

The Rengen Grassland Experiment: relationship between soil and biomass chemical properties, amount of elements applied, and their uptake

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Abstract The Rengen Grassland Experiment (RGE) was established in the Eifel Mountains (Germany) on a low productive *Nardetum* in 1941. Since then, the following fertilizer treatments have been applied with a late two-cut system: unfertilized control, Ca, CaN, CaNP, CaNPKCl and CaNPK₂SO₄. We aimed to understand how concentrations of macro (N, P, K, Ca and Mg), micro (Cu, Fe, Mn and Zn) and trace (As, Cd, Cr, Ni and Pb) elements in the plant biomass were affected by long-term fertilizer application, soil chemical properties and biomass production. In 2008, biomass samples from the first cut (early July)

and the second cut (mid-October) were collected and analyzed. The simultaneous application of N, P and K decreased nitrogen concentration in the aboveground biomass, but substantially increased biomass production. Late cutting management decreased forage quality in highly productive more than in low productive plant communities. The concentrations of P and K in the plant biomass were positively related to P and K application and, therefore, to plant available P and K concentrations in the soil. The concentrations of some micro (Fe, Mn and Zn) and trace (As, Cd, Cr, Ni and Pb) elements in the plant

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biomass were negatively correlated with the amount of elements supplied by fertilizers and biomass production, probably because of the dilution effect. Long-term fertilizer application resulted in the accumulation of macro (P, Ca and Mg), micro (Fe and Mn) and trace (As and Cr) elements in the soil, but in many cases this accumulation was not connected with an increase in the concentrations of these elements in the plant biomass. Nutritional status, as indicated by the biomass N:P ratio, was consistent with N or P limitation as indicated by the nitrogen and phosphorus nutrition indices. Furthermore, additional K (co-) limitation was indicated by the N:K and K:P ratios in the biomass from the NP treatment. The results from the RGE indicate that there is no simple positive relationship between the applied elements and their concentrations in the plant biomass.

Keywords Biomass production · Dilution effect · Forage quality · Heavy metal concentrations · Long-term fertilizer experiment · Nitrogen and phosphorus nutrition index · N:P, N:K and K:P ratios

Abbreviations

DM	Dry matter yield of aboveground biomass
NNI and PNI	Nitrogen and phosphorus nutrition indices
RGE	The Rengen Grassland Experiment

Introduction

Nutrient concentrations and the N:P, N:K and K:P ratios in plant biomass have been used widely for the diagnosis of plant nutrient status to identify nutrient limitations in biomass production. This approach was originally developed for field crops to make recommendations for appropriate fertilizer application (Walworth et al. 1986; Bailey et al. 2000). Recently, the biomass nutrient concentration and ratio approach has even been used for natural and semi-natural species-rich grasslands and wetlands to identify growth limitation by nutrients. This approach aims to predict the effects of nitrogen deposition, desiccation or defoliation management on the development of plant communities (Güsewell and Koerselman 2002; Wassen et al. 2005; Klauisová et al. 2009; Olde Venterink et al. 2009).

An alternative approach to identifying nutrient limitation is using nutrition indices for the nitrogen (NNI) and phosphorus (PNI; Duru and Ducrocq 1997; Lemaire 1997) used widely for arable crops (Bélanger et al. 2001; Ziadi et al. 2008) and grasslands (Jouany et al. 2004; Garnier et al. 2007; Schnyder et al. 2010). The major advantage of this approach is that it reflects the typical dilution effect, i.e., the decrease in N and P concentrations during the progressive accumulation of aboveground standing biomass. The comparison of biomass N:P, N:K and K:P ratios together with NNI and PNI can reveal if the nutrient ratio and nutrient index approaches provide comparable results.

Furthermore, concentrations of elements in the biomass are relevant to animal nutrition and can determine forage quality (Tallowin and Jefferson 1999; Donath et al. 2004). For example, optimal N and P concentrations in forage for dairy cattle range from 19.2 to 25.6 mg kg⁻¹ and from 2.3 to 3.7 mg kg⁻¹ (Whitehead 2000). The imbalance of macro elements, especially the Ca:P and K:Ca+Mg ratios, can result in serious health problems, explaining why forage quality is additionally evaluated regarding the ratios of macro elements (Hopkins et al. 1994; Pelletier et al. 2008). The concentration of trace elements, especially As, Cd, Cr and Pb, must be as low as possible in forage because these elements are toxic for animals as well as humans. Many applied fertilizers contain these elements, which accumulate in the soil in considerable amounts if fertilizers are applied over many years (Chen et al. 2007; Lipoth and Schoenau 2007; Čásová et al. 2009; Francois et al. 2009; Pérez and Anderson 2009; Uprety et al. 2009).

This raises the question of whether the uptake of elements by the aboveground biomass and its concentration is related to the amount of elements supplied by fertilizers, and how this interacts with soil chemical properties. Multivariate data analysis techniques such as ordinations (Lepš and Šmilauer 2003) enable the simultaneous analysis of huge amounts of soil and biomass chemical data together. Another advantage of the ordinations approach is that results can be visualized in the form of one ordination diagram, enabling the simple identification of relationships among all analyzed data.

In this paper, biomass chemical properties and their relationships to the amount of applied elements, biomass production and soil chemical properties were analyzed in the Rengen Grassland Experiment (RGE)

established in the Eifel Mountains of Germany in 1941 (Schellberg et al. 1999). The RGE is probably the oldest properly designed live grassland fertilizer experiment worldwide.

The objective of this paper was to investigate the interaction of biomass production, soil and biomass chemical properties, amount of applied elements and their uptake after 67 years of permanent Ca, N, P and K fertilizer application on grasslands. Within this context, we tried to answer the following questions: (1) how is the concentration of macro (N, P, K, Ca and Mg), micro (Cu, Fe, Mn and Zn) and trace (As, Cd, Cr, Ni and Pb) elements in the plant biomass affected by long-term fertilizer application?; (2) how is the concentration of all elements in the plant biomass affected by the soil chemical properties and nutrient-driven biomass production?; (3) how is the nutritional status of the vegetation indicated by the N:P, N:K and K:P ratios in the plant biomass and NNI and PNI affected by a fertilizer regime?; and (4) what is the difference between the uptake and application rate for all analyzed elements?

Materials and methods

Study site and treatments

In 1941, the fertilizer experiment was set up at the Rengen Grassland Research Station of the University of Bonn in the Eifel Mountains (Germany, 50°13'N, 6°51'E) at 475 m a.s.l. At the study site, the mean annual precipitation is 811 mm and mean annual temperature is 6.9°C (Rengen meteorological station).

The soil is a pseudogley as indicated by the temporarily wet conditions in the turf layer after rainfall in winter and spring and the slow vertical rise of capillary water in summer. The experiment is arranged in a completely randomized block design with five fertilizer treatments (treatment B=Ca; C=Ca/N; D=Ca/N/P; E=Ca/N/P/KCl; F=Ca/N/P/K₂SO₄) and the same number of replicates (see Fig. 1 and Table 1 for details).

As an unlimed control was originally missing in the block structure of the RGE, five replicates of a treatment without any fertilizer input (referred to as treatment A) were added in 1998. The treatments were introduced next to the existing plots on an area exhibiting the same soil properties. This area was

protected as an unfertilized control, has never been fertilized and has been cut at the same time that the experimental plots were harvested. In the past 45 years, all experimental plots, including the unfertilized A treatment, have been cut twice a year in late June or early July and in mid-October. The soil chemical properties in A–F treatments analyzed recently by Hejcman et al. (2009) are given in Table 2. The classification of plant communities into alliances (Chytrý et al. 2009) together with tree main graminoid, legume and forb species in each treatment are given in Table 3.

Aboveground biomass production and sample collection

In 2008, aboveground fresh biomass was measured by cutting the sward in each plot at a height of approximately 2 cm in swaths of 1 m by 5 m in early July and mid-October. Sub-samples of approximately 0.5 kg were then taken from the cut material and oven-dried at 60°C for 48 h to determine the dry matter content of the harvest and its total yield. These samples were used for chemical analyses. As an average throughout the last 20 years, above ground biomass harvested with the first cut was 1.79 (0.69), 2.07 (0.48), 2.69 (0.53), 3.17 (0.68) and 3.22 (0.92) t ha⁻¹ in the treatments Ca, CaN, CaNP, CaNPKCl and CaNPK₂SO₄, respectively (SD in brackets). Hence, growth conditions were comparable with other years and therefore results of chemical analysis were suggested sufficiently representative to give reliable estimation of element budget within the RGE.

Chemical analysis of biomass

The results of the total content of N in plant samples was determined by the Kjeldahl method after wet ashing in an electrically heated block (Digestion system 20–1015 Digester) with concentrated H₂SO₄ (98%) and a mixed catalyst at 420°C using a Kjeltac Auto 1030 analyzer. For the determination of P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn, the plant samples were decomposed using the dry ashing procedure as follows: an aliquot (~1 g) of the dried and powdered aboveground biomass and roots were weighed to 1 mg, placed into a borosilicate glass test tube and decomposed in a mixture of oxidizing gases (O₂ + O₃+NO_x) at 400°C for 10 h in a Dry Mode

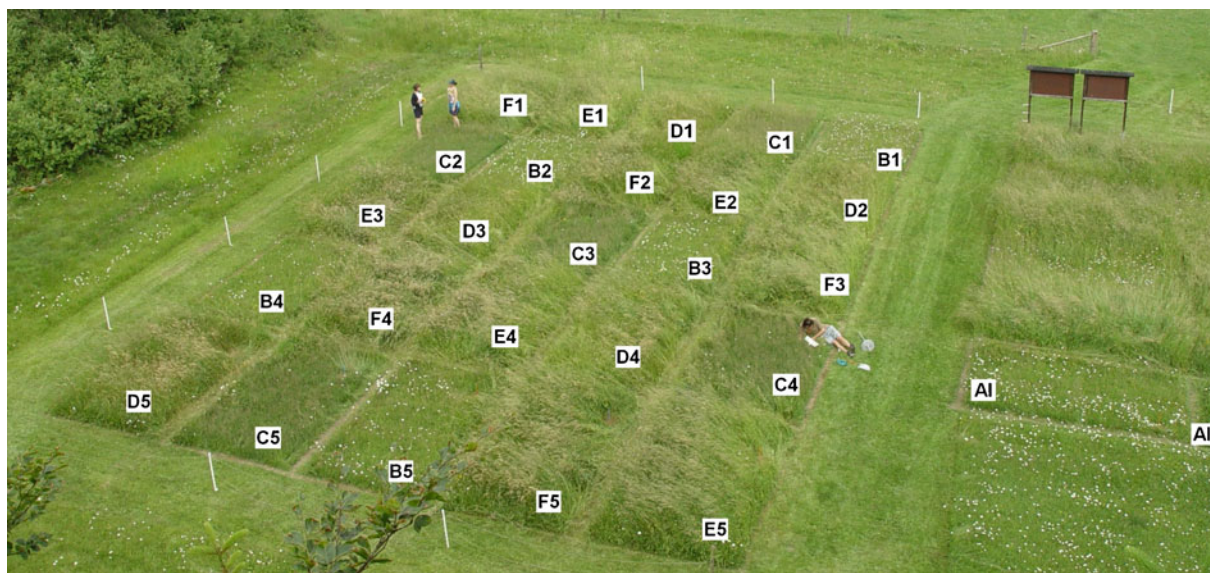


Fig. 1 Aerial photograph of the Rengen Grassland Experiment (RGE) taken in late June 2006 (photo M. Hejzman[©]). Treatment abbreviations are given in Table 1. Letters indicate

type of fertilizer treatment and numbers represent randomized plot replicates in blocks 1–5. Four control plots without fertilization (AII–AV) are outside the area photographed

Mineralizer Apion (Tessek, Prague, Czech Republic). The ash was dissolved in 20 mL 1.5% HNO₃ (electronic grade purity, Analytika, Prague, Czech Republic) and kept in glass tubes until analysis (Miholová et al. 1993). Aliquots of the certified

reference material RM NCS DC 73350 poplar leaves (purchased from Analytika) were mineralized under the same conditions of the total arsenic concentrations in experimental plants for quality assurance. The ash was then heated for 5–10 min at 100°C in 3 ml aqua

Table 1 Mean amounts of elements supplied annually to the soil of treatments in the Rengen Grassland Experiment (RGE)

Element	Treatment					
	A Control	B Ca	C CaN	D CaNP	E CaNPKCl	F CaNPK ₂ SO ₄
N (kg ha ⁻¹)	–	–	100	100	100	100
P (kg ha ⁻¹)	–	–	–	35	35	35
K (kg ha ⁻¹)	–	–	–	–	133	133
Ca (kg ha ⁻¹)	–	715	752	936	936	936
Mg (kg ha ⁻¹)	–	67	67	75	90	75
As (g ha ⁻¹)	–	1.2	1.4	4	4	4
Cd (g ha ⁻¹)	–	0.1	0.1	0.3	0.4	0.4
Cr (g ha ⁻¹)	–	0.3	1	319	321	319
Cu (g ha ⁻¹)	–	9	11	34	36	37
Fe (g ha ⁻¹)	–	4	149	2,591	2,652	2,594
Mn (g ha ⁻¹)	–	0.2	34	744	745	745
Ni (g ha ⁻¹)	–	2	2	4	5	4
Pb (g ha ⁻¹)	–	1	12	15	15	15
Zn (g ha ⁻¹)	–	0.1	10	12	14	14

