

Original article

Co-limitation of plant primary productivity by nitrogen and phosphorus in a species-rich wooded meadow on calcareous soils

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Abstract

High small-scale species richness of calcareous grasslands is generally thought to result from evening of species competitive potentials by limited N availability, because of relatively low herb N/P ratios in these communities. However, P mobility is low in alkaline soils as well. We studied soil chemistry and productivity of herb and moss layers in a very diverse calcareous meadow (up to 76 vascular plant species per m²) to test the hypotheses of a co-limitation of herb productivity by both soil N and P availabilities and moss productivity primarily by P availability. The effect of nutrient supply on productivity was investigated using both a natural productivity gradient as well as fertilization experiments. We observed strong positive correlations of soil P availability and total soil N with the above-ground productivity of herb layer. A long-term fertilization experiment demonstrated that P alone and N and P together increased productivity of vascular species, and that the productivity continuously declined after cessation of fertilization with the effect of previous fertilization occasionally visible even 14 years after treatment termination. A short-term fertilization experiment further demonstrated that N and P when supplied alone increase productivity of vascular plants, suggesting that both elements were limiting. Furthermore, there was a significant interaction between N and P on productivity, indicating that simultaneous N and P supply increased productivity more than separate nutrient additions. Moss productivity was negatively associated with vascular plant productivity. In particular, N addition decreased moss productivity, but moss productivity did not decline in P addition treatments. P requirements of mosses were larger than those of vascular plants. Our data indicate co-limitation of herb productivity by both soil N and P in this highly diverse grassland, while limitation of moss productivity mainly by P. We suggest that N and P co-limitations are common in calcareous diverse grasslands, and may partly explain the extreme small-scale species diversity in these communities.

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1. Introduction

Species-rich communities are generally characterized by nutrient limitations, in particular, nitrogen is considered as the nutrient most universally limiting plant productivity (Chapin et al., 1987; Schulze et al., 1994). Therefore, studies on vegetation potential productivity and dynamics have been heavily focused on nitrogen acquisition and use physiology. A general understanding is that nitrogen-rich habitats favor species with inherently higher growth rates that outcompete slow-growing species, and that this may lead to decreases in community diversity (e.g. Berendse, 1993; Tilman et al.,

2001). Many N fertilization experiments demonstrate a dramatic decline in community diversity and recovery after cessation of fertilization (Berendse, 1993; Inouye and Tilman, 1995), indicating that developing N limitations do even species competitive capacities.

In primarily N-limited diverse grasslands, species richness is on the order of 30–40 vascular plant species per m² (Olf and Bakker, 1991; Willems and van Nieuwstadt, 1996; Huovinen-Hufschmid and Körner, 1998), while the top three species richness values are 88 vascular species per m² in weakly grazed meadows in Sierra Cordoba Mountains, Argentina (J.J. Cantero, personal communication), 87 species per m² in regularly mown steppes in Strelets, Russia (Afanasyeva and Golubev, 1962) and 76 species per m² in Esto-

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nian wooded meadows (Kull and Zobel, 1991; Kukk and Kull, 1997; Anonymous, 2001). This suggests that multiple factors rather than nitrogen alone limit plant productivity in extremely species-rich communities.

All these very diverse grasslands are on calcareous soils, where in addition to N, phosphorus may limit productivity due to lowered mobility of P that is bound to calcium phosphates (Frossard et al., 1995) as well as due to possible presence of Al and Fe oxides in calcareous sediments that limit P desorption as in acidic soils (Lajtha and Harrison, 1995). Such nutrient co-limitations are occasionally observed (DeLucia et al., 1989; Verhoeven and Schmitz, 1991; Morecroft et al., 1994; Whitehead et al., 1997), but are difficult to identify conclusively. Productivity limitations are often inferred from plant N/P ratios that have been derived from the work in European wetlands (e.g. Wassen et al., 1995; Koerselman and Meuleman, 1996; Tessier and Raynal, 2003; Drenovsky and Richards, 2004). Because the vegetation N/P ratios are relatively low in calcareous meadows (Morecroft et al., 1994; Kooijman et al., 1998), most of these communities would be considered N-limited according to N/P criterion of Koerselman and Meuleman (1996). However, protein synthesis decreases in P-limited conditions, often resulting in less efficient uptake and use of N (Lajtha and Klein, 1988; Reich and Schoettle, 1988; Reich et al., 1994; Raaimakers et al., 1995).

In P-deficiency conditions, plant resources are optimally distributed if plant P and N concentrations are in a balance such that photosynthetic carbon acquisition is co-limited by both P and N (Chapin et al., 1987). Thus, knowledge of foliar P and N concentrations alone is not always sufficient to draw conclusions on which of them is limiting, especially because plants widely differ in N and P requirements (McJannet et al., 1995; Ryser and Lambers, 1995; Gordon et al., 2001; Tomassen et al., 2003, 2004; Drenovsky and Richards, 2004). In fact, P-limited plant growth may be much more frequent in natural ecosystems than is currently acknowledged. Because P and N differently modify plant photosynthetic (Conroy et al., 1988; Dietz and Harris, 1997; Lovelock et al., 1997; Niinemets et al., 1999) and productivity (Conroy et al., 1988; Whitehead et al., 1997; Stöcklin et al., 1998) responses to elevated CO₂, separation between N and P-limitations is central to project global change and diversity effects on primary production.

Using chemical composition of woody species as a proxy of nutrient availability, we previously suggested that plant productivity in calcareous wooded meadows is limited by both N and P (Niinemets and Kull, 2003). In the current study, we conducted a series of fertilization experiments to investigate the intrinsic community response to added nutrients and quantitatively test the hypothesis of the productivity co-limitation by P and N in these communities. According to previous studies, grassland species may have a strong potential to use early-season pulses of nutrients (Bilbrough and Caldwell, 1997), while maintaining slow growth during the rest of the year. Yet, it is not clear whether the community potential to respond to fertilization depends on initial productivity. As more pro-

ductive sites support species with inherently higher growth rates (Lambers and Poorter, 1992), more productive sites may be more responsive to added nutrients. We also tested the hypothesis that the sites with higher initial productivity respond more strongly to fertilization than the less productive plots using a gradient of productivity that had resulted from a long-term fertilization treatment.

Apart from the vascular plants, mosses often significantly contribute to the overall ecosystem carbon stocks, especially in low-stature communities such as meadows, but the contribution of moss productivity to total ecosystem response is often ignored. Moss contribution to productivity may be especially significant in nutrient-poor habitats where more light penetrates through the sparse herb canopy to the moss layer, while the significance of mosses gradually decreases with nutrient, especially N, additions (Ingerpuu et al., 1998; Strengbom et al., 2001). Because of active soil respiration, mosses photosynthesize on average at higher atmospheric CO₂ concentrations than the herb layer. Given that P requirements for photosynthesis are relatively larger at higher CO₂ (Barrett and Gifford, 1995; Dietz and Harris, 1997; Lovelock et al., 1997; Niinemets et al., 1999), moss productivity may be more strongly limited by low soil P availability than the productivity of herbaceous vegetation. We tested the hypotheses that P requirements on a tissue concentration basis and relative to tissue N concentration are larger for mosses than for vascular plants and that enhanced N availabilities reduce more the contribution of mosses to total ecosystem productivity than enhanced P availabilities.

2. Materials and methods

2.1. Laelatu wooded meadow

The study was conducted in a wooded meadow on calcareous alkaline soils in Laelatu (58°36'N, 23°35'E; elevation 2–5 m above sea level), Estonia. The wooded meadows are formed as the result of regular mowing and silvicultural treatments that maintain the tree and shrub coverage at a constant and moderate level (ca. 40%). These communities were widespread in the coastal regions of the Baltic sea (Hægström, 1983; Kukk and Kull, 1997), but have drastically decreased in coverage as the result of land-use changes. Laelatu wooded meadow has been mown for at least 300 years, but it has probably not been subject to cattle grazing (Kukk and Kull, 1997). Studies demonstrate that the removal of mineral nutrients by hay and the supply by weathering of parent rock minerals are roughly in balance in this community such that the productivity of woody meadow vegetation is constant over the long-term despite regular mowing (Pork, 1979).

The herb layer (average height 30–50 cm) with exceptionally high small-scale species richness (Kull and Zobel, 1991) is dominated by *Briza media* L., *Carex flacca* Schreb., *Festuca rubra* L., *Filipendula hexapetala* Gil., *Schorzoneria humilis* L. and *Sesleria caerulea* (L.) Ard. (Krall and Pork,

1970; Kukk and Kull, 1997). Solitary clumps of *Cornus sanguinea* L. and *Corylus avellana* L. characterize the shrub layer (height 2–4 m), and *Betula pendula* Roth., *Fraxinus excelsior* L. and *Quercus robur* L. constitute the sparse tree layer (height 6–12 m). The soil is a rendzic leptosol with a thin (17–22 cm) humus horizon (A) that lays directly on calcareous marine sediments and glacial moraines (C horizon). The roots of herbs and grasses are primarily in the A horizon, while the roots of shrubs and trees are in A and C horizons. Bedrock, a Silurian limestone, is generally deeper than 1 m and does not significantly contribute to soil formation processes (Sepp and Rooma, 1970).

