Alchemila* sp.; AlopPra, *Alopecurus pratensis*; AnthOdo, *Anthoxanthum odoratum*; AnthSyl, *Anthriscus sylvestris*; ArrhEla, *Arrhenatherum elatius*; BetoOff, *Betonica officinalis*; CampPat, *Campanula patula*; CardPra, *Cardamine pratensis*; CeraHol, *Cerastium holosteoides*; ChaeHir, *Chaerophyllum hirsutum*; CirsArv, *Cirsium arvense*; CirsPal, *Cirsium palustre*; CirsVul, *Cirsium vulgare*; CynoCri, *Cynosurus cristatus*; DactGlo, *Dactylis glomerata*; DescCes, *Deschampsia caespitosa*; ElytRep, *Elytrigia repens*; EquiPal, *Equisetum palustre*; FestRub, *Festuca rubra*; FicaVer, *Ficaria verna*; GaliVer, *Galium verum*; GlecHed, *Glechoma hederacea*; HeraSph, *Heracleum sphondylium*; HolcLan, *Holcus lanatus*; LathPra, *Lathyrus pratensis*; LuzuCam, *Luzula campestris*; LychF-C, *Lychnis flos-cuculi*; LysiNum, *Lysimachia nummularia*; PlanLan, *Plantago lanceolata*; PoaPra, *Poa pratensis*; PoaTri, *Poa trivialis*; RanuAcr, *Ranunculus acris*; RanuAuri, *Ranunculus auricomus*; RanuRep, *Ranunculus repens*; RumeAce, *Rumex acetosa*; StelGra, *Stellaria graminea*; TaraSp, *Taraxacum* sp.; TrifDub, *Trifolium dubium*; TrifHyb, *Trifolium hybridum*; TrifPra, *Trifolium pratense*; TrifRep, *Trifolium repens*; TrisFla, *Trisetum flavescens*; VeroCha, *Veronica chamaedrys*.

4.1. Soil chemical properties

The most prominent was the continuing high concentration of plant-available P and K concentrations in soil of the PK treatment. It must be stressed that increased P availability in this treatment was not a hidden effect of soil pH, because soil pH ranged from 5.5 to 5.7 in all treatments in the experiment. The long-term residual effect of P application is in accordance with many studies from various ecosystems (Silvertown et al., 1994; Malhi et al., 2003; Chiarucci and Maccherini, 2007; Vojta, 2007; Prietzel et al., 2008). The most interesting fact was that the higher P concentration was only observed in the PK treatment, although the N_{100}PK–N_{400}PK treatments received the same amount of P. The same result was recorded in the subplots of this experiment with continuous application of fertilizers (Hrevušová, unpublished data). It is very probable that the higher concentration of plant-available P was present during the period of fertilizer application before 1992. A possible explanation seems to be that N application promoted extraction of P by plant biomass. This explanation is in accordance with results from other long-term studies in which the soil concentration of P was extremely low in treatments with long-term application of N (Shiel and Batten, 1988; van der Woude et al., 1994; Schellberg et al., 1999; Hejcman et al., 2007b). There is no direct explanation for the higher K concentration in the PK treatment because plant-available K is highly mobile in the soil profile and is taken up in high quantities by plants (Kayser and Isselstein, 2005). Conditions for release of K from the soil substratum (minerals) were probably the most favorable in the PK treatment. It is hard to believe that the high K concentration was the direct effect of K application terminated 16 years earlier.

4.2. Biomass chemical properties

Although the effect of treatment on the concentration of nutrients in the plant biomass was not significant 16 years after the last fertilizer application, the lowest concentration of P recorded in biomass from the control was consistent with the lowest plant-available P concentration in the soil. Evidently, the effect of previous fertilizer application had not completely disappeared as P extraction by harvested biomass was still lowest in the control in 2007. The N/P ratio was below 9 in the biomass even in the control, which had at least 40 years without any fertilizer P input. In the investigated alluvial grassland, biomass production was not limited by P, and P limitation can hardly be achieved by long-term two-cut management. The biomass production was N-limited, as shown by the steep decrease in hay yields recorded after termination of N application. Limitation of alluvial grasslands by N rather than P is a well-known phenomenon (Lamers et al., 2006; Beltman et al., 2007).