Table 2 Soil chemical properties in a 0–10 cm layer

	Element	Treatment					F CaNPK ₂ SO ₄
		A Control	B Ca	C CaN	D CaNP	E CaNPKCl	
	P (mg kg ⁻¹) ^a	15	6	4	311	226	222
	K (mg kg ⁻¹) ^a	43	25	23	32	94	105
	pH	4.9	6.5	6.5	6.6	6.5	6.6
	N (%) ^b	0.373	0.35	0.364	0.363	0.363	0.367
	C (%) ^b	4.9	4.2	4.4	4.3	4.4	4.5
	C:N	13.1	12	12	11.8	12	12.3
	Mg (mg kg ⁻¹) ^c	132	199	195	199	207	207
	As (mg kg ⁻¹) ^c	0	0	0	0.004	0.009	0.014
^a Plant available portion extracted with a calcium-acetate-lactate solution	Cd (mg kg ⁻¹) ^c	0.044	0.020	0.011	0.011	0.010	0.013
	Cr (mg kg ⁻¹) ^c	0.029	0.033	0.025	0.031	0.043	0.026
	Cu (mg kg ⁻¹) ^c	0.4	0.4	0.7	0.6	0.3	0.7
^b Total contents determined by elemental analyses (Carlo Erba, Italy)	Fe (mg kg ⁻¹) ^c	5.2	2.2	1.7	1.8	1.3	2.6
	Mn (mg kg ⁻¹) ^c	4.6	2.1	2.2	0.9	3.0	1.7
^c Plant available portion extracted with 0.01 mol l ⁻¹ of a calcium chloride solution	Ni (mg kg ⁻¹) ^c	0.3	0.4	0.4	0.3	0.3	0.3
	Pb (mg kg ⁻¹) ^c	0.2	0.1	0.3	0.3	0.3	0.2
	Zn (mg kg ⁻¹) ^c	0.19	0.04	0.04	0.03	0.07	0.05

regia, transferred quantitatively to test tubes and filled up to 20 ml with deionized water before being kept until the analysis (Street et al. 2006).

The determination of Cd, Cr, Cu, Fe, Mn, Ni, P, Pb and Zn in plant digests was carried out by inductively coupled plasma optical emission spectrometry with axial plasma configuration (ICP-OES–Varian VistaPro, Mulgrave, Australia), equipped with an autosampler SPS-5, at spectral lines $\lambda=226.5$ nm for Cd, $\lambda=205.6$ nm for Cr, $\lambda=327.4$ nm for Cu, $\lambda=238.3$ nm for Fe, $\lambda=257.6$ nm for Mn, $\lambda=231.6$ nm for Ni, $\lambda=214.9$ nm for P, $\lambda=220.4$ nm for Pb and $\lambda=206.2$ nm for Zn. For the determination of Ca, K and Mg, atomic absorption spectroscopy (Faas, Varian SpectraAA=300) was used. The total arsenic concentrations in the digests were determined by hydride generation atomic absorption spectrometry (Varian AA280Z), equipped with continuous hydride generator VgA-77, where a mixture of potassium iodide and ascorbic acid was used for the pre-reduction of the sample and the extract was acidified by HCl before measurement.

Calculation of N and P nutrition indices

The dry matter yield (DM in t ha⁻¹) and herbage N and P concentrations were used to calculate the NNI

and PNI indices. Both these indices are derived from the ratio, on a percentage basis, of the actual (measured) nutrient content to the critical content, which corresponds to the minimal concentration in the sward needed to produce the maximum amount of dry matter, i.e., the nutrient content allowing potential growth. For N, the critical curve given by Lemaire (1997) is as follows: $NNI = \%N_{\text{measured}} / \%N_{\text{critical}} * 100$, with $\%N_{\text{critical}} = 4.8 * (DM)^{-0.32}$. For P, the critical curve given by Duru and Ducrocq (1997) is as follows: $PNI = (P\%_{\text{measured}} / P\%_{\text{critical}}) * 100$, with $P\%_{\text{critical}} = 0.15 + 0.065 N\%_{\text{measured}}$. A value of NNI or PNI of 100 indicates optimal N or P nutrition, whereas values below 60 indicate severe N and P deficiency that limit biomass production.

Data analysis

All univariate analyses were performed using Statistica 8.0 software (StatSoft, Tulsa, OK). One-way ANOVA followed by post-hoc comparison using Tukey's test was used to identify significant differences among treatments. The relationship between biomass production and the concentration of N in the biomass was analyzed by linear regression. A redundancy analysis (RDA) in the CANOCO 4.5

Table 3 Classification of plant communities into alliances and cover (in %) of three main graminoids, forbs and legumes species in each treatment in 2006

Treatment	B		C		D		E		F	
	Ca		CaN		CaNP		CaNPKCl		CaNPK ₂ SO ₄	
Alliance	<i>Violin caninae</i>		<i>Polygono-Trisetion</i>		<i>Arrhenatherion</i>		<i>Arrhenatherion</i>		<i>Arrhenatherion</i>	
Graminoids	<i>Briza media</i> (14%) <i>Festuca sp.</i> (13%) <i>Helictotrichon pubescens</i> (4%)		<i>Briza media</i> (43%) <i>Festuca sp.</i> (3%) <i>Carex panicea</i> (3%)		<i>Briza media</i> (65%) <i>Carex panicea</i> (10%) <i>Carex flacca</i> (4%)		<i>Trisetum flavescens</i> (30%) <i>Arrhenatherum elatius</i> (17%) <i>Alopecurus pratensis</i> (13%)		<i>Trisetum flavescens</i> (19%) <i>Arrhenatherum elatius</i> (16%) <i>Alopecurus pratensis</i> (15%)	
Forbs	<i>Plantago lanceolata</i> (17%) <i>Rhinanthus minor</i> (9%) <i>Leucanthemum vulgare</i> (6%)		<i>Plantago lanceolata</i> (7%) <i>Leucanthemum vulgare</i> (7%) <i>Succisa pratensis</i> (7%)		<i>Plantago lanceolata</i> (5%) <i>Succisa pratensis</i> (4%) <i>Taraxacum sp.</i> (3%)		<i>Galium album</i> (12%) <i>Plantago lanceolata</i> (5%) <i>Rumex acetosa</i> (2%)		<i>Holcus lanatus</i> (7%) <i>Galium album</i> (13%) <i>Plantago lanceolata</i> (2%)	
Legumes	<i>Lathyrus linifolius</i> (9%) <i>Trifolium pratense</i> (6%) <i>Lotus corniculatus</i> (4%)		<i>Lotus corniculatus</i> (10%) <i>Trifolium pratense</i> (8%) <i>Trifolium repens</i> (2%)		<i>Lotus corniculatus</i> (8%) <i>Trifolium pratense</i> (3%) –		<i>Taraxacum sp.</i> (3%) <i>Lotus corniculatus</i> (1%) <i>Trifolium pratense</i> (1%)		<i>Crepis biennis</i> (2%) <i>Trifolium hybridum</i> (2%) <i>Lotus corniculatus</i> (1%) <i>Lathyrus pratensis</i> (1%)	

program (ter Braak and Šmilauer 2002) was applied to evaluate all data together. RDA was used because environmental variables (treatments) were in the form of categorical predictors (dummy variables). Standardization by species (dependent variables) was used because the data analyzed were of various types and units. Furthermore, a Monte Carlo permutation test with 999 permutations was used to reveal whether the treatments had a significant effect on all analyzed data. The results of the multivariate analyses were visualized in the form of a bi-plot ordination diagram by the CanoDraw program (<http://www.canodraw.com/>).