2.2. Long-term fertilization experiment

A fertilization experiment was started in 1961 in 12, 10 × 30 m plots that were free of woody vegetation and was continued until 1981 (Pork, 1979). Four treatments with three randomized replications included: control (no fertilizer), PK, PKN1 and PKN2 (supplied as potassium phosphate and ammonium nitrate). P and K addition rates (2.6 and 5 g m⁻² year⁻¹, respectively) were the same for all fertilization treatments, PKN1 received additionally 3.5 g N m⁻² year⁻¹, and PKN2 10 g N m⁻² year⁻¹.

Since the cessation of fertilization in 1981, the above-ground productivity of vascular plants has been examined yearly to determine the longevity of the fertilization effect and study the succession. According to Kull and Zobel (1991), the productivity of some fertilized plots was still significantly higher 8 years after termination of fertilization treatments. For the current analyses, above-ground biomass of the herb layer was harvested in 0.1 × 0.5 m areas from all treatment plots in three replications at the end of June in years 1989, 1991, 1993 and 1995.

2.3. Short-term fertilization experiment

The long-term fertilization experiment did not distinguish between N and P controls on plant productivity. The long-term fertilization also led to significant changes in composition of vascular (Kull and Zobel, 1991) and moss species (Ingerpuu et al., 1998) that complicate the interpretation of nutritional controls on productivity. To overcome these difficulties we conducted an additional fertilization experiment in 1998 on the permanent plots of the long-term fertilization study using a split-plot design (Sokal and Rohlf, 1995). To check for an interaction between short- and long-term fertilization, and the effect of initial productivity on short-term fertilization response, we selected two of each control, PK and PKN1 plots that covered the full range of herb layer productivities observed in the first part of the study. On these main plots, experimental treatments were randomly assigned to each of the sub-plot of 0.5 × 0.5 m size. Due to well-developed turf layer, the below-ground plant parts were concentrated in the immediate vicinity of 10–20 cm from plant stems even in rhizotomous species (Niinemets, 2004). To fur-

ther reduce the edge effects, all experimental sub-plots were separated by 0.5 × 0.5 m untreated areas.

The experimental treatments applied were P additions (as Na₂HPO₄) of 1 g m⁻² (P1), 2 g m⁻² (P2), 4 g m⁻² (P3) and 6 g m⁻² (P4), N additions (as NH₄NO₃) of 2 g m⁻² (N1), 5 g m⁻² (N2), 10 g m⁻² (N3) and 20 g m⁻² (N4), and combined N and P additions (P2N2 and P4N4). Every P or/and N fertilized plot also received 5 g m⁻² of K as KCl. The additional nutrients were supplied in three events during the growing season (25 April, 15 May and 28 May). The nutrient salts were dissolved in local artesian water, and the nutrient concentrations were adjusted such that every treatment plot received 1 l of water with dissolved nutrients in every fertilization occasion. In each of the main plots, four sub-plots that were watered with equal amounts of artesian water only were also selected (control treatment). Thus, the experiment included altogether 84 sub-plots on six main plots [6 × (4P treatments + 4N treatments + 2P × N combined treatments + 4 controls)].

Above-ground biomass of herb and moss layers was separately harvested from all treatment and control plots on 12 July. Herb layer was removed by scissors, and the height of remaining turf was 0.4 cm. Green parts of moss layer were harvested thereafter.

2.4. Plant analyses

Biomass samples were dried at 90 °C for at least 48 h, and the dry mass was determined. Total nitrogen and phosphorus concentrations (concentration per unit dry mass) of the samples were determined after standard Kjeldahl digestion by indophenol and molybdenum blue methods (Grimshaw et al., 1989).

2.5. Soil characteristics

From every plot included in the long-term fertilization experiment, duplicate soil samples of A horizon were taken from the depth between 5 and 10 cm at peak vegetation period during 5–10 July, 1989. Soil pH was measured in 1 M KCl solution (pH_{KCl}). Ammonium lactate–acetate-soluble K was determined by flame emission spectroscopy and P by molybdenum blue method after extraction with the mixture of lactic (0.1 M) and acetic (0.3 M) acids, and ammonium acetate (0.1 M; pH 3.75). This extraction method estimates the amount of K and P that is available to the plants and is widely used to characterize arable soils and meadows in northern Europe (AL-method, Swedish standard SS 02 83 10; Egner et al., 1960; Eriksson et al., 1997).

Total Ca was analyzed by flame emission spectroscopy and Mg by atom absorption spectroscopy after sample dry ashing and mineral solubilization with concentrated HCl. Soil N concentration was measured as for plant material. Organic carbon concentration was determined colorimetrically according to potassium dichromate oxidation method (Zyrina and Orlova, 1980).

2.6. Statistical analyses

Linear regression analyses were used to test for the relationships between soil variables, and foliage chemical and biomass variables. To improve linearity and/or normality, natural logarithmic transformation was applied where appropriate. In the long-term fertilization experiment, differences in the means of soil parameters and net above-ground productivity between various fertilization treatments were separated by Bonferroni test after one-way ANOVA.

In the preliminary statistical analysis of the short-term fertilization experiment, we first tested for the effect of previous fertilization regime on productivity and nutrient concentration of moss and herb layers in two ways. First, we used only the control treatments that received artesian water but no nutrients, and tested for the effect of previous fertilization regime on plant productivity by one-way ANOVA followed by Bonferroni test. Second, we analyzed the entire split-plot design using PROC MIXED of SAS (SAS Institute Inc., 1990), where the main plot (previous fertilization regime), $M(F)$, was the random variable and all others fixed effects (MODEL $X = F + N + P + N \times P$; random $M(F)$). In this model, F is the previous fertilization regime, N is nitrogen and P phosphorus fertilization level. The model was fitted by restricted maximum likelihood method, the degrees of freedom were calculated by the Satterthwaite procedure and the fixed effects were tested using Type III F -tests to assess the differences between the adjusted means (SAS Institute Inc., 1990; Potvin, 1993). Both analyses demonstrated that the previous fertilization regime did not affect any of the tested plant variables (above-ground productivity and N and P concentrations of herb and moss layers; $P > 0.2$ – 0.99). Thus, a simplified model was constructed by eliminating the non-significant effect of previous fertilization treatment (MODEL $X = M + N + P + N \times P$, where M is the main plot).

In our design, N and P levels were not fully factorial. Although it is known that the maximum likelihood method is relatively insensitive to empty cells (Potvin, 1993), we tested for the $N \times P$ interaction also in a dataset where $N1$, $N3$, $P1$ and $P3$ treatments were removed, resulting in fully factorial design. The results of this analysis were qualitatively identical to those obtained with the entire dataset. In addition, because N and P additions are quantitative variables, we also used covariance analyses to test for the $N \times P$ interaction term.

To gain detailed insight into the main plot differences in productivity and nutrient concentrations, we used plot-specific plant biomass (average for 1989–1995 years) and soil

N and P concentrations as plot-specific explaining variables in the regression analysis. All relationships were considered significant at $P < 0.05$ (SAS Institute Inc., 1990).

3. Results

3.1. Soil characteristics

Soil available phosphorus concentrations in the order of 15–35 mg kg⁻¹ (Table 1) were very low (< 20 mg kg⁻¹) to low (20–40 mg kg⁻¹) according to the standards for North-European arable and grassland soils. Soluble K concentrations in the order of 0.13–0.16 g kg⁻¹ (Table 1) were average (0.08–0.16 g kg⁻¹) compared with a series of North-European agricultural soils (Eriksson et al., 1997). Soil pH and Ca and Mg concentrations were high (Table 1) compared with the soils in the region (Eriksson et al., 1997). Soil total N and carbon concentrations were high for a mineral soil layer, suggesting a low soil turnover rate (Table 1). Soil N and C concentrations were strongly correlated (inset in Fig. 1C).

Plot-to-plot variability in soil characteristics was largest for P , but during soil sampling in 1989, the previous long-term fertilization during years 1961–1981 had a significant effect on soil P availability only in plot PKN1 (Table 1).

3.2. Above-ground productivity of herbaceous plants in relation to soil nutrients

There was a strong correlation between above-ground productivity and soil phosphorus availability (Fig. 1A). Although this relationship was biased by one exceptionally high soil P /herbaceous productivity pair, P versus productivity relationship was significant without this data point as well (Fig. 1A). Plant above-ground productivity was also correlated with soil total N concentration (Fig. 1B) and soil carbon concentration (Fig. 1C).

3.3. Responses of productivity to cessation of fertilization

The plot-specific values of above-ground productivity of herb layer measured in various years after cessation of fertilization were correlated (Fig. 2). However, there was a significant year-to-year variability in the standing biomass pools of herb layer (Table 2 and Fig. 2). After cessation of fertilization, the productivity of fertilized plots was always lower than during the period of fertilization (average data for 1961–

Table 1
Characteristics of the soil A horizon in the fertilized and unfertilized plots (average \pm S.E.)

Treatment	Soluble P (mg kg ⁻¹)	Soluble K (g kg ⁻¹)	Total N (%)	Total C (%)	Total Ca (%)	Total Mg (%)	pH _{KCl}
Control	14.9 \pm 1.6a ^a	0.127 \pm 0.009a	0.548 \pm 0.025a	4.98 \pm 0.08a	0.720 \pm 0.029a	0.425 \pm 0.037a	6.932 \pm 0.027a
PK	25.5 \pm 2.9ab	0.155 \pm 0.007a	0.565 \pm 0.023a	4.946 \pm 0.026a	0.732 \pm 0.026a	0.388 \pm 0.041a	6.912 \pm 0.025a
PKN1	34.6 \pm 6.4b	0.133 \pm 0.011a	0.527 \pm 0.025a	5.06 \pm 0.10a	0.707 \pm 0.014a	0.383 \pm 0.015a	6.922 \pm 0.017a
PKN2	22.9 \pm 2.2ab	0.144 \pm 0.006a	0.580 \pm 0.015a	5.157 \pm 0.041a	0.718 \pm 0.011a	0.400 \pm 0.017a	6.862 \pm 0.025a

Soil measurements were conducted in 1989.

^a Values denoted by the same letter are not significantly different ($P > 0.05$). Means are separated by Bonferroni test following one-way ANOVA.

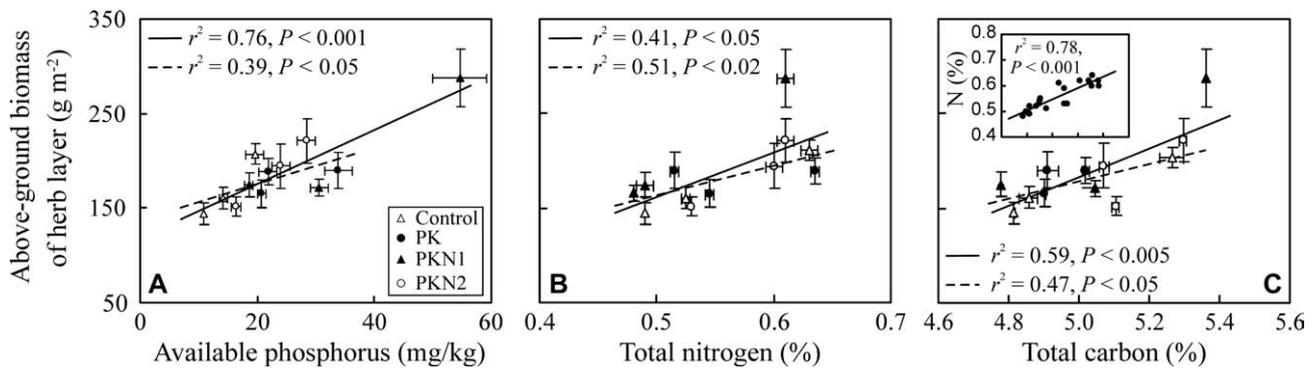


Fig. 1. Dependence of the average (1989–1995) above-ground productivity of the herbaceous layer on soil available P concentration (A), on total soil N concentration (B) and on total soil carbon concentration (C) in the wooded meadow. Various symbols stand for different long-term fertilization treatments. The long-term fertilization experiment continued from 1961 till 1981. During fertilization, P addition rate of 2.6 g m⁻² year⁻¹ and K addition rate of 5 g m⁻² year⁻¹ were the same for all treatments, while PKN1 treatment received additionally 3.5 g N m⁻² year⁻¹, and PKN2 10 g N m⁻² year⁻¹. Error bars give ± S.E. Data were fitted by linear regressions. All regressions were fitted with (solid lines) or without (dashed lines) an influential (largest) data point observed at a PKN1 plot. Inset in C demonstrates the correlation between total soil N and carbon concentrations for all plots sampled.

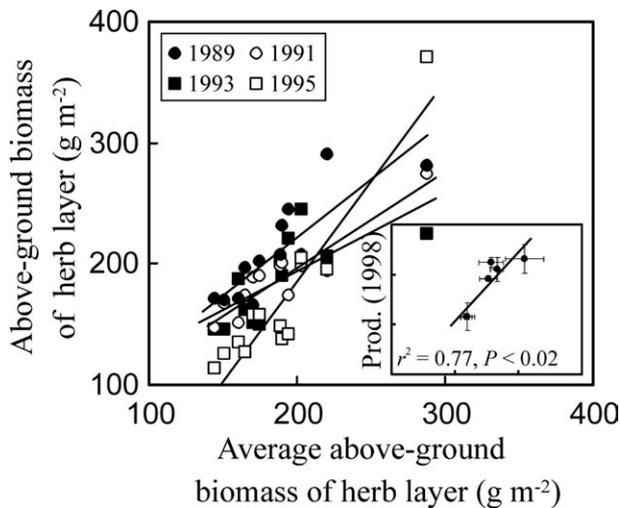


Fig. 2. Correlations between the above-ground productivity of herb layer measured at a specific year following the cessation of fertilization treatment and the average productivity (average of 1989–1995). Data were fitted by linear regressions. The explained variances (r^2) were 0.73 ($P < 0.001$) for 1989, 0.84 ($P < 0.001$) for 1991, 0.52 ($P < 0.02$) for 1993 and 0.87 ($P < 0.001$) for 1995. The inset demonstrates the correlation between the productivity of control plots (1998) of the short-term fertilization treatment (irrigated with artesian water only) with average productivity (1989–1995, error bars provide ± S.E.).

1977 in Table 2). Across the years 1989 (8 years since the cessation of treatment) to 1995 (14 years since the cessation

of fertilization) there was still a time-dependent decrease in herb productivity ($r^2 = 0.92$, $P < 0.05$ for the correlation between the year of sampling and average productivity for all plots sampled). In addition, the fertilization treatment × Year interaction was marginally significant for the period 1989–1995 ($P = 0.07$), further suggesting that the differences in productivity between fertilized and non-fertilized plots gradually decreased.

The effect of previous fertilization was statistically not significant for years 1989–1993 (Table 2), but the above-ground herb biomass pools in PKN1 plots were significantly larger than the standing biomass in control plots in 1995 (Table 2). No such effect of previous fertilization was evident in the control plots of the short-term fertilization treatment (Table 2). These data also indicate that plot-to-plot differences in nutrient availability (Table 1 and Fig. 1) may provide the primary explanation of temporarily consistent variations in productivity among the plots (Fig. 2).

3.4. Responses of herb and moss productivity to short-term fertilization

Productivity of herb layer increased with both N ($P < 0.001$) and P addition ($P < 0.001$; Fig. 3A). Furthermore, there was a significant interaction between N and P treatments ($P < 0.03$), implying that herb productivity increased more when both N and P were added simultaneously than when these nutrients were supplied alone (Fig. 3A).

Table 2

Above-ground productivity of the herb layer (average ± S.E. g m⁻²) during fertilization (1961–1981), and at various years after cessation of fertilization

Treatment	Productivity during fertilization	Productivity after fertilization at various years				
		1989	1991	1993	1995	1998
Control	126.2 ± 5.3a	183 ± 12a	165 ± 16a	192 ± 29a	124.8 ± 5.7a	267 ± 12a
PK	293 ± 17b	211 ± 10a	191 ± 9a	185 ± 12a	137.5 ± 9.8a	204 ± 21a
PKN1	323 ± 11c	216 ± 34a	218 ± 28a	175 ± 25a	229 ± 39b	223 ± 28a
PKN2	431 ± 16d	234 ± 35a	178 ± 8a	190 ± 23a	153.6 ± 19ab	n.d.

The productivity has been determined every year during the peak of herb layer productivity at the end of June or beginning of July. Values denoted by the same letter are not significantly different ($P > 0.05$). The average for the fertilization period was calculated from Pork (1979), and the means were compared by paired *t*-tests (for each year an average value for four plots per treatment was available). In all other cases, means are separated by Bonferroni test following one-way ANOVA.

