

Changes in chalk-grassland structure and species richness resulting from selective nutrient additions

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Abstract. A series of fertilization experiments was carried out over a 5-yr period in a chalk grassland in Limburg (The Netherlands) as part of a study of the maintenance of species richness in species-rich grasslands. Phosphorus and nitrogen were shown to be the most limiting nutrients. Addition of both elements doubled above-ground production, and species richness dropped ca. 50 % in 0.01-m² subplots, relative to controls. However, neither the above-ground production nor plant growth-forms were sufficient to explain the observed changes in species richness. Small-scale structural heterogeneity of the vegetation is probably critical for maintaining high levels of richness. Historically, high nitrogen, low phosphorus conditions were rarely encountered in the Dutch landscape and few species appear adapted to these conditions. Among the chalk grassland species, *Brachypodium pinnatum* seems well adapted to these conditions, where it dominates and excludes most other species. A detailed understanding of the small-scale processes responsible for maintenance of species richness is critically important in efforts to maintain the biodiversity of natural ecosystems.

Keywords: Biomass; *Brachypodium pinnatum*, Canopy structure; Light (PAR); Nitrogen; Phosphorus; Plant nutrient content; Primary production.

Nomenclature: Tutin et al. (1964-1980).

Introduction

At scales between 0.001 and 10 m², the most species-rich plant communities in the world are temperate grasslands (Peet, Glenn-Lewin & Walker Wolf 1983; Walker & Peet, 1983; Shmida & Ellner 1984). Among the best known and most studied examples of species-rich grassland are the chalk grasslands of western Europe. These communities, once widespread but now greatly diminished in extent owing largely to changes in land-use practices (Keymer & Leach 1990; Willems 1990), usually have species numbers exceeding 30, and

occasionally 40 per m² (Willems 1978, 1982).

While species-rich grasslands are found in many locations and under a broad range of ecological conditions, two factors appear to be important: low fertility and episodic removal of foliage, usually by grazing, mowing or fire. In this paper we examine the importance of low fertility in maintaining high species richness by examining the structural consequences to chalk grassland vegetation of selective nutrient addition.

Experimental fertilization of chalk grasslands in southern Limburg with nitrogen, phosphorus and potassium had previously shown this vegetation to be strongly nutrient limited (Willems 1980; During & Willems 1984; Bobbink, Bik & Willems 1988). In addition, these earlier experiments had caused significant declines in species richness, a result commonly observed following grassland fertilization (see Peet, Glenn-Lewin & Walker Wolf 1983). However, the mechanism(s) by which nutrient additions cause species richness to decline has remained unclear. For instance, Grime and co-workers (Al Mufti et al. 1977; Grime 1979, 1990) have proposed that increased above-ground production results in increased rates of competitive exclusion and ultimately in decreases in species richness in much the same way that an increased extinction rate can be expected to lower the equilibrium number of species on an island (MacArthur & Wilson 1967). This argument can be extended to predict that species richness should decline directly with the increase in above-ground production, and should, therefore, be independent of the specific nutrients inducing the increase in production. Tilman (1982, 1985) has proposed an alternative theory based on resource ratios that also predicts richness to be the highest at low levels of productivity. In this study we employ addition of selected nutrients to explicitly test the prediction that changes in richness are tied directly to changes in above-ground production.

Methods

Study site

The study site is located on the Wrakelberg Nature Reserve in Limburg, The Netherlands (50° 52' N, 5° 55' E). The reserve is situated on a south-facing slope (15–20° inclination) between 130 and 170 m a.s.l. The underlying rocks are Upper-Senonian (Cretaceous) calcareous deposits; the shallow (ca. 30 cm) soil is a Rendzina type. Average daily temperatures vary from –1° to +4 °C in the coldest month (January), and from 13 to 23 °C in the warmest month (July). The mean annual precipitation is ca. 750 mm, evenly distributed over the year. For several centuries the slope was part of a common sheep-grazing area and supported a chalk grassland belonging to the *Mesobrometum erecti* (class *Festuco-Brometea*). Around 1900 the lower part of the area was plowed and converted to an arable field. The area was set aside in 1961 as a state-owned nature reserve, and subsequently has been managed by the National Forestry Commission. The vegetation is mown annually in the autumn to prevent spontaneous shrub encroachment and natural succession to woodland. The experimental plots were located on a site where cultivation ended around 1945, immediately down slope from the residual unplowed area. At this site a species-rich *Mesobrometum* grassland had again developed, originating from seed produced in the adjacent unplowed grassland strip. (see Willems & Bobbink 1990).