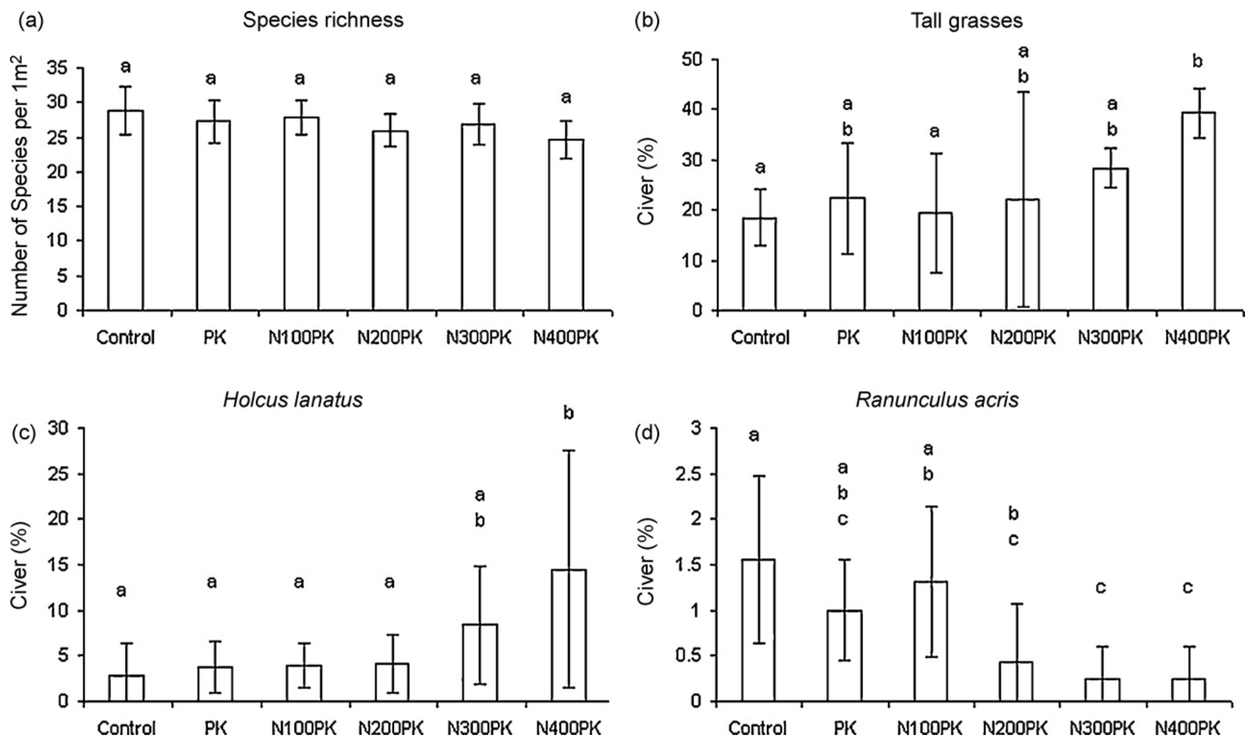

Fig. 5. Effect of treatment on species richness (a), cover of tall grasses (b), cover of *Holcus lanatus* (c) and cover of *Ranunculus acris* (d) 16 years after the last fertilizer application. Using Tukey post hoc test, treatments with the same letter were not significantly different. Error bars indicate S.D.

4.3. Biomass production

The high fluctuation in biomass production and proportions of total biomass of individual cuts after cessation of fertilizer application is in accordance with studies from formerly intensively used grasslands (Willems and van Nieuwstadt, 1996; Niinemets and Kull, 2005). The effect of previous fertilizer treatment on biomass production was significant in selected years only, not in all investigated seasons. In several years, the effect of treatment was obviously overruled by winter or spring flood events. In 2002 for example, an early spring flood occurred and the effect of treatment on biomass production was not significant. This is in accordance with Beltman et al. (2007), who concluded that flood events can completely overrule the effect of fertilizers on biomass production in alluvial grasslands.

4.4. Plant species composition

The longevity and severity of the residual effect on plant species composition are probably a function of fertilizer rates. This was highly apparent in the N_{400}PK treatment where the cover of tall grasses, especially *H. lanatus*, was still significantly increased compared to the control after 16 years without fertilizer. On the other hand, the cover of *R. acris* was still reduced in N_{200}PK–N_{400}PK treatments. This is in accordance with experiment by Královec and Prach (1997) in which increased biomass production of *H. lanatus* was still being recorded 16 years after termination of NPK application. Within the 16 years following termination of fertilizer application, species richness increased substantially in NPK treatments to a level comparable to the control. This is why no significant effect of former fertilizer treatments on species richness was recorded in 2007. The substantial increase in species richness during 16 years without any fertilizer input was apparent from comparison of species richness with the still-fertilized half of the experiment, where species richness was reduced by 50% in fully fertilized treatments in 2005 (Honsová et al., 2007). The increase in species richness was probably due to the decrease in biomass production and saturation by species from the control and areas surrounding the experiment. Similar results were recorded by other authors, who concluded that a decrease in biomass production is a necessary prerequisite for an increase in species richness (Olff and Bakker, 1991; Willems and van Nieuwstadt, 1996).

5. Conclusions

It was concluded that 16 years was not long enough to achieve resilience in plant-available soil P and K concentrations, the N/P ratio in the plant biomass or plant species composition, but it was long enough to achieve resilience in species richness. In the case of biomass production the effect of former fertilizer treatments was still apparent in 10 of the 16 seasons investigated, indicating that resilience in biomass production must be evaluated with data from more than one vegetation season. The results of the study stress the necessity for long-term research because of high year-to-year variability in biomass production and the long after-effects of fertilizer treatments in alluvial grassland.

Acknowledgements

The authors are deeply indebted to Prof. Ing. Jiří Velich, DrSc., the initiator of the experiment. Thanks go to the editor and two anonymous referees for their useful comments. The research was financially supported by grant MŠMT 6046070901 and MŠMT 2B06012. M. Hejcman and V.V. Pavlů were supported by projects MA 0002700601 and GAČR 521/08/1131.

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