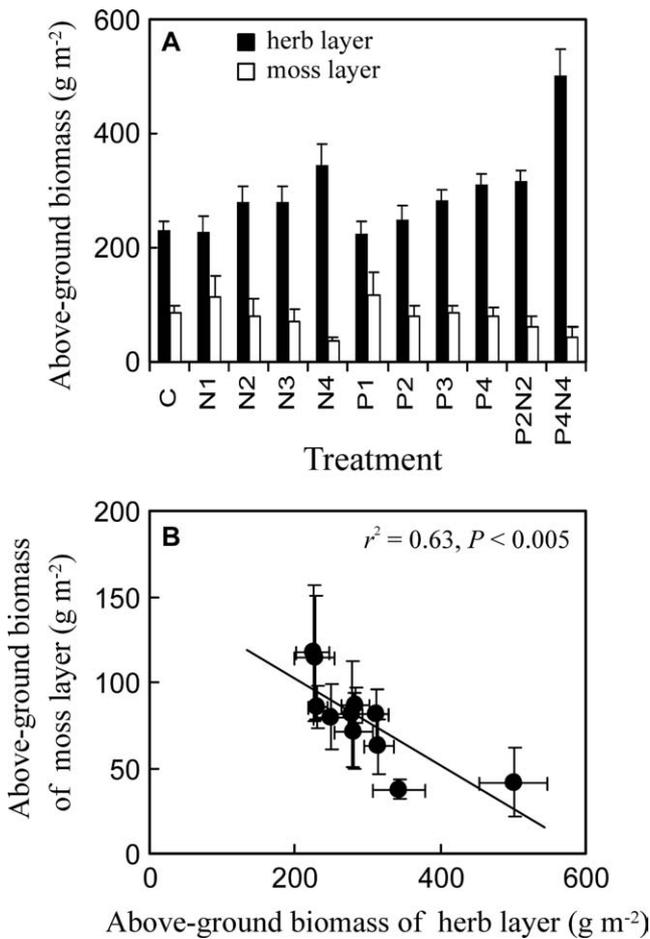


Fig. 3. Variation in above-ground biomass of herb (closed bars) and moss (open bars) layers due to short-term fertilization treatment (A), and the relationship between the above-ground biomass of moss and herb layers (B, averages of all treatments pooled). The treatments were control (C, irrigation with artesian water only), and irrigation with nutrient solutions giving phosphorus additions of 1 g m⁻² (P1), 2 g m⁻² (P2), 4 g m⁻² (P3) and 6 g m⁻² (P4), and N additions of 2 g m⁻² (N1), 5 g m⁻² (N2), 10 g m⁻² (N3) and 20 g m⁻² (N4), and combined N and P additions (P2N2 and P4N4). Data in B were fitted by linear regression. Error bars provide + S.E. in A and ± S.E. in B.

Table 3
Effects of added nitrogen and phosphorus and initial herb productivity^a on the above-ground productivity of herb and moss layers (g m⁻²) and the total (moss + herb) productivity: results of multiple linear regression analyses

Layer	Intercept (g m ⁻²)	Slopes		r ²
		Added N (g m ⁻²)	Initial productivity (g m ⁻²)	
Herb	-108 ^b	5.64	1.66	0.75
Moss	-5.22	-2.60	0.486	0.15
Herb + moss	-113	3.05	2.15	0.63

Layer	Intercept (g m ⁻²)	Slopes		r ²
		Added P (g m ⁻²)	Initial productivity (g m ⁻²)	
Herb	-31.9	13.7	1.27	0.69
Moss	-75.9	-1.33	0.819	0.22
Herb + moss	-108	12.4	2.08	0.66

^a Average (1989–1995) plot-specific above-ground productivity of herb layer.

^b Values in bold font are significantly different from zero ($P < 0.05$).

Moss productivity was independent of P addition ($P > 0.4$), but decreased significantly with increasing N addition ($P < 0.01$), while the interaction between N and P was not significant ($P > 0.6$; Fig. 3A). For all treatments pooled, there was a negative correlation ($P > 0.6$; Fig. 3B). Despite this negative correlation, the total above-ground productivity was positively affected by both N ($P < 0.02$) and P additions ($P < 0.01$), and the interaction between N and P additions was also significant ($P < 0.04$).

The plot (block) effect was significant for both the herb and moss layer above-ground productivities ($P < 0.001$ for both) as expected on the basis of productivity measurements during 1989–1995 (Figs. 1 and 2). The average (1989–1995) plot-specific herb productivity (I_p) that is a proxy for plot inherent nutrient availability explained together with N addition level (Fig. 4A) 75% of total variability in herb productivity, and together with P addition level (Fig. 4B) close to 70% of variability (Table 3). Total (moss + herb) above-

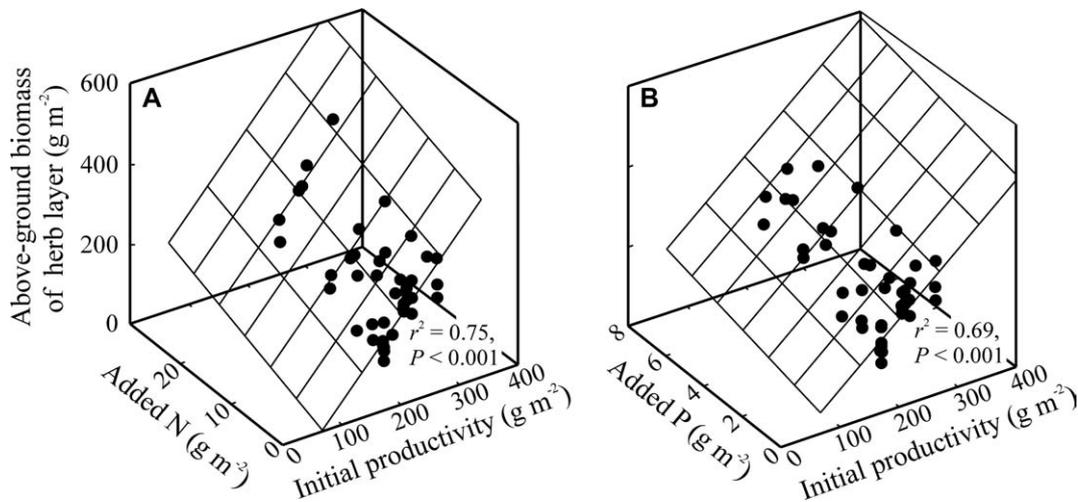


Fig. 4. Dependence of above-ground biomass of herb layer on initial productivity (average plot-specific productivity during 1989–1995) and on added N (A) and P (B) concentrations. Every point corresponds to specific short-term N or P treatment (Fig. 3, combined N and P treatments not included). Data were fitted by multiple linear regressions.

ground productivity was positively correlated with both I_P and N addition (Table 3) and I_P and P addition (Table 3). The interaction terms (N addition) \times I_P and, (P addition) \times I_P were in all cases not statistically significant ($P > 0.3$), demonstrating that the plots with different initial productivity did not respond more strongly to the added nutrients than the less productive plots.

When soil P concentration was used as the explaining variable together with the degree of fertilization, the percentages of explained variation in herb and total above-ground productivities were also 70–75% (data not shown).

3.5. Fertilization effects on vascular plant N and P concentrations

Nitrogen concentration (% of dry matter) of above-ground herb biomass was significantly and positively affected by both N ($P < 0.001$) and P ($P < 0.01$) treatments (Fig. 5A). The interaction between N and P was also significant ($P < 0.001$), demonstrating that above-ground biomass N concentrations increased less if N and P were added simultaneously (Fig. 5A).

Above-ground biomass P concentrations decreased with N addition ($P < 0.005$) and increased with P addition ($P < 0.001$), but the interaction was non-significant ($P > 0.3$; Fig. 5B). Biomass N/P ratio increased with increasing N addition ($P < 0.001$), and decreased with increasing P addition ($P < 0.001$; Fig. 5C). In this case, N and P interaction was highly significant ($P < 0.001$), indicating more balanced nutrition when both nutrients were jointly added.

3.6. Differences between vascular plant and moss nutrient concentrations

The concentrations of N and P in mosses responded similarly to N and P additions as the nutrient concentrations in vascular plants (Fig. 5). However, the N concentration of mosses was unaffected by P addition ($P > 0.3$), and moss P concentration was independent of N addition ($P > 0.2$).

N and P concentrations of mosses and herbs were correlated (Fig. 6) with several discrepancies. Specifically, N and P concentrations of mosses varied less than those in herbs (Fig. 6A, B), and N concentrations were larger over most of the range of variation in vascular plants (Figs. 5 and 6A), while P concentrations were generally larger in mosses (Figs. 5 and 6B). This resulted in smaller N/P ratio in mosses relative to vascular plants (Fig. 6C).