Field and laboratory methods

A 10 m × 30 m area of maximally homogeneous vegetation was located using detailed (1:200) vegetation maps (Willems & Bobbink 1990) and field inspection. In this designated area, 15 permanently marked 2 m × 6 m experimental plots were established in April 1985 (Fig. 1). These plots consisted of three replicates each of five treatments: (1) control; (2) complete fertilization: NPM = Nitrogen, Phosphorus, Micronutrients; (3) complete fertilization, except for nitrogen: PM; (4) complete fertilization, except for phosphorus: NM; (5) complete fertilization, except for nitrogen and phosphorus: M. Complete fertilization included regular addition of all potentially limiting elements (Table 1).

All nutrients except phosphorus were dissolved in water and distributed evenly over the plot by watering-can twice per year, once in spring (April) and once after mowing of the preserved area as a whole in the autumn (September–October). Nutrient additions were initiated in April 1985 and continued through 1989. All plots, including the controls, received a total of 12 l of tap water at each fertilization. Phosphorus was added

Table 1. Annual nutrient additions to the complete fertilization treatments.

Element	Addition (g/m ²)	Product
N	10.0	NH ₄ NO ₃
P	4.2 (10 P ₂ O ₅)	Triple super phosphate
K	4.2 (5.0 K ₂ O)	KCl
Ca	3.0	CaCl ₂
S	> 2.5	In other products
Mg	2.5	MgSO ₄
Zn	0.5	ZnSO ₄ ·7H ₂ O
Mn	0.5	MnSO ₄ ·H ₂ O
Cu	0.2	CuSO ₄ ·H ₂ O
Fe	0.2	FeSO ₄ ·H ₂ O
B	0.02	Solubor
Mo	0.005	Na ₂ MoO ₄ ·2H ₂ O

in the form of small pellets (ca. 1 mm) to provide slower release.

For each 2 m × 6 m plot, a central 1 m × 5 m area was marked for observation, with the remaining area serving as a buffer zone. The 1 m × 5 m area was divided into ten 0.5 m × 0.5 m subplots designated for inventory of species composition and eight 0.5 m × 0.5 m subplots for biomass harvesting. In each of the ten 0.5 m × 0.5 m inventory subplots, a transect of five 10 cm × 10 cm subplots was located along the outer edge, and in the corner of each of these a 3.1 m × 3.1 cm subplot was established (Fig. 1). For each of the 15 experimental plots, this provided a total of 50 0.001-m² subplots, 50 0.01-m² subplots, and 10 0.25-m² subplots. Species presence was recorded annually in July in each permanently marked subplot of each size for 5 yr. Species were considered present only if a shoot was rooted within the subplot. The nested-plot design was chosen in order to observe the impact of fertilizer treatment on the vegetation at different scales.

Above-ground biomass was harvested in July at the time of peak standing crop. The plants were clipped by hand shears a few cm above the soil surface, in order to prevent any lethal effects. There appeared to be hardly any regrowth on the plots until the following growing season; peak standing crop effectively equals yearly productivity. The harvested biomass was sorted into five categories: (*Brachypodium pinnatum*, other graminoids including *Carex* spp., legumes, other forbs, and standing dead), dried at 70 °C for 48 h, and weighed. Soil samples were collected in duplicate from the buffer area surrounding the inventory and harvest subplots to a depth of 10 cm immediately after the harvest.

Nitrogen and phosphorus concentrations of biomass samples were determined by digesting ca. 150 mg ground material in 5 ml 30N sulfuric acid, together with salicