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Long-term residual effect of short-term fertilizer application on Ca, N and P concentrations in grasses *Nardus stricta* L. and *Avenella flexuosa* L.

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Abstract The resilience (the ability of an ecosystem to recover after termination of perturbation) of plant species composition after short-term fertilizer application can take decades in sub-alpine grassland, but little attention has been paid to the resilience of nutrient concentrations in the biomass of individual plant species. In 2004, an abandoned experiment in which phosphorus, nitrogen and calcium had been applied from 1965 to 1967 was identified in the Giant Mts. (Krkonoše/Karkonosze, Czech Republic). The biomass of two dominant grasses, *Nardus stricta* and *Avenella flexuosa*, was analyzed for Ca, N and P concentrations 37 years after the last fertilizer application. In treatments with P application, the P concentration was still significantly increased in both species. The N concentration was higher in treatments with N or Ca application and the lowest in P treatments. The N:P ratio ranged from 7.7 to 16.6 and from 6.2 to 16.3 in *N. stricta* and *A. flexuosa*, respectively, and was lowest in P treatments where *A. flexuosa* predominated. *N. stricta* dominated in treatments where the biomass N:P ratio was higher than 13, whereas lower ratios were more favourable for *A. flexuosa*. In the case of *N. stricta*, the Ca concentration was increased in Ca treatments. Ca and P concentrations in *N. stricta* biomass were significantly positively correlated with soil plant available P and Ca concentrations, but this was not recorded for *A. flexuosa*. In environments with different P availability, the competitive ability of investigated species was predetermined by the N:P ratio in their biomass. As in the case of plant species composition and soil chemical properties, the resilience of Ca, N and P concentrations in the biomass of individual species can take decades in sub-alpine grassland.

Keywords Calcium · Krkonoše/Karkonosze · Mat grass · Nitrogen · N:P ratio · Phosphorus · Sub-alpine grassland

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Introduction

Various fertilizer experiments have shown that short-term responses of vegetation to changes in nutrient supply can differ markedly from long-term responses (Tilman 1982; Chapin et al. 1995; Inouye and Tilman 1995). According to Tilman (1982), short-term responses may mainly reflect the type of nutrient deficiency before fertilizer application, whereas long-term responses are primarily driven by changes in competitive interactions among species in the plant community. Short-term fertilizer experiments are therefore frequently used to detect the nutrients that limit increased biomass production in species present immediately after application (Güsewell et al. 2002; Niinemets and Kull 2005; Soudzilovskaia et al. 2005) whereas long-term experiments, in which indirect effects also have time to develop, are extremely important for studying nutrient availability as a driving force of plant species composition (Kopeć 2002; Sammul et al. 2003; Silvertown et al. 2006).

Determining the limiting nutrient is important for conservation of low productivity plant communities or for rational use of fertilizers. If P is the limiting nutrient, low productivity grassland can persist even under high levels of N availability. For example short grasses, sedges, and orchids persisted during 64 years of N application in the Rengen Grassland Experiment in Germany (Schellberg et al. 1999; Hejcman et al. 2007a) and *Nardus stricta,* a stress-tolerant species well equipped to grow in acid soils where P availability is low (Güsewell et al. 2005), dominated during 30 years of N application, but not when N was applied together with P in the Krinica experiment in Poland (Kopeć and Gondek 2002). To simply detect the limiting nutrient without complicated experiments, Koerselman and Meuleman (1996) proposed the aboveground biomass N:P ratio as a quantitative tool to assess the nutrient limitation in plant communities. Recent papers have demonstrated that the critical N:P value is species and/or plant community specific although it is affected by many factors (Tomassen et al. 2003; Drenovsky and Richards 2004; Güsewell 2004). The ability of ecosystems to recover after termination of perturbation is called resilience (Lepš et al. 1982; Spiegelberger et al. 2006; Hrevušová et al. 2009). In an alpine *N. stricta* grassland, Hegg et al. (1992) recorded increased concentrations of N and P in leaves of selected plant species almost 40 years after the last N and P application. They showed that it is not only the resilience of plant species composition after fertilizer application that can be a long-term process, but also the resilience of nutrient concentrations in the biomass of individual species.

An abandoned experiment that was fertilized from 1965 to 1967 was identified by Hejcman et al. (2007b) in the Giant Mts. (Krkonoše/Karkonosze, Riesengebirge) in 2004. The effect of selected fertilizer treatments on plant species composition was still visible 37 years after the last fertilizer application. *Avenella flexuosa* and *Anthoxanthum alpinum* were the dominant species in former P treatments whereas *N. stricta* was the dominant species in the control as well as in the Ca and N fertilized treatments. In the bulk biomass, the concentration of nutrients (Ca and P) was still increased in P and Ca treatments, respectively. It was speculated that these differences were caused predominately by different plant species composition, but the biomass of individual species was not analyzed. Further, species with an inherently low concentration of P in the biomass and a high N:P ratio are better adapted to environments with low P availability (Vance et al. 2003; Güsewell 2004). Analysis of the dominant species *A. flexuosa* and *N. stricta* for N and P concentrations and the N:P ratio in the biomass may help to explain why they show different competitive abilities under different P availability in the soil.

Within this context, the aim of this paper was to answer the following questions: (1) Is there any detectable residual effect of fertilizer application on Ca, N and P concentrations and the N:P ratio in the biomass of individual plant species after almost 40 years? (2) Is the different competitive ability of *A. flexuosa* and *N. stricta* connected with the N:P ratio in their biomass?

Methods

Site description

In 1965, a fertilizer experiment investigating the response of *Nardus* grassland (*Nardo strictae–Caricion bigelowii* alliance (Chytrý 2007)) to increases in Ca, N and P availability was established in the border land between the Czech Republic and Poland (Hejcman et al. 2007b). The altitude of the experimental grassland is 1,430 m, the average annual precipitation within the area is 1,380 mm, and the long-term mean annual temperature is 2°C (Vrbatova Bouda meteorological station). The geological substratum is granite underlying low deep humus podzols. The mineral Ah horizon is 4 cm thick and dark, with 50% organic matter and a pH (H_2O) of 3.8. Before primary fertilizer application, the sub-alpine grassland was dominated by *N. stricta* followed by *A. flexuosa*, *A. alpinum* and *Carex bigelowii*. After setting up the experiment, biomass was collected to investigate the effect of treatments on sward structure only in the initial phase of the experiment, and then the plots were abandoned.

Design of the experiment

The experiment consisted of three nutrients applied at three rates (treatment abbreviations: P1, P2, P3, N1, N2, N3, Ca1, Ca2 and Ca3) and a control without any fertilizer application (Table 1). Each treatment was replicated three times. Individual plot size was 1 m^2 delimited by steel nails in the corners and surrounded by a 1 m wide buffer zone without fertilization (see Hejcman et al. (2007b) for details). The abandoned experiment was identified using a metal detector in summer 2004. P fertilized plots were clearly visible due to the dark green color of the vegetation (Fig. 1). Fertilizers were applied five times: in autumn 1965, spring 1966, autumn 1966, spring 1967 and in autumn 1967. Ca was also applied together with N and P, and S together with P, in commercial fertilizers (Table 1).

Data collection and analysis

N. stricta and *A. flexuosa* were selected as model species because of their characteristic dominance and competition in sub-alpine grasslands in the Giant Mts. In August 2004 aboveground biomass samples were collected from the permanent 1 m^2 plots of all three replicates of each treatment (in total, 30 samples for each species). The sward was clipped to a target height of 3 cm and living (green) biomass of *N. stricta* and *A. flexuosa* was separated manually. The biomass was dried at 85°C until complete desiccation and ground in a titanium centrifugal mill. Total N concentration was determined spectrophotometrically after Kjeldahl digestion, and total Ca and P concentrations were determined spectrophotometrically or by emission flame spectrometry after digestion in sulphuric acid. All analyses were performed in an accredited national laboratory (Ekolab Žamberk). The concentrations of plant available nutrients in the upper soil layer (extracted according to the Mehlich III method) were taken from Hejcman et al. (2007b).