4. Discussion

4.1. Plant above-ground productivity and soil chemistry in the wooded meadow

Our hypothesis of the ecosystem-level P-limitations in these calcareous soils was supported by low soil P availabili-

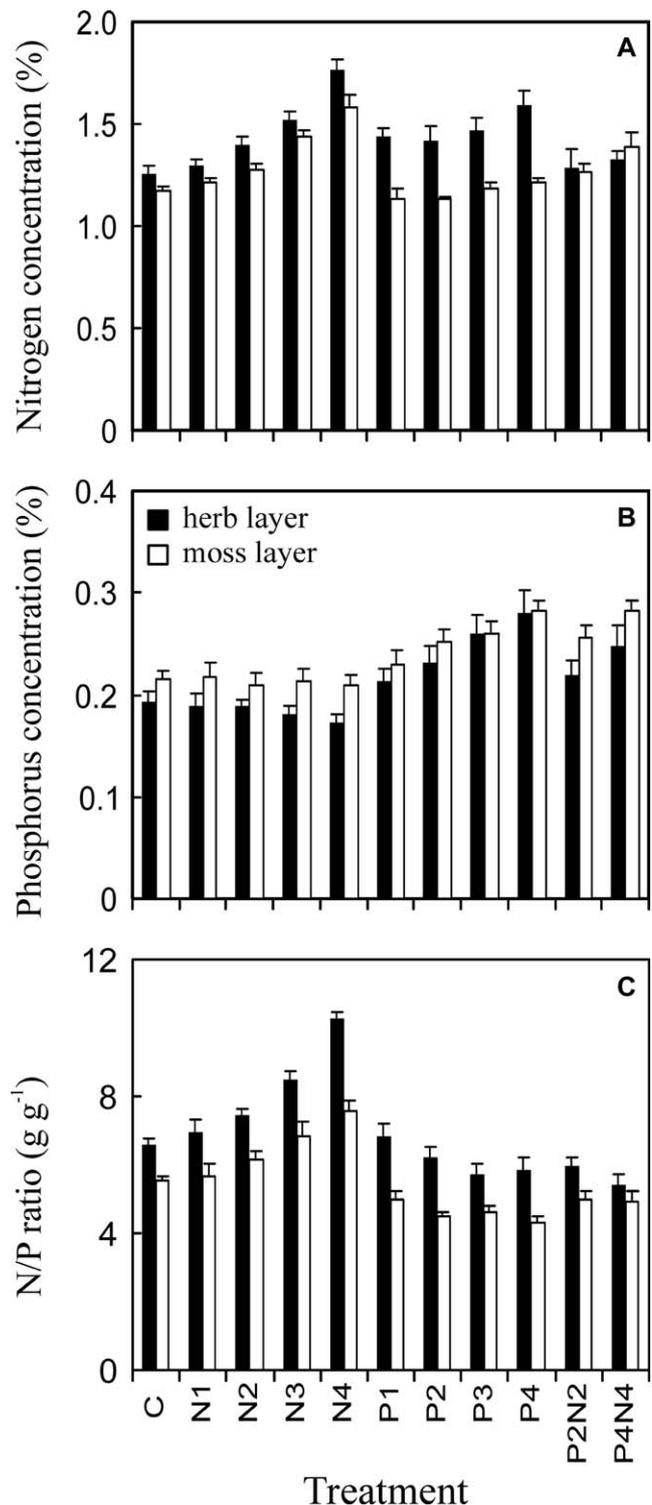


Fig. 5. Variation in above-ground herb (filled bars) and moss (open bars) biomass nitrogen (A) and phosphorus (B) concentrations, and N/P ratio (C) due to short-term fertilization treatment. Treatment codes as in Fig. 3A. Error bars provide + S.E.

ties compared with the soils in this region (Table 1, Eriksson et al., 1997), and an overall strong effect of soil P availability on above-ground productivity of herb layer (Fig. 1A). This correlation alone does not rule out possible N limitations in this system, because the soil N and P cycles are often strongly

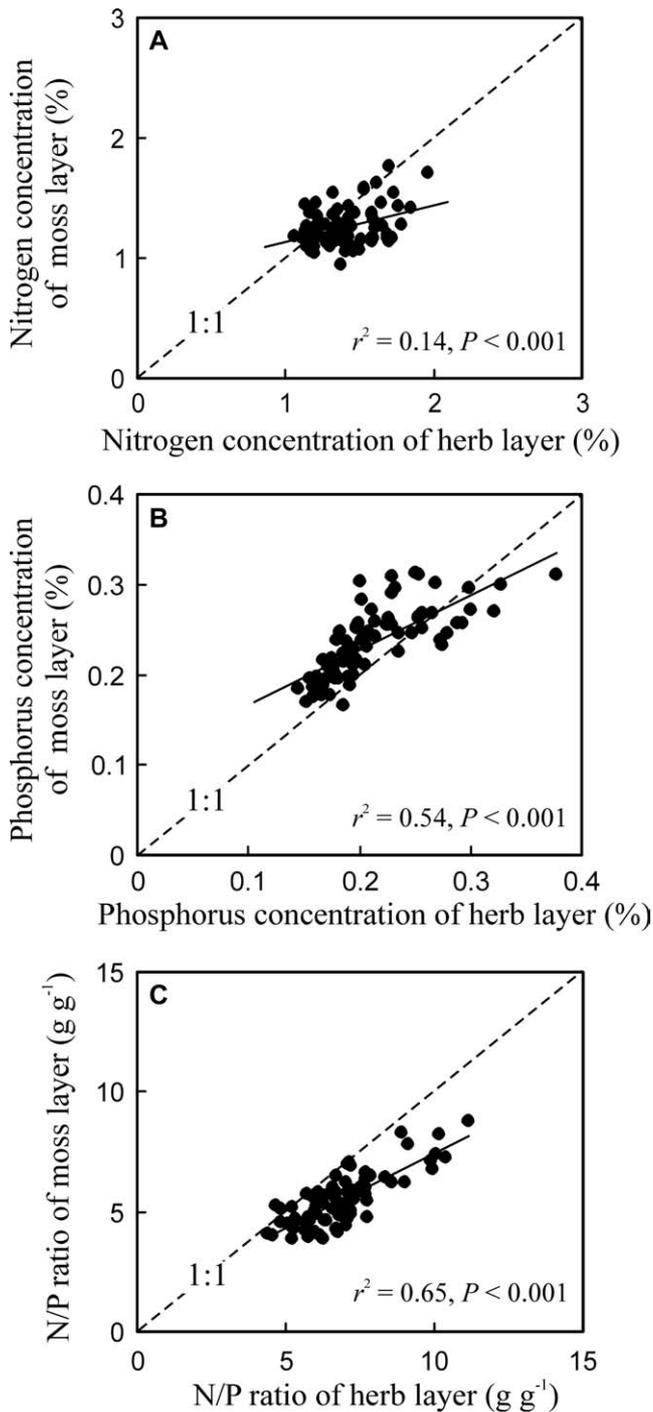


Fig. 6. Correlations between the nitrogen (A), and phosphorus (B) concentrations and N/P ratios (C) of above-ground biomass of herbs and mosses from the short-term fertilization experiment (all data points pooled). Data were fitted by linear regressions (solid lines). Dashed lines provide the 1:1 relations.

coupled. A large fraction of soil P is in organic compounds, and the organic plant-unavailable P may even exceed the plant-available P that is present as soluble phosphates (Binkley and Vitousek, 1989). Accordingly, low N supply due to low mineralization rate and limited N fixation may limit the turnover of organic P, and be the cause of low soil P concentrations. At the same time, low P concentrations may limit N

mineralization and fixation leading to low N availabilities as well. There is evidence that N and P additions increase soil microbial activity and nutrient release from soil organic matter (Scheu, 1990; Prescott et al., 1992b; Olf et al., 1994; Aber et al., 1995; Lovell and Hatch, 1998; Grierson et al., 1999) and P addition increase N fixation (Drevon and Hartwig, 1997; Niklaus et al., 1998).

We found that total soil N was positively correlated with productivity (Fig. 1B). However, it is unclear whether this relationship reflects N availability effects on productivity per se. In our study, both soil N and C concentrations were high (Table 1), and C/N ratio on the order of 9–10 (mass/mass) was also high. Total soil N may also be large in productive sites that form more litter with a lower turnover rate. Large humus N concentrations are often indicative of decreased litter turnover (Scheu, 1990; Berg et al., 1995; Saetre et al., 1999). This suggestion is partly supported by the positive correlations between total soil carbon concentration and productivity (Fig. 1C) and soil total C and N concentrations (inset in Fig. 1C). Overall, such large soil N contents and low C/N ratios are typical for calcareous soils, and characterize the high N immobilization capacity of these soils (Kooijman et al., 1998). Thus, the positive correlations between productivity and total soil C and N concentrations may reflect an overall higher accumulation of plant litter and soil humus in more productive plots rather than demonstrate a true effect of enhanced soil N availability on biomass production.

4.2. Long-term fertilization experiment and productivity responses after cessation of fertilization

The long-term fertilization experiment conducted during 1961–1981 further demonstrated a strong effect of P on productivity (PK treatment in Table 2, recalculated from the data of Pork, 1979). This experiment also suggested a significant N limitation to productivity (PKN1 and PKN2 treatments in Table 2).

According to the fertilization experiments in Estonian rendzic soils, excess P is bound in the upper soil horizons in relatively available forms, resulting in a good positive correlation between total soil P and lactate-soluble P ($r^2 = 0.79$, $P < 0.001$, recalculated from Raudväli, 1995). Therefore, enhanced soil P concentrations are maintained for several years after the fertilization in rendzic soils (Raudväli, 1995). We observed a continuous decline in productivity during the period of 8–14 years after cessation of treatment such that the effect of previous fertilization on production gradually vanished with time (Table 2), but also that the fertilization effect was occasionally evident in productivity even 14 years after termination of fertilization.