Results

Aboveground biomass production

Dry matter biomass of the first cut ranged from 1.4 to 4.1 t ha⁻¹ (Table 4), whereas biomass of the second cut ranged from 1.2 to 4.1 t ha⁻¹ (Table 5). In both cuts, there were significant differences among the A, B and C treatments without P against the D, E and F treatments with N and P application. Total biomass production (both cuts together) was affected significantly by treatments, and ranged from 2.6 to 8.1 t ha⁻¹ in A and E treatments, respectively (Table 6).

Concentration of elements in the biomass

Concentrations of elements in the biomass are presented in Tables 4 and 5 for first and second cut, respectively. Noteworthy significant differences in many elements were recorded, mainly between highly productive P fertilized (D, E and F) against low productive treatments without P application (A, B and C). This explains why the concentrations of N, Ca, Mg, As, Fe, Mn, Ni and Zn were negatively related to biomass production (N especially, Fig. 2) and the concentration of P was positively related to it. Generally, higher concentrations of N, Ca, Mg, As, Fe, Mn, Ni and Zn were recorded in treatments without P application (A–C), whereas a higher concentration of P was recorded in treatments with P application. Concentrations of Cd and Mn in both cuts and Cr, Cu and Ni in the second cut were highest in the never fertilized control (A). The concentration of K was highest in treatments with K application (E and F).

Table 4 Mean biomass yield, concentration of elements, nitrogen nutrition index (NNI), phosphorus nutrition index (PNI) and main nutrients ratios in first cut biomass. Treatment

abbreviations are given in Table 1. Using Tukey's post-hoc test, treatments with the same letter were not significantly different

Analyzed variable	Treatment						
	<i>P</i> value	A Control	B Ca	C CaN	D CaNP	E CaNPKCl	F CaNPK ₂ SO ₄
Yield (t ha ⁻¹)	<0.001*	1.4 a	1.9 ab	2.0 ab	2.6b c	4.1 d	3.2 cd
N (g kg ⁻¹)	<0.001*	14 ab	14 ab	17 a	10b c	7 c	8 c
NNI	<0.001*	33 abc	36 ab	43 a	28 bc	23 c	23 c
P (g kg ⁻¹)	<0.001*	0.98 ab	1.0 ab	0.93 a	2.1 d	1.5 c	1.6 cd
PNI	<0.001*	40 a	42 a	36 a	96 b	76 c	80 bc
N:P	<0.001*	14.9 ab	13.6 b	17.7 a	4.8 c	4.8 c	4.8 c
K (g kg ⁻¹)	<0.001*	11 ab	14 ad	8 bc	6 c	16 d	15 d
N:K	<0.001*	1.4 abc	1.0 bc	2.2 a	1.8 ab	0.4 c	0.5 c
K:P	<0.001*	11.9 a	14 a	8.5 ab	3.3 b	10.9 a	9.7 a
Ca (g kg ⁻¹)	<0.001*	8.9 ab	10 b	6.7 abc	5.2 bc	4.0 c	5.3 c
Ca:P	0.027*	2.8 ab	2.6 ab	2.3 a	2.4 a	2.7 ab	3.6 b
Mg (g kg ⁻¹)	<0.001*	3.2 ab	4.0 a	3.0 bc	2.2 cd	1.5 d	1.5 d
As (mg kg ⁻¹)	<0.001*	0.18 a	0.13 a	0.43 b	0.07 a	0.11 a	0.06 a
Cd (mg kg ⁻¹)	<0.001*	0.46 a	0.25 b	0.20 b	0.27 b	0.24 b	0.25 b
Cr (mg kg ⁻¹)	0.170	0.88	1.02	0.56	0.78	0.76	0.63
Cu (mg kg ⁻¹)	0.099	8.1	7.8	8.1	4.8	6.5	6.7
Fe (mg kg ⁻¹)	<0.001*	337 a	335 a	120 b	86 b	73 b	95 b
Mn (mg kg ⁻¹)	<0.001*	151 a	48 b	35 b	31 b	31 b	32 b
Ni (mg kg ⁻¹)	<0.001*	0.18 a	0.13 a	0.43 b	0.07 a	0.11 a	0.06 a
Pb (mg kg ⁻¹)	0.445	0.6	0.4	0.4	0.3	0.3	0.5
Zn (mg kg ⁻¹)	<0.001*	43 a	27 b	29 b	17 bc	14 c	18 bc

**P* value significant (probability value obtained from one-way ANOVA)

Nutritional status and elements ratios

In the first cut biomass, NNI ranged from 23 in the E and F treatments to 43 in the C treatment, and the effect of treatment was significant (Table 4). In the second cut biomass, NNI ranged from 36 to 50 in the A and C treatments, respectively, and the effect of treatment was significant as well (Table 5). The effect of treatment on PNI was significant in both cuts—PNI ranged from 36 to 55 in the A–C treatments and from 76 to 149 in the D–F treatments. In both cuts, a highly contrasting N:P ratio in the plant biomass was recorded between treatments with and without P application. The N:P ratio ranged from 12.6 to 18.9 in the A–C treatments and from 4.4 to 5.5 in the D–F treatments. The N:K ratio was highly affected by K application; it was below 1 in the E and F treatments

(with K application) and above 1 in all other treatments. The K:P ratio was significantly affected by treatment. In the first cut biomass, the K:P ratio ranged from 3.3 to 14 in the D and B treatments, respectively, and in the second cut biomass, the K:P ratio varied between 2 and 8.7 in the D and B treatments, respectively. The Ca:P ratio ranged from 2.3 to 3.6 in the first cut biomass and from 2.1 to 10 in the second cut biomass.

Annual uptake of elements by aboveground biomass

As expected, the uptake of N, P, K, Mg, As, Cd, Cr, Cu and Mn by harvested aboveground biomass was significantly affected by treatment (Table 6). The uptake of N was between 39 and 77 kg ha⁻¹ in accordance with N application in the A and E

Table 5 Mean biomass yield, concentration of elements, NNI, PNI and main nutrients ratios in second cut biomass. Treatment abbreviations are given in Table 1. Using Tukey's post-hoc test,

treatments with the same letter were not significantly different (probability value of 0.05)