Treatment effects for both selected species were evaluated by one-way ANOVA using the STATISTICA 5.0 software (StatSoft 1995). To determine differences among treatments, the Tukey HSD test was applied after obtaining the ANOVA results. Regression was used to evaluate relations between

Table 1 Description of the investigated treatments

Treatment abbreviation	Nutrient				Fertilizer used
	Ca	N	P	S	
	Amount applied kg ha^{-1}				
C					Unfertilized control
P1	300		120	150	$CaSO_4 + Ca[H_2PO_4]_2$ (20% Ca; 8% P; 10% S)
P2	1,500		600	750	$CaSO_4 + Ca[H_2PO_4]_2$ (20% Ca; 8% P; 10% S)
P3	3,000		1,200	1,500	$CaSO_4 + Ca[H_2PO_4]_2$ (20% Ca; 8% P; 10% S)
N1	100	250			$NH_4NO_3 + CaCO_3$(25% N; 10% Ca)
N2	500	1,250			$NH_4NO_3 + CaCO_3$(25% N; 10% Ca)
N3	1,000	2,500			$NH_4NO_3 + CaCO_3$(25% N; 10% Ca)
Ca1	1,065				CaO (71% Ca) + MgO (5% Mg)
Ca2	5,325				CaO (71% Ca) + MgO (5% Mg)
Ca3	10,650				CaO (71% Ca) + MgO (5% Mg)

Doses shown in the table correspond to the total amount of nutrients applied in the years 1965–1967

Fig. 1 Effect of P applied on color of the vegetation was still clearly visible in 2004 even after 37 years after the last P application. ©Michal Hejcman

concentrations of Ca and P in plant biomass and their concentrations in the soil.

Results

The concentration of P and Ca was generally higher in *A. flexuosa* than in *N. stricta* but the opposite was the case for the N:P ratio. A significant effect of treatment on concentrations of P, N and the N:P ratio was recorded in *N. stricta* biomass (Table 2; Fig. 2). In the case of Ca, the effect of treatment was of borderline significance ($P = 0.06$). P concentration was the highest in the P2 and P3 treatments and differed significantly from the control, N1, N2, N3, Ca1, Ca2 and Ca3 treatments. Mean Ca concentration was still increased in the Ca2 and Ca3 treatments and these treatments contrasted with the N2 and control treatments. High variability in the concentration of N in *N. stricta* biomass was recorded. The lowest N concentration was in all P treatments and in the control, which significantly differed from treatments N2, N3, Ca2 and Ca3. The N:P ratio in *N. stricta* biomass ranged from 7.7 to 16.6 and was significantly affected by treatment (Fig. 2). In general, in all P treatments the N:P ratio was lower and in all Ca and N treatments higher than in the control. Ca and P concentrations in *N. stricta* biomass were significantly positively affected by soil plant available P and Ca concentrations (Table 3).

The effect of treatment on concentrations of N and P and the N:P ratio in *A. flexuosa* was significant (Table 2; Fig. 2). In the case of Ca the effect of treatment was not significant and no trend within the data was recorded. P concentration was the highest in the P3 treatment, followed by the P2 treatment, and significantly differed from the control, N1, N2, N3, Ca1, Ca2 and Ca3 treatments. High inter-treatment variability in the concentration of N was recorded as for *N. stricta*. The lowest N concentration was in the P3 treatment, which significantly differed from the N3, Ca1, Ca2 and Ca3 treatments. The highest N concentration was in the Ca2 treatment, which differed significantly from all P treatments and the control. Ca and P concentrations in *A. flexuosa* biomass were not significantly related to soil plant available Ca and P concentrations (Table 3). The N:P ratio in *A. flexuosa* biomass ranged from 6.2 to 16.4 and was significantly affected by treatment (Table 2; Fig. 2). In general, the N:P ratio was the lowest in all P treatments.

Discussion

The significant effect of treatment on N, P and the N:P ratio in biomass of *N. stricta* and *A. flexuosa* almost

Table 2 Mean concentration of Ca, N and P in biomass of *Nardus stricta* and *Avenella flexuosa*