The time needed for complete ecosystem recovery after cessation of fertilization varies from study-to-study (Berdendse and Elberse, 1990; Olf and Bakker, 1991; Strengbom et al., 2001), partly because of variation in community capacity for nutrient retention (Prescott et al., 1992b), and possibly also because different species have different kinetics of dis-

persal and establishment (Berendse and Elberse, 1990; Olf and Bakker, 1991). Moreover, even in apparently stable phases of succession, there may be fluctuations in species number and productivity (Olf and Bakker, 1991).

Previous studies following the above-ground productivity and soil activity in calcareous grasslands after cessation of fertilization demonstrate that there are large time-dependent decreases in soil N mineralization (Olf et al., 1994) that lead to a significant decline in productivity (Olf and Bakker, 1991; Olf et al., 1994). However, these studies also demonstrate that decreases in N mineralization also result in decreased soil P availabilities that may further constrain productivity and N release from soil organic matter (Olf et al., 1994), further strengthening the argument of a tight coupling of N and P cycles in such grasslands.

4.3. Short-term fertilization treatment: further evidence of the co-limitation of productivity by N and P

Our data demonstrate that both N and P additions alone enhanced the above-ground productivity of the herb layer (Fig. 3A). Furthermore, there was a significant interaction between N and P additions on productivity (Fig. 3A), suggesting a co-limitation of productivity by both nutrients in our site on calcareous soil. Such interactive effects of N and P on productivity have been previously observed in mire (Verhoeven and Schmitz, 1991) and forest (DeLucia et al., 1989; Scheu, 1990; Prescott et al., 1992a; Whitehead et al., 1997) ecosystems on acidic soils, and together with our results suggest that N and P co-limitation often occurs in natural ecosystems. In fact, whenever P mineralization from soil organic matter rather than P desorption from soil colloids and/or soil mineral weathering limits P availability, one always expects N and P co-limitations on productivity (Parton et al., 1988). As in our study, co-limitation of productivity by N and P has been found in British (Morecroft et al., 1994) and Dutch calcareous grasslands (Kooijman et al., 1998). However, contrary to our and these studies, only the legume productivity was found to be P-limited in calcareous pasture in the foothills of Jura at ca. 500 m elevation in Switzerland (Niklaus et al., 1998; Stöcklin et al., 1998; Stöcklin and Körner, 1999), possibly because of stronger limitations on N mineralization than on P weathering in these soils. A greenhouse bioassay using a soil from Swiss calcareous non-grazed grassland demonstrated that both N and P appear to limit herb productivity in these alkaline soils as well (Köhler et al., 2001).

Examination of nutrient concentrations provide further insight into the nature of nutrient limitations in our study (Fig. 5). Our data demonstrate that N uptake of the herb layer was positively affected by enhanced P availability (Fig. 5A). Such a positive effect of P addition may result either because N uptake was limited by P availability in unfertilized soil or because P additions enhanced soil N mineralization. In our study, N/P ratios significantly decreased in P addition treatments (Fig. 5C), suggesting that there were unlikely excess mineral N pools in the soil. Thus, a short-term stimulation of

soil turnover by P seems a more likely explanation for P-related increases in plant N. A similar tendency for enhanced herb N concentrations in response to P fertilization has been observed in wetland (Güsewell et al., 2003b) and tundra (Bowman, 1994) grassland fertilization experiments.

4.4. N and P concentrations, N/P ratio and co-limitation

On the basis of a literature review of wetland fertilization experiments, Koerselman and Meuleman (1996) suggested that the vegetation above-ground biomass N/P ratios (mass/mass) less than 14 indicate N limitations, while the ratios N/P > 16 indicate P-limitations. In our study, N/P ratio for the control plots exhibiting co-limitation of N and P ranged from 5.6 to 7.5 and averaged 6.61 ± 0.17 (\pm S.E.; Fig. 5C). Although the criterion of Koerselman and Meuleman (1996) has been almost universally applied to various ecosystems, recent work demonstrates that the critical N/P values may be species- or ecosystem-specific (Güsewell et al., 2003b; Tomassen et al., 2003, 2004; Drenovsky and Richards, 2004). The productivity of certain species may be P-limited even at N/P ratios of 5–10 (Drenovsky and Richards, 2004), while some species may still be responsive to N fertilization at N/P ratios >40 (Tomassen et al., 2004).

To put these conflicting results in a context, it is important that the community-level above-ground N/P ratio is the weighted average of the N/P ratios of composite species, and therefore, critically depends on long-term changes in vegetation composition. In particular, enhanced N deposition leads to dominance of species with lower P requirements, and overall modification in critical N/P ratios (Gordon et al., 2001; Tomassen et al., 2003). The review of Koerselman and Meuleman (1996) was compiled using the western European sites that have been exposed to high N deposition loads, in the order of $1.5\text{--}6 \text{ g m}^{-2}$ during the last century (Phoenix et al., 2003; Van Heerwaarden et al., 2003). Thus, in addition to strong immobilization capacity of calcareous soils for both N and P, the overall dominance of species with larger P requirement at our site with low N-depositions, in the order of $0.3\text{--}0.5 \text{ g m}^{-2}$ (Bartnicki et al., 2003) may explain why the critical N/P ratio for N and P co-limitation was lower. Similarly to the critical N/P ratio in our site, N and P co-limitation has been observed for N/P ratios between 6.7 and 26.3 in a calcareous grassland in Sahel (Penning de Vries et al., 1980).

4.5. Grassland productivity response to fertilization versus the initial productivity

The short-term fertilization experiment was conducted in plots with widely varying initial productivities (Fig. 2). We initially hypothesized that the short-term fertilization effect is stronger in plots with higher initial productivity, because more productive sites support species with inherently higher growth rates and enhanced nutrient use efficiency (Chapin et al., 1986b; Aerts, 1990; Berendse, 1993), and such plot-to-plot differences in species composition have been described

for the study area (Kull and Zobel, 1991). We tested for these possible interactions using the interaction terms [(N addition) \times (initial productivity and (P addition) \times (initial productivity))] that characterize the change of the slope of the regression plane in Fig. 4. All interaction terms for the regressions depicted in Table 3 were statistically not significant, demonstrating that all plots fitted the same general response surfaces (Fig. 4A, B). Although this result contrasts with our hypothesis, it does indicate that even the most productive plots were strongly nutrient-limited. In addition to our study, there is further evidence that species from nutrient-richer sites are not necessarily more responsive to fertilization, especially if P also limits productivity (Keddy et al., 2001; Güsewell et al., 2003a, 2003b) as was the case in our study.

4.6. Nutrient co-limitation: a possible explanation for high species diversity?

Nutrient limitation is one factor evening species competitive potentials, and numerous experimental data indicate that nutrient enrichment results in significant decreases in grassland species richness (Olf and Bakker, 1991). While the species-rich grasslands that are mainly N-limited support 30–40 species per m², the most species-rich communities including our site support ca. 70–90 species per m² (see Section 1). All these extremely species-rich grasslands have neutral to slightly alkaline soils, they are prone to drought, are subject to frequent disturbance due to mowing or grazing and have essentially the same values of primary productivity of 80–150 g m⁻² as our study site (Kull and Zobel, 1991). Similarly to N, species widely differ with respect to the degree of growth rate adjustment to improved P nutrition (Chapin et al., 1983; Aerts, 1994), whereas the species ranking according to growth rate responses to N and P is not necessarily the same (Chapin et al., 1986a; Lajtha, 1994; Ryser and Lambers, 1995; Ryser et al., 1997). Thus, N and P co-limitations should equalize species competitive potentials more than each limitation acting separately. We suggest that strong nutrient limitations imposed on plant primary productivity, in particular the co-limitation of productivity by both N and P, can partly explain the high small-scale species richness in these communities.

4.7. Differences between moss and vascular plant responses to fertilization

We observed that the productivity of mosses decreased in response to enhanced N addition (Fig. 3A), and that there was an overall negative correlation between moss and vascular species productivity (Fig. 3B). Such a negative effect of enhanced vascular plant biomass on moss productivity is consistent with previous observations (Brown, 1982; Ingerpuu et al., 1998; Strengbom et al., 2001), and can be explained by more severe light competition at the bottom of dense vascular plant canopies as well as by fewer regeneration niches due to extensive cushions of productive grass species (Rydin, 1997).

Importantly, P additions did not decrease moss productivity, even though the effect of P fertilization on vascular plant productivity was of similar magnitude than that of N fertilization (Fig. 3A). Our data in general suggest that the moss layer requires a larger P concentration relative to N than the herb layer (Figs. 5 and 6). Larger P requirement of mosses may be associated with the circumstance that their photosynthesis essentially always functions at CO₂-saturated conditions due to low irradiances and high CO₂ concentrations at the bottom of vascular plant layer. Because of the high fraction of cytosolic P bound in sugar esters, photosynthesis in high CO₂ is generally more sensitive to low P availabilities than photosynthesis at current ambient CO₂ atmospheres (Dietz and Harris, 1997). Thus, maintenance of moss productivity with increasing P addition may be associated with more efficient light and N usage in photosynthesis in P-fertilized mosses. These data collectively demonstrate important contribution of mosses to the overall ecosystem productivity, but also indicate that vascular plant and moss responses may be different for N and P fertilizations, significantly modifying the whole-ecosystem response.

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References

- Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air Soil Pollut.* 85, 1665–1670.
- Aerts, R., 1990. Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* 84, 391–397.
- Aerts, R., 1994. The effect of nitrogen supply on the partitioning of biomass and nitrogen in plant species from heathlands and fens: alternatives in plant functioning and scientific approach. In: Roy, J., Garnier, E. (Eds.), *A Whole Plant Perspective on Carbon–Nitrogen Interactions*. SPB Academic Publishing bv, The Hague, pp. 248–265.
- Afanasyeva, E.A., Golubev, V.N., 1962. A description of soils and vegetation of Streletsk steppe. *Kurskoye Knizhnoye Izdatelstvo*, Kursk, in Russian.
- Anonymous, 2001. Biologists observe a record species richness. *Postimees*, June 29, 1 (in Estonian).
- Barrett, D.J., Gifford, R.M., 1995. Photosynthetic acclimation to elevated CO₂ in relation to biomass allocation in cotton. *J. Biogeogr.* 22, 331–339.
- Bartnicki, J., Gusev, A., Barrett, K., Simpson, D., 2003. Atmospheric supply of nitrogen, lead, cadmium, mercury and lindane to the Baltic Sea in the period 1996–2000. *EMEP Centres Joint Report for HELCOM*. Norwegian Meteorological Institute, Oslo.

- Berendse, F., 1993. Ecosystem stability, competition, and nutrient cycling. In: Schulze, E.-D., Mooney, H.A. (Eds.), *Biodiversity and Ecosystem Function*, Ecological Studies, 99. Springer Verlag, Berlin, pp. 409–431.
- Berendse, F., Elberse, W.T., 1990. Competition and nutrient availability in heathland and grassland ecosystems. In: Grace, J.B., Tilman, D. (Eds.), *Perspectives on Plant Competition*. Academic Press, Inc., San Diego, pp. 93–116.
- Berg, B., McClaugherty, C., Virzo de Santo, A., Johansson, M.B., Ekbohm, G., 1995. Decomposition of litter and soil organic matter - can we distinguish a mechanism for soil organic matter buildup? *Scand. J. Forest Res.* 10, 108–119.
- Bilbrough, C.J., Caldwell, M.M., 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. *Ecology* 78, 231–243.
- Binkley, D., Vitousek, P., 1989. Soil nutrient availability. In: Percy, R.W., Ehleringer, J.R., Mooney, H.A., Rundel, P.W. (Eds.), *Plant Physiological Ecology. Field Methods and Instrumentation*. Chapman and Hall, London, pp. 75–96.
- Bowman, W.D., 1994. Accumulation and use of nitrogen and phosphorus following fertilization in two alpine tundra communities. *Oikos* 70, 261–270.
- Brown, D.H., 1982. Mineral nutrition. In: Smith, A.J.E. (Ed.), *Bryophyte Ecology*. Chapman and Hall, London, pp. 383–444.
- Chapin III, F.S., Tyron, P.R., Van Cleve, K., 1983. Influence of phosphorus on growth and biomass distribution of Alaskan taiga tree seedlings. *Can. J. For. Res.* 13, 1092–1098.
- Chapin III, F.S., Van Cleve, K., Tryon, P.R., 1986a. Relationship of ion absorption to growth rate in taiga trees. *Oecologia* 69, 238–242.
- Chapin III, F.S., Vitousek, P.M., Van Cleve, K., 1986b. The nature of nutrient limitation in plant communities. *Am. Nat.* 127, 48–58.
- Chapin III, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. *BioScience* 37, 49–57.
- Conroy, J.P., Küppers, M., Küppers, B., Virgona, J., Barlow, E.W.R., 1988. The influence of CO₂ enrichment, phosphorus deficiency and water stress on the growth, conductance and water use of *Pinus radiata* D. Don. *Plant Cell Environ.* 11, 91–98.
- DeLucia, E.H., Schlesinger, W.H., Billings, W.D., 1989. Edaphic limitations to growth and photosynthesis in Sierran and Great Basin vegetation. *Oecologia* 78, 184–190.
- Dietz, K.-J., Harris, G.C., 1997. Photosynthesis under nutrient deficiency. In: Pessaraki, M. (Ed.), *Handbook of Photosynthesis, Books in Soils, Plants, and the Environment*. Marcel Dekker, New York, pp. 951–975.
- Drenovsky, R.E., Richards, J.H., 2004. Critical N:P values: predicting nutrient deficiencies in desert shrublands. *Plant Soil* 259, 59–69.
- Drevon, J.-J., Hartwig, U.A., 1997. Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa. *Planta* 201, 463–469.
- Egner, H., Riehm, H., Domingo, W., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. *Ann. Royal Agricult. Coll. Sweden* 26, 1–199.
- Eriksson, J., Andersson, A., Andersson, R., 1997. Tillståndet i svensk åkermark. Naturvårdverket, Rapport 4778. Naturvårdverket förlag, Uppsala.
- Frossard, E., Brossard, M., Hedley, M.J., Metherell, A., 1995. Reactions controlling the cycling of P in soils. In: Tiessen, H. (Ed.), *Phosphorus in the Global Environment. Transfers, Cycles and Management*, Scope, 54. Wiley, Chichester, pp. 107–137.
- Gordon, C., Wynn, J.M., Woodin, S.J., 2001. Impacts of increased nitrogen supply on high Arctic heath: the importance of bryophytes and phosphorus availability. *New Phytol.* 149, 461–471.
- Grierson, P.F., Comerford, N.B., Jokela, E.J., 1999. Phosphorus mineralization and microbial biomass in a Florida spodosol: effects of water potential, temperature and fertilizer application. *Biol. Fertil. Soils* 28, 244–252.
- Grimshaw, H.M., Allen, S.E., Parkinson, J.A., 1989. Nutrient elements. In: Allen, S.E. (Ed.), *Chemical Analysis of Ecological Materials*, (2nd ed.). Blackwell Scientific Publications, Oxford, pp. 81–159.
- Güsewell, S., Bollens, U., Ryser, P., Klötzli, F., 2003a. Contrasting effects of nitrogen, phosphorus and water regime on first- and second-year growth of 16 wetland plant species. *Funct. Ecol.* 17, 754–765.
- Güsewell, S., Koerselman, W., Verhoeven, J.T.A., 2003b. Biomass N:P ratios as indicators of nutrient limitation for plant populations in wetlands. *Ecol. Appl.* 13, 372–384.
- Hægglström, C.-A., 1983. Vegetation and soil of the wooded meadows in Nätö, Åland. *Acta Bot. Fenn.* 120, 1–66.
- Huovinen-Hufschmid, C., Körner, C., 1998. Microscale patterns of plant species distribution, biomass and leaf tissue quality in calcareous grassland. *Bot. Helv.* 108, 69–83.
- Ingerpuu, N., Kull, K., Vellak, K., 1998. Bryophyte vegetation in a wooded meadow: relationships with phanerogam diversity and responses to fertilisation. *Plant Ecol.* 134, 163–171.
- Inouye, R.S., Tilman, D., 1995. Convergence and divergence of old-field vegetation after 11 years of nitrogen addition. *Ecology* 76, 1872–1887.
- Keddy, P., Fraser, L.H., Keogh, T.A., 2001. Responses of 21 wetland species to shortages of light, nitrogen and phosphorus. *Bull. Geobot. Inst. ETH* 67, 13–26.
- Köhler, B., Ryser, P., Güsewell, S., Gigon, A., 2001. Nutrient availability and limitation in traditionally mown and in abandoned limestone grasslands: a bioassay experiment. *Plant Soil* 230, 323–332.
- Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* 33, 1441–1450.
- Kooijman, A.M., Dopheide, J., Sevink, J., Takken, I., Verstraten, J., 1998. Nutrient limitations and their implications for the effects of atmospheric deposition in coastal dunes: lime-poor and lime-rich sites in the Netherlands. *J. Ecol.* 86, 511–526.
- Krall, H., Pork, K., 1970. Laelatu wooded meadow. In: Kumari, E. (Ed.), *Lääne-Eesti rannikualade loodus*. Valgus, Tallinn, in Estonian, pp. 115–128.
- Kukk, T., Kull, K., 1997. Wooded meadows. Estonia Maritima. Publications of the West-Estonian Archipelago Biosphere Reserve. 2. West-Estonian Archipelago Biosphere Reserve, Haapsalu, in Estonian.
- Kull, K., Zobel, M., 1991. High species richness in an Estonian wooded meadow. *J. Veg. Sci.* 2, 711–714.
- Lajtha, K., 1994. Nutrient uptake in eastern deciduous tree seedlings. *Plant Soil* 160, 193–199.
- Lajtha, K., Harrison, A.F., 1995. Strategies of phosphorus acquisition and conservation by plant species and communities. In: Tiessen, H. (Ed.), *Phosphorus in the Global Environment. Transfers, Cycles and Management*, Scope, 54. Wiley, Chichester, pp. 139–147.
- Lajtha, K., Klein, M., 1988. The effect of varying nitrogen and phosphorus availability on nutrient use by *Larrea tridentata*, a desert evergreen shrub. *Oecologia* 75, 348–353.
- Lambers, H., Poorter, H., 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. In: Begon, M., Fitter, A.H. (Eds.), *Advances in Ecological Research* 23. Academic Press, London, pp. 187–261.
- Lovell, R.D., Hatch, D.J., 1998. Stimulation of microbial activity following spring applications of nitrogen. *Biol. Fertil. Soils* 26, 28–30.
- Lovelock, C.E., Kyllö, D., Popp, M., Isopp, H., Virgo, A., Winter, K., 1997. Symbiotic vesicular-arbuscular mycorrhizae influence maximum rates of photosynthesis in tropical tree seedlings grown under elevated CO₂. *Aust. J. Plant Physiol.* 24, 185–194.
- McJannet, C.L., Keddy, P.A., Pick, F.R., 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. *Funct. Ecol.* 9, 231–238.
- Morecroft, M.D., Sellers, E.K., Lee, J.A., 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *J. Ecol.* 82, 475–483.
- Niinemets, Ü., 2004. Adaptive adjustments to light in foliage and whole-plant characteristics depend on relative age in the perennial herb *Leontodon hispidus*. *New Phytol.* 162, 683–696.
- Niinemets, Ü., Kull, K., 2003. Leaf structure vs. nutrient relationships vary with soil conditions in temperate shrubs and trees. *Acta Oecol.* 24, 209–219.