Analyzed variable	Treatment						
	<i>P</i> value	A Control	B Ca	C CaN	D CaNP	E CaNPKCl	F CaNPK ₂ SO ₄
Yield (t ha ⁻¹)	<0.001*	1.2 a	1.5 a	1.5 a	2.9 b	4.0 c	4.1 c
N (g kg ⁻¹)	<0.001*	16 a	19 ab	21 b	16 a	12 c	12 c
NNI	0.009*	36 a	44 ab	50 b	46 ab	39 ab	39 ab
P (g kg ⁻¹)	<0.001*	1.2 a	1.5 a	1.1 a	3.5 b	2.3 c	2.2 c
PNI	<0.001*	47 a	55 a	40 a	140 b	102 c	98 c
N:P	<0.001*	13.6 a	12.6 a	18.9 b	4.4 c	5.2 c	5.5 c
K (g kg ⁻¹)	<0.001*	8.7 ab	13 cd	9.2 abc	7.2 a	15 d	12 bcd
N:K	<0.001*	1.9 ab	1.5 ac	2.4 b	2.3 b	0.8 c	1 c
K:P	<0.001*	7.2 ab	8.7 b	8.0 ab	2.0 c	6.3 ad	5.3 d
Ca (g kg ⁻¹)	<0.001*	11.5 a	14.8 b	9.2 a	8.8 a	4.7 c	5.0 c
Ca:P	<0.001*	9.4 ab	10 a	8.1 b	2.5 c	2.1 c	2.3 c
Mg (g kg ⁻¹)	<0.001*	3.2 a	4.0 b	3.1 a	3.0 a	1.6 c	1.3 c
As (mg kg ⁻¹)	0.013*	0.26 a	0.24 ab	0.25 ab	0.20 ab	0.10 b	0.13 ab
Cd (mg kg ⁻¹)	0.005*	0.23 a	0.14 ab	0.13 ab	0.10 b	0.17 ab	0.07 b
Cr (mg kg ⁻¹)	<0.001*	4.3 a	1.7 b	0.9 b	0.6 b	0.6 b	0.4 b
Cu (mg kg ⁻¹)	<0.001*	5.4 a	5.6 a	4.6 a	4.2 a	4.5 b	2.6 b
Fe (mg kg ⁻¹)	0.413	195	327	304	137	148	120
Mn (mg kg ⁻¹)	0.008*	153 a	82 b	69 b	99 ab	73 b	68 b
Ni (mg kg ⁻¹)	<0.001*	2.8 a	1.7 b	0.79 c	0.78 c	0.65 c	0.62 c
Pb (mg kg ⁻¹)	0.453	1.14	0.48	0.26	0.17	0.38	0.25
Zn (mg kg ⁻¹)	<0.001*	38 a	30 ba	27 b	24 bc	15 c	16 c

**P* value significant (probability value obtained from one-way ANOVA)

treatments, respectively, whereas that of P was substantially increased by P application, and ranged from 2.8 to 15.6 kg ha⁻¹ in the A and D treatments, respectively. The same pattern, a highly positive effect of K application, was observed for the uptake of K. Magnesium had not been applied as a major fertilizer element but differed with the amount applied together with lime. The uptake of Mg ranged from 8.1 to 14.3 kg ha⁻¹ in the A and D treatments, respectively. No significant effect of treatment on the uptake of Ca, Fe, Ni, Pb and Zn was recorded.

The effect of treatment on the difference between applied and removed elements, i.e., simplified nutrient balance, was significant for all analyzed elements (Table 7). For example, a negative simplified balance was recorded for all analyzed elements in the A and B treatments, whereas it was positive

for the majority of analyzed elements in the F treatment.

Relationship among biomass production, soil and biomass chemical properties, amount of applied elements and their uptake

Calculated by RDA, the effect of treatment on all analyzed data measured on soil and biomass together was significant ($F=8.9$; $P=0.001$) and explained 65% of the total data variability. The relationships among all analyzed data and their responses to fertilizer treatments are clearly visible from the ordination diagram in Fig. 2. In that diagram, the length and direction of the vectors relating to the individual elements indicate the association of elements with their respective treatments. Furthermore, the angle between the two

Table 6 Annual biomass yield and uptake of macro, micro and toxic elements by harvested biomass (first and second cut together). Treatment abbreviations are given in Table 1. Using

Tukey's post-hoc test, treatments with the same letter were not significantly different (probability value of 0.05)

Analyzed variable	Treatment						
	P value	A Control	B Ca	C CaN	D CaNP	E CaNPKCl	F CaNPK ₂ SO ₄
Yield (t ha ⁻¹)	<0.001*	2.6 a	3.4 a	3.5 a	5.5 b	8.1 c	7.3 c
N (kg ha ⁻¹)	<0.001*	39 a	54 ab	64 b	72 b	77 b	74 b
P (kg ha ⁻¹)	<0.001*	2.8 a	4.1 a	3.6 a	15.6 b	15.3 b	14.3 b
K (kg ha ⁻¹)	<0.001*	26 a	45 a	30 a	37 a	125 b	98 c
Ca (kg ha ⁻¹)	0.051	26	42	27	38	35	38
Mg (kg ha ⁻¹)	<0.001*	8.1 a	13.6 bc	10.3 ac	14.3 b	12.4 bc	10.3 ac
As (g ha ⁻¹)	0.030*	0.58 a	0.6 a	1.24 b	0.76 ab	0.87 ab	0.76 ab
Cd (g ha ⁻¹)	<0.001*	0.91 a	0.66 a	0.58 a	0.98 a	1.67 b	1.05 a
Cr (g ha ⁻¹)	0.016*	6.5 a	4.5 ab	2.4 b	3.8 ab	5.6 ab	3.8 ab
Cu (g ha ⁻¹)	0.017*	18 a	23 ab	23 ab	25 ab	37 b	31 ab
Fe (g ha ⁻¹)	0.277	1,555	1,163	677	630	880	801
Mn (g ha ⁻¹)	<0.001*	392 a	213 ab	169 b	359 ab	407 a	382 a
Ni (g ha ⁻¹)	0.187	3.6	2.7	2.0	2.4	3.0	2.7
Pb (g ha ⁻¹)	0.117	2.3	1.6	1.1	2.2	2.8	2.5
Zn (g ha ⁻¹)	0.345	105	94	98	113	118	122

*P value significant (probability value obtained from one-way ANOVA)

selected elements indicates their mutual relationship, whereas vector length indicates the importance for the result of the analysis. For example, there was a negative correlation between the amount of applied Zn (ZnA) and plant available Zn concentration in the soil (ZnS) because the vectors for these two Zn concentrations point in opposite directions (ZnA and ZnS vectors included an angle bigger than 90°). ZnA was negatively correlated with Zn concentration in the first and second cut biomass (Zn1 and Zn2). Both were highly positively correlated because their vectors were located close together, pointing in the same direction, whereas the angle between them was acute. Furthermore, Zn1 and Zn2 were highest in the A, B and C treatments because the Zn1 and Zn2 vectors were directed towards the A, B and C treatments.

Similar patterns of negative correlation between the amounts of applied elements and concentration in the biomass were recorded for many other elements (As, Cd, Cr, Cu, Fe, Mn, N, Ni and Pb). Zn uptake (ZnU) was predetermined more by yield rather than the concentration of Zn in the biomass. This becomes clear from the negative correlation between ZnU and the Zn concentration in the biomass (Zn1 and Zn2), and the positive

correlation between ZnU and yield (DM). For several other elements (As, Cd, Cu, Mn, N and Pb), this pattern of element uptake was predetermined more by biomass yield rather than the concentration of the element in the biomass. In the case of Cr and Ni, uptake was predetermined by the concentration of elements in the biomass rather than by biomass yield.

In the case of P, there was a highly positive correlation between the amount of applied P (PA), plant available concentration of P in the soil (PS), concentration of P in the biomass (P1 and P2) and P uptake (PU). All P data were highly positively correlated with yield because vectors for all P data and yield (DM) were close together, making acute angles in the ordination diagram. A similar “pattern” was also recorded for K.