Treatment	P** $g\ kg^{-1}$	SD	Ca $g\ kg^{-1}$	SD	N** $g\ kg^{-1}$	SD
Nardus stricta						
P1	1.10a	0.1	0.50a	0.2	12.70a	0.7
P2	1.67b	0.23	0.60a	0.1	12.57a	0.6
P3	1.60b	0.44	0.50a	0.1	12.93a	0.21
N1	0.87a	0.06	0.33a	0.1	13.30ab	0.26
N2	0.93a	0.06	0.30a	0.1	14.80c	0.66
N3	0.93a	0.06	0.50a	0.1	14.47bc	0.21
Ca1	0.87a	0.06	0.43a	0.1	13.83abc	0.15
Ca2	0.90a	0	0.97a	0.4	14.93c	0.49
Ca3	0.97a	0.12	1.40a	1.1	14.60bc	0.26
Control	1.07a	0.06	0.47a	0.1	13.13a	0.57
Avenella flexuosa						
P1	1.33ab	0.01	1.90a	0.0	13.07ac	0.06
P2	1.83bc	0.01	2.00a	0.1	13.50abc	0.07
P3	1.97c	0.01	1.60a	0.1	12.23c	0.03
N1	1.23a	0.03	1.53a	0.1	13.77abc	0.03
N2	1.13a	0.01	1.23a	0.1	13.97abc	0.05
N3	1.1a	0.01	1.37a	0.2	15.50bd	0.05
Ca1	1.23a	0.02	1.57a	0.5	15.13ab	0.03
Ca2	1.03a	0.01	1.53a	0.3	16.93d	0.20
Ca3	1.30ab	0.04	2.30a	1.1	14.83abd	0.07
Control	1.07a	0.01	1.50a	0.4	13.70abc	0.07

Values with the same letter were not significantly different

** Result of one-way ANOVA significant at 0.001 probability

SD standard deviation

40 years after final fertilizer application is consistent with the results presented by Hegg et al. (1992). In subalpine conditions, it is not only the resilience of plant species composition that is slow, but also the resilience of nutrient concentration in the biomass of individual plant species. The significant effect of treatments on N, P, and Ca concentrations and the N:P ratio in bulk biomass (Hejcman et al. 2007b) was therefore caused by two factors: (1) the higher proportion of plant species with inherently higher nutrient concentrations in the biomass, especially in P treatments (*A. flexuosa*, cover data in Fig. 2), and (2) higher nutrient concentrations in the biomass of individual plant species, as demonstrated in *N. stricta* in this study.

The most surprising result was the significantly higher concentrations of N in all N and Ca treatments in comparison to the control. Due to the generally known high mobility of mineral N in grassland ecosystems (Schellberg et al. 2006), increased N concentrations in the N treatments can probably be ascribed to the effect of Ca applied together with commercial N fertilizer (Table 1). This possibility is supported by the fact that N concentrations were higher in Ca treatments as well. Ca probably increased microbial activity in the soil and therefore mineralization of organic matter and N uptake by both study species. On the other hand, this explanation is not consistent with conclusions made by Hegg et al. (1992), who used N fertilizer free of Ca (ammonium sulphate) and also found increased N concentrations in leaves of selected plant species after 40 years. According to this study, part of the applied N can quickly be incorporated into fungal protein and stored there, and therefore could affect ecosystem functions for a long time.

Although the experimental grassland had not been harvested for many years, transport of nutrients occurred in some treatments. For example the selective grazing by red deer was observed in P treatments as in other abandoned fertilizer experiment in the locality (Semelová et al. 2008). Preferential grazing of P-rich biomass by red deer was also revealed in the Alps and reflected the P shortage for ruminants in the total consumed biomass (Schutz et al. 2006). Despite grazing by wild animals, P depletion had not occurred even after almost 40 years. The increased P concentration in *N. stricta* biomass collected in treatments P2 and P3 and its positive correlation with soil P concentrations indicated some level of flexibility of this stress-tolerant species in response to increased P availability. On the other hand, the flexibility of *N. stricta* was not high enough because of its immediate death after P application in 1966, in contrast to *A. flexuosa*, which quickly expanded in the late 1960s (Hejcman et al. 2007b). This supports the conclusion made by Hawkins et al. (2008) that the substantial increase in P availability may be toxic for stress tolerant species that are adapted to extremely P poor conditions. Adaptation of *N. stricta* to P limited conditions was indicated by the generally lower P concentration and higher N:P ratio in its biomass than in *A. flexuosa*, as reported previously (Thompson et al. 1997; Semelová et al. 2008). Further, *A. flexuosa* was the dominant species in P treatments while *N. stricta* dominated in N, Ca and control treatments (Fig. 2).