- Niinemets, Ü., Tenhunen, J.D., Canta, N.R., Chaves, M.M., Faria, T., Pereira, J.S., Reynolds, J.F., 1999. Interactive effects of nitrogen and phosphorus on the acclimation potential of foliage photosynthetic properties of cork oak, *Quercus suber*, to elevated atmospheric CO₂ concentrations. *Glob. Change Biol.* 5, 455–470.
- Niklaus, P.A., Leadley, P.W., Stöcklin, J., Körner, C., 1998. Nutrient relations in calcareous grassland under elevated CO₂. *Oecologia* 116, 67–75.
- Oloff, H., Bakker, J.P., 1991. Long-term dynamics of standing crop and species composition after the cessation of fertilizer application to mown grassland. *J. Appl. Ecol.* 28, 1040–1052.
- Oloff, H., Berendse, F., De Visser, W., 1994. Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *J. Ecol.* 82, 611–620.
- Parton, W.J., Stewart, J.W.B., Cole, C.V., 1988. Dynamics of C, N, P and S in grassland soils: a model. *Biogeochem.* 5, 109–131.
- Penning de Vries, F.W.T., Krul, J.M., Van Keulen, H., 1980. Productivity of Sahelian rangelands in relation to the availability of nitrogen and phosphorus from the soil. In: Rosswall, T. (Ed.), *Nitrogen cycling in West African Ecosystems*. SCOPE/UNEP International Nitrogen Unit, Royal Swedish Academy of Sciences, Uppsala, Sweden, pp. 95–113.
- Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P., Lee, J.A., 2003. Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. *Glob. Change Biol.* 9, 1309–1321.
- Pork, K., 1979. The state of meadows and problems on their utilization at present in the Estonian S.S.R. In: Kalamees, K. (Ed.), *Eesti NSV niitude uurimise bioloogilisi probleeme, Eesti Looduseuurijate Seltsi aastaraamat*, 67. Valgus, Tallinn, in Estonian, pp. 7–37.
- Potvin, C., 1993. ANOVA: experiments in controlled environments. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*. Chapman and Hall, New York, pp. 46–68.
- Prescott, C.E., Corbin, J.P., Parkinson, D., 1992a. Availability of nitrogen and phosphorus in the forest floors of Rocky Mountain coniferous forests. *Can. J. For. Res.* 22, 593–600.
- Prescott, C.E., Corbin, J.P., Parkinson, D., 1992b. Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. *Plant Soil* 143, 1–10.
- Raaimakers, D., Boot, R.G.A., Dijkstra, P., Pot, S., Pons, T., 1995. Photosynthetic rates in relation to leaf phosphorus content in pioneer versus climax tropical rainforest trees. *Oecologia* 102, 120–125.
- Raudväli, E., 1995. Über die Beweglichkeit des Phosphors in rendzina. In: Kuldkepp, P. (Ed.), *Soil and Fertilization*, Transactions of the International Working Group of Soil Fertility of International Society of Soil Science (ISSS). Estonian Agricultural University - IOSDV, Tartu, pp. 96–103.
- Reich, P.B., Schoettle, A.W., 1988. Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient-use efficiency in eastern white pine. *Oecologia* 77, 25–33.
- Reich, P.B., Walters, M.B., Ellsworth, D.S., Uhl, C., 1994. Photosynthesis-nitrogen relations in Amazonian tree species. I. Patterns among species and communities. *Oecologia* 97, 62–72.
- Rydin, H., 1997. Competition among bryophytes. *Adv. Bryol.* 6, 135–168.
- Ryser, P., Lambers, H., 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant Soil* 170, 251–265.
- Ryser, P., Verduyn, B., Lambers, H., 1997. Phosphorus allocation and utilization in three grass species with contrasting response to N and P supply. *New Phytol.* 137, 293–302.
- Saetre, P., Brandtberg, P.-O., Lundkvist, H., Bengtsson, J., 1999. Soil organisms and carbon, nitrogen and phosphorus mineralisation in Norway spruce and mixed Norway spruce-birch stands. *Biol. Fertil. Soils* 28, 382–388.
- SAS Institute Inc, 1990. *SAS/STAT User's guide*, Version 6. (Vols. 1–2. 4th ed.). SAS Institute, Inc., Cary, NC.
- Scheu, S., 1990. Changes in microbial nutrient status during secondary succession and its modification by earthworms. *Oecologia* 84, 351–358.
- Schulze, E.-D., Kelliher, F.M., Körner, C., Lloyd, J., Leuning, R., 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. *Annu. Rev. Ecol. Syst.* 25, 629–660.
- Sepp, R., Rooma, I., 1970. The soils of Virtsu-Laelatu-Puhtu nature reserve. In: Kumari, E. (Ed.), *Lääne-Eesti rannikualade loodus*. Valgus, Tallinn, in Estonian, pp. 83–107.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry. The Principles and Practice of Statistics in Biological Research*. (3rd ed.) W. H. Freeman and Company, New York.
- Stöcklin, J., Körner, C., 1999. Interactive effects of elevated CO₂, P availability and legume presence on calcareous grassland: results of a glasshouse experiment. *Funct. Ecol.* 13, 200–209.
- Stöcklin, J., Schweizer, K., Körner, C., 1998. Effects of elevated CO₂ and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. *Oecologia* 116, 50–56.
- Strengbom, J., Nordin, A., Näsholm, T., Ericson, L., 2001. Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Funct. Ecol.* 15, 451–457.
- Tessier, J.T., Raynal, D.J., 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *J. Appl. Ecol.* 40, 523–534.
- Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. *Science* 294, 843–845.
- Tomassen, H.B., Smolders, A.J.P., Lamers, L.P.M., Roelofs, J.G.M., 2003. Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. *J. Ecol.* 91, 357–370.
- Tomassen, H.B., Smolders, A.J.P., Limpens, J., Lamers, L.P.M., Roelofs, J.G.M., 2004. Expansion of invasive species on ombrotrophic bogs: desiccation or high N deposition? *J. Appl. Ecol.* 41, 139–150.
- Van Heerwaarden, L.M., Toet, S., Aerts, R., 2003. Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *J. Ecol.* 91, 1060–1070.
- Verhoeven, J.T.A., Schmitz, M.B., 1991. Control of plant growth by nitrogen and phosphorus in mesotrophic fens. *Biogeochem.* 12, 135–148.
- Wassen, M.J., Olde Venterink, H.G.M., de Swart, E.O.A.M., 1995. Nutrient concentrations in mire vegetation as a measure of nutrient limitation in mire ecosystems. *J. Veg. Sci.* 6, 5–16.
- Whitehead, S.J., Caporn, S.J.M., Press, M.C., 1997. Effects of elevated CO₂, nitrogen and phosphorus on the growth and photosynthesis of two upland perennials: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytol.* 135, 201–211.
- Willems, J.H., Van Nieuwstadt, M.G.L., 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. *J. Veg. Sci.* 7, 177–184.
- Zyryna, N.G., Orlova, D.S. (Eds.), 1980. *Fiziko-himicheskiye metody issledovaniya pochv. (Physical and Chemical Methods for the Study of Soils)*. Izdatelstvo Moskovskogo Universiteta, Moskva.