Discussion

Aboveground biomass production

Biomass production in the treatments B–F was comparable, with mean values from 1989–1995

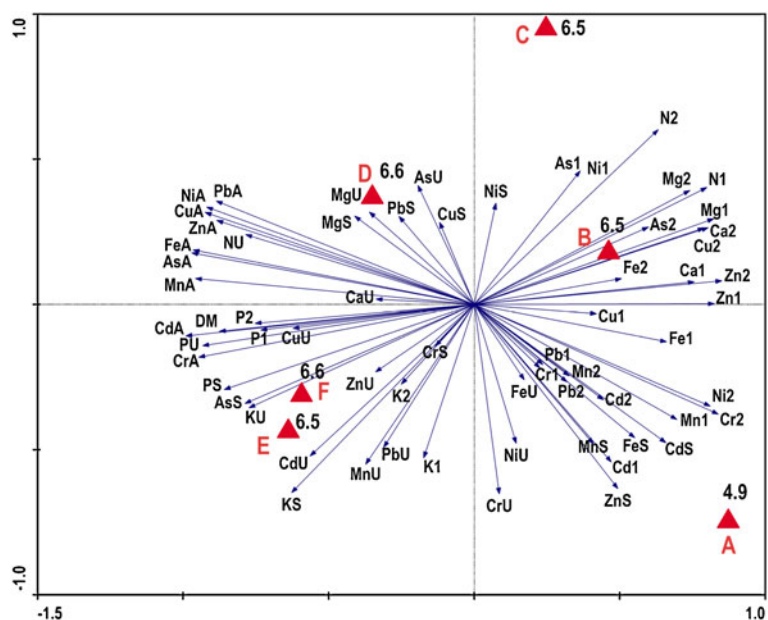


Fig. 2 Ordination diagram showing the result of the redundancy analysis (RDA) of plant available concentration of elements in a 0–10 cm soil layer (element abbreviation supplemented by letter *S*=soil), concentration of elements in the first and second cut biomass (element abbreviation supplemented by number *1* or *2* for the first and second cut, respectively), uptake of elements by

harvested biomass (element abbreviation supplemented by *U*=uptake), amount of elements applied by fertilizers (element abbreviation supplemented by *A*=applied) and dry matter biomass production (*DM*). Treatment abbreviations (A – F) are given in Table 1. Numbers above triangles indicate pH (CaCl₂) in a 0–10 cm soil layer in each treatment

Table 7 Annual balance of elements. Negative values indicate the depletion of the element by harvested biomass uptake, whereas positive values indicate a surplus and possible leaching or accumulation of the element in the RGE. Treatment

abbreviations are given in Table 1. Using Tukey's post-hoc test, treatments with the same letter were not significantly different (probability value of 0.05)

Analyzed variable	Treatment						
	<i>P</i> value	A Control	B Ca	C CaN	D CaNP	E CaNPKCl	F CaNPK ₂ SO ₄
N (kg ha ⁻¹)	<0.001*	-39 a	-54 a	36 b	28 b	23 b	26 b
P (kg ha ⁻¹)	<0.001*	-2.8 a	-4.1 a	-3.6 a	19.4 b	19.7 b	20.7 b
K (kg ha ⁻¹)	<0.001*	-26 a	-45 a	-30 a	-37 a	8 b	35 b
As (g ha ⁻¹)	<0.001*	-0.6 a	0.6 b	0.2 b	3.2 c	3.1 c	3.2 c
Cd (g ha ⁻¹)	<0.001*	-0.9 ab	-0.6 a	-0.5 a	-0.7 a	-1.3 b	-0.7 a
Cr (g ha ⁻¹)	<0.001*	-6.5 a	-4.2 ab	-1.8 b	316 c	315 c	316 c
Cu (g ha ⁻¹)	<0.001*	-18 a	-14 ab	-12 ab	9 c	-1 bc	6 c
Fe (g ha ⁻¹)	<0.001*	-1,555 a	-1,159 a	-528 a	1,961 b	1,772 b	1,793 b
Mn (g ha ⁻¹)	<0.001*	-392 a	-213 ab	-135 b	385 c	339 c	362 c
Ni (g ha ⁻¹)	<0.001*	-3.6 a	-1 b	0.3 bc	1.1 c	1.5 c	0.9 c
Pb (g ha ⁻¹)	<0.001*	-2.3 a	-1 a	10.4 b	13.4 c	12.2 bc	12.4 bc
Zn (g ha ⁻¹)	0.345	-105	-94	-89	-100	-104	-108

**P* value significant (probability value obtained from one-way ANOVA)

published by Schellberg et al. (1999) and biomass production for all treatments in 2005 published by Hejzman et al. (2010a). In the current study, biomass production used for the calculation of nutrient uptake was sufficiently steady to provide a reliable basis for nutrient budget estimation in the RGE. Results from the treatment A, the control, indicate that upper biomass production limit for *Violin caninae* grassland is approximately 3 t ha^{-1} . This is consistent with results of other authors from *Violin caninae* or the similar *Nardus stricta* grasslands (Bakker et al. 2002; Hejzman et al. 2007a; Badía et al. 2008).

The RGE impressively demonstrates that fertilizer application induces not only a change in soil chemical properties, and thereby in biomass production, but also a significant transformation of plant community type. In the B and C treatments, an increase in biomass production with the application of Ca or Ca+N was connected with a change towards a more productive mountain *Polygono-Trisetion* meadow (Table 3). Based on the results from the RGE and other studies (Mašková et al. 2009), the upper biomass production limit for the *Polygono-Trisetion* meadows was estimated to be 4 t ha^{-1} . An additional increase in biomass production was again connected with a considerable change in plant community from *Polygono-Trisetion* towards a more productive mesotrophic *Arrhenatherion* meadow in the D, E and F treatments (Chytrý et al. 2009). Based on the current results and results of other studies (Oomes and Mooi 1981; Donath et al. 2004; Beltman et al. 2007), the typical annual biomass production of *Arrhenatherion* meadows can be estimated to be $4\text{--}9 \text{ t ha}^{-1}$.

Concentration of elements in the biomass

With the exception of the C treatment (CaN) in the second cut, forage obtained from all treatments was of low quality because the concentration of N, and thus digestible protein was below the nutritional requirements of dairy cattle (ranging from 1.92 to 2.56% N; Whitehead 2000). Nitrogen application substantially decreased N concentration in the biomass from the D, E and F treatments compared with the control, although many researchers have recorded the opposite result, i.e., an increase in biomass N concentration with N application (Pelletier et al. 2006; Pontes et al. 2007; Aavola and Kärner 2008; Brum et al. 2009; Liu et al. 2010; Malhi et al. 2010; Pan et al. 2010). This

can be explained in three possible ways. Firstly, by the completely different plant species composition among treatments because a predominance of stress-tolerant short grasses (*Briza media*) and sedges (*Carex* spp.) with long-living leaves (Ryser and Urbas 2000; Duru et al. 2009) was recorded in the A, B and C treatments in contrast with the tall competitive grasses (*Alopecurus pratensis*, *Arrhenatherum elatius* and *Trisetum flavescens*) with short-living leaves recorded in the D, E and F treatments (Hejzman et al. 2007b; Chytrý et al. 2009). The first and second cuts in this experiment were performed relatively late, the first cut being performed in early July and the second cut in mid-October. Thus, at the time of cutting, the D, E and F treatments contained substantially higher amounts of senescent stem material and older leaves than the A, B and C treatments did. It must further be noted that the concentration of N is substantially lower in senescent than in living biomass because of N partitioning from old into living organs, especially in N-deficient ecosystems (Kobe et al. 2005).