The N:P ratio in both species was below 10 in the P treatments. Competition between the investigated

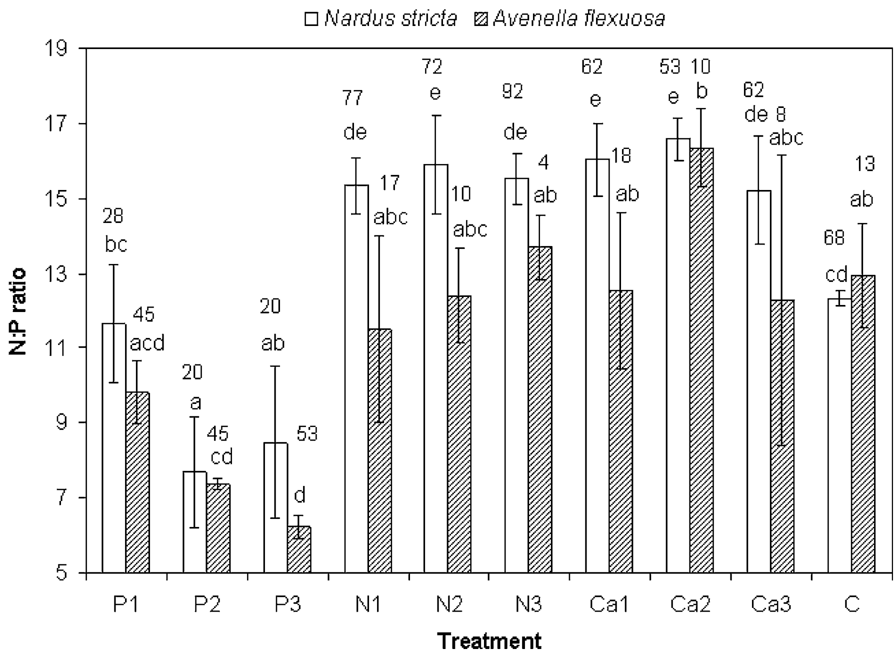

Fig. 2 Effect of treatment on N:P ratio in *Nardus stricta* and *Avenella flexuosa* biomass. Treatment abbreviations are given in Table 1. Columns with the same letter for the same species are not significantly different. Error bars represent SD. Values above each column represent mean cover of each species in percentages (according to Hejcman et al. 2007b)

Table 3 Results of regression analyses of nutrient concentrations in *Nardus stricta* and *Avenella flexuosa* biomass on soil P and Ca concentrations

Independent variable	Dependent variable	*R*	*F* value	*P* value
Soil P concentration	P concentration in *N. stricta*	0.41	5.74	0.023
Soil Ca concentration	Ca concentration in *N. stricta*	0.83	62.04	<0.001
Soil P concentration	P concentration in *A. flexuosa*	0.33	3.42	0.075
Soil Ca concentration	Ca concentration in *A. flexuosa*	0.24	1.74	0.198

grasses was therefore probably driven by P availability as well as the N:P ratio in the biomass and it seems that *N. stricta* loses its dominance when the N:P ratio is below ten. This conclusion is in accordance with Güsewell et al. (2005), who recorded a biomass N:P ratio lower than ten only in plant communities dominated by *Agrostis capillaris* and *Festuca rubra*, but not in the neighboring sward dominated by *N. stricta*. In the Giant Mts., dominant *N. stricta* is replaced by *A. flexuosa* in many micro-localities in *Nardo strictae–Caricion bigelowii* sub-alpine grassland. Based on the results of the study, this may be due to increased P availability in the soil, thus decreasing the competitive ability of *N. stricta*. The higher concentration of Ca in *A. flexuosa* than *N. stricta* reflects the higher Ca requirements of *A. flexuosa* and better adaptability of *N. stricta* to environments with a limited Ca supply.

In conclusion, the results of this study stress the importance of really long-term studies in plant ecology and nutrition. In environments with different P availability, the competitive ability of the investigated species was predetermined by the N:P ratio in their biomass. As in the case of plant species composition and soil chemical properties, the resilience of Ca, N and P concentrations in the biomass of individual species can take decades in sub-alpine grassland.

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