Secondly, in comparison to the abovementioned C treatment with N application and low P availability, the simultaneous application of N and P in the D, E and F treatments substantially increased sward height (Hejzman et al. 2007b). This led to a decreased proportion of N-rich leaves for the benefit of N-poor stems and thereby decreased N concentration in the total biomass. The decrease in N concentration with an increase in standing biomass during the phenological development of sward (dilution effect) has been recorded by other authors (Duru and Ducrocq 1997; Lemaire 1997). In this study, the dilution of N concentration with an increase in standing biomass was recorded when sampled at the same time from different plant communities that had developed after long-term fertilizer application.

Thirdly, the low biomass N concentration in treatments with NP application can be explained by the relatively low N rate (50 kg N ha^{-1} before each cut) applied in the RGE compared with authors who applied $150\text{--}350 \text{ kg N ha}^{-1}$. According to Whitehead (2000), low N rates in N-deficient grasslands cannot increase N concentration in the total biomass but can promote biomass growth. A substantially higher concentration of N in the second cut than in the first cut biomass was probably connected with the higher proportion of dicotyledonous species.

The concentration of P in the biomass reflected the plant available (CAL) P content in the soil when this was increased by P application. In this respect, we found consistency with the results from other studies (Pelletier et al. 2006; Stroia et al. 2007; Semelová et al. 2008). In treatments without P application, P concentration was below the nutritional requirements for dairy cattle (ranging from 0.23 to 0.37% P; Whitehead 2000). As with N, the concentration of P in the first cut biomass was below the nutritional requirements for dairy cattle. This was also the case in treatments with P application, probably because of the late harvest, which led to P resorption from senesced biomass and to a high proportion of P-poor stems in the total biomass. We also observed higher P concentration in the D treatment (with CaNP application) than in the E and F treatments (with CaNPK application). This was probably initiated by a dilution effect because a higher biomass production was recorded in the E and F treatments than in D.

It is important to mention that high quality forage grasses with a characteristic high concentration of P in the biomass (Singer 2002) were dominant only in the D, E and F treatments, but not in treatments without P application. A low concentration of P in the biomass collected from the control is consistent with other studies from similar vegetation types (Tallowin and Jefferson 1999; Hejcman et al. 2007a; Badia et al. 2008; Klaudivová et al. 2009). In addition to the low plant available P content in the soil (Janssens et al. 1998; Marini et al. 2007; Wellstein et al. 2007), a low concentration of P in the aboveground biomass can be seen as characteristic feature of low productive semi-natural grasslands.

With the exception of the D treatment in both cuts and the A treatment in the second cut, K concentration was above the nutritional requirements for dairy cattle (ranging from 0.65 to 0.9% K; Whitehead 2000). A higher K concentration in grassland biomass than is required by livestock is a common observation in many grasslands types (Hopkins et al. 1994; Kayser and Isselstein 2005; Badia et al. 2008; Hrevušová et al. 2009). Similar to P, plant available K concentration in the soil was a function of K application, which substantially increased K concentration in the biomass. The positive effect of K fertilizer on the K concentration in the biomass observed in this experiment is consistent with results provided by other authors (Pelletier et al. 2008;

Madaras and Lipavský 2009; Liu et al. 2010). By contrast, a dilution of K concentration in the biomass occurred in the D treatment with Ca, N and P application, which is consistent with the results of the fertilizer experiment by Beltman et al. (2007).

The optimal Ca concentration for the nutrition of dairy cattle (ranging from 0.29 to 0.58% Ca; Whitehead 2000) was recorded only in the fully fertilized E and F treatments. In the remaining treatments, Ca concentration was substantially higher than the required optimum, especially in the second cut biomass. In the RGE, higher Ca and Mg concentrations in the second cut than in the first cut biomass was recorded previously by Schellberg et al. (1999). A substantially higher Ca concentration in the last cut than in the first was also recorded by Hopkins et al. (1994). This is at least partly because of the higher proportion of dicotyledonous species in the total biomass late in the vegetation season.

The optimal Mg concentration for dairy cattle (ranging from 0.16 to 0.2% Mg; Whitehead 2000) was recorded only in the E and F treatments. In the other treatments, Mg concentration was higher.

The biomass harvested from the unlimed control plots (pH 4.1) contained relatively high amounts of Ca and Mg compared with the E and F treatments. Across the whole experiment, we found no direct relationship between the application of lime and concentrations of Ca and Mg in the biomass. However, we have long observed a substantially higher proportion of dicotyledonous species compared with grasses (Hejcman et al. 2007b) in the control plots. This explains these differences well because it is well known that dicots, especially legumes, are naturally rich in Ca and Mg (Thompson et al. 1997; Durková and Jančovič 2003; Ngai and Jefferies 2004).

With respect to the toxic elements in both cuts, the concentration of As in the biomass was far below the EU legislation limit for forage crops (4.5 mg kg^{-1} in DM; Directive 2002/32/EC). There was a clear negative correlation between the application rate of As and its concentration in the biomass, as well as between plant available As concentration in the soil and its concentration in the biomass (Fig. 2). This was because of the dilution effect because the biomass production in the D, E and F treatments with elevated As loads was substantially higher than in treatments without P application. The highest As concentration

recorded in the first cut biomass in the C treatment was the same as the As concentration from the grass biomass of polluted sites reported by Váradiová et al. (2006). Similarly, the concentration of Cd in the biomass was below the EU legislation limit (1.1 mg kg^{-1}). The highest Cd concentration in the biomass from the control receiving no Cd input was connected with the highest plant available Cd concentration in the soil because of soil acidity (Hejcman et al. 2009). This result indicates that the concentration of Cd in the biomass can be more affected by soil pH than by Cd application with fertilizer (Clemente et al. 2008; Hanč et al. 2009).

Although the application rate of Cr was high in the D, E and F treatments, there was a strict negative correlation between Cr application and Cr concentration in the biomass. This can be explained by the low plant availability of applied Cr as well as by the dilution effect. Furthermore, species adapted to low soil fertility as in the control treatment generally invest a higher proportion of energy into root development to be highly efficient in nutrient acquisition. That way, the acquisition of toxic elements by plants can substantially increase along with low fertility compared with high fertility grasslands.

The higher concentration of Cu in the A, B and C treatments than in the D, E and F treatments with a higher Cu application was again connected with the dilution effect. Similar results were recorded by Christie (1990). In all treatments in the RGE, Cu concentration was below the potentially toxic level for sheep, which was estimated to be 10 mg kg^{-1} (Christie 1990). In the first cut biomass, we estimated Cu concentration as being relatively high (above 8 mg kg^{-1}) as reported from areas contaminated by Cu through applied slurry (Christie 1990) and air deposition (Váradiová et al. 2006). An increased concentration of Fe, Mn and Zn in the control was connected with low soil pH and high Fe, Zn and Mn mobility in the soil, but not with Fe, Mn and Zn application. By contrast, the concentrations of Fe, Mn and Zn in the D, E and F treatments were substantially lower, probably because of differences in species composition, neutral soil reaction and dilution effects. A high concentration of Zn in the biomass from the control is consistent with values published by Christie and Beattie (1989) from moderately acidic soil contaminated by Zn from long-term pig slurry application. The concentration of Pb in the biomass

was far below the EU legislation limit (34 mg kg^{-1}) and was similar to values revealed by Váradiová et al. (2006).

Nutritional status and element ratios

In general, low NNI in the RGE was affected partly by the late biomass harvest and, therefore, a low N concentration in the biomass. By contrast, the N limitation of biomass production in all treatments could be expected because the N application rate in the RGE was only 100 kg ha^{-1} . The most severe P limitation of biomass production was recorded in the C treatment with N application because PNI was the lowest overall in this treatment. This is consistent with conclusions based on the analysis of plant species compositions. Stress-tolerant species well adapted to low P availability were most frequent in the C treatment (Hejcman et al. 2007b). Furthermore, the most severe P limitation of biomass production in the C treatment was indicated by the lowest plant available P concentration in the soil compared with all other treatments and by the lowest P concentration and highest N:P ratio in the bryophyte biomass (Hejcman et al. 2010b).

Biomass production was co-limited by P in the A and B treatments, but not in the D, E and F treatments. The highest PNI in the D treatment is consistent with the highest concentration of plant available P in the soil (Schellberg et al. 1999; Hejcman et al. 2009). The N and P co-limitation of biomass production was indicated by NNI and PNI in the A and B treatments. This is consistent with the results indicated by the biomass N:P ratio in this study. The same holds for the severe P limitation in the C treatment on the one hand and the non-limiting P but N limitation in the D, E and F treatments on the other. According to Koerselman and Meuleman (1996) and Güsewell (2004), a N:P ratio in the aboveground grassland biomass below 14 indicates N limitation, an N:P ratio of 14–16 indicates N and P co-limitation and an N:P ratio above 16 indicates P limitation.

Additionally, biomass production in the C treatment was probably moderately K co-limited because the N:K ratio was above 2.1 (Olde Venterink et al. 2003). In the D treatment, the K co-limitation was additionally indicated by a K:P ratio below 3.4 (Olde Venterink et al. 2003). Furthermore, the K limitation

in the D treatment was experimentally confirmed by a significant increase in aboveground biomass production with the addition of K in the E and F treatments compared with the D treatment.

In the first cut biomass, the Ca:P ratio was slightly higher than optimum (between the level of 1–2 proposed for livestock nutrition; Tallowin and Jefferson 1999). By contrast, it was four to five times higher than the optimum for livestock in the A, B and C treatments in the second cut. This can be harmful for livestock because the absorption and utilization of both Ca and P depends on supplying the correct Ca:P ratio into the diet. However, this observation is common because a high Ca:P ratio is a characteristic feature of biomass from many semi-natural grasslands.

Annual uptake of elements by aboveground biomass

The negative values for the simplified balance of all elements in control A and for the majority of elements in the B and C treatments indicate a continuous decrease in soil fertility. A negative nutrient balance is in agreement with the results of repeated soil analyses indicating a continuous decrease in plant available P concentration in these treatments (Hejerman et al. 2010a). The negative values for the N balance in the A and B treatments must be taken with caution because additional N was delivered by atmospheric deposition (at least 10 kg N ha⁻¹ annually, Solga et al. 2006) and by N₂ fixation by legumes (Soussana and Tallec 2010). In the A and B treatments, the cover of legumes was more than 20% (Hejerman et al. 2007b) with an estimated annual N fixation of more than 30 kg ha⁻¹. These values indicate a balanced annual N input-output relationship in the A and B treatments. This agrees with the results of other authors that have shown that N availability in low productive grasslands can be stabilized but not decreased by cutting and biomass removal management (Olde Venterink et al. 2002, Hejerman et al. 2010c).

In plots where N, P and K were applied, the positive balances of these elements indicated a substantial surplus. This holds for N in the treatments C, D and F, for P in the treatments D, E and F and for K in the treatments E and F. Positive balances are partly driven by the inefficient use of applied nutrients because of late cutting in the extensive grassland system in the RGE. To increase N, P and K

removal through the biomass and forage quality, both cuts should be performed substantially earlier in the season, although this would interfere with the desired floristic composition as well as the unique ecological value of the RGE.

Based on the positive values for element balance, substantial accumulation of Cr, Fe and Mn in the soils of treatments with P application occurred for the duration of the experiment. This is consistent with the results of soil chemical analyses performed by Hejerman et al. (2009). Despite Cr, Fe and Mn accumulation in the D, E and F treatments, the highest uptake of these elements was recorded in the control. This indicates the low effect of Fe and Mn application and the highly positive effect of low soil pH on the uptake of Fe and Mn. Furthermore, applied Cr is barely removed from the ecosystem by biomass extraction.

Conclusion

Nitrogen application can decrease N concentration in aboveground grassland biomass. This is the case when, for example, medium N rates are applied and when extended growth phases during spring and summer allow the full reproductive development of grasses and herbs, leading to increased ratios of N-poor stems to N-rich leaves. Late cutting management, therefore, decreases forage quality in highly productive more than in low productive plant communities. Concentrations of P and K in the plant biomass were positively related to P and K application and, therefore, to plant available P and K concentrations in the soil.

Furthermore, the concentration of many micro and trace elements in the plant biomass cannot be positively related to the amount of elements supplied by fertilizers. Negative relationship even appear because of high biomass production and the consequent high dilution of elements in aboveground biomass under high productivity. Based on the highest concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in the biomass from low productive *Violin caninae* grasslands in the control, and the lowest in highly productive *Arrhenatherion* grasslands in the NPK treatments, it was concluded that the concentration of trace elements in the aboveground grassland biomass can be negatively related to grassland productivity. However, this relationship will require further testing

at an experimental and a landscape level because the concentrations of trace elements in the grassland biomass and their relationship to biomass production have rarely been studied. In soils polluted by trace elements, a neutral soil reaction and high biomass production maintained by an adequate mineral fertilizer application seems to be the best measure to prevent excessive concentrations of trace elements in the grassland biomass.

Nutritional status, as indicated by the biomass N:P ratio, was consistent with N or P limitation indicated by NNI or PNI. Furthermore, additional K (co-) limitation was indicated by the N:K and K:P ratios in the biomass from NP treatment. Long-term fertilizer application can result in the accumulation of macro (P, Ca and Mg), micro (Fe and Mn) and trace (As and Cr) elements in the soil, but this accumulation cannot be connected with the increase in the concentrations of elements in the plant biomass. The results from the RGE indicate that there is no simple positive relationship between the applied elements and their concentrations in the plant biomass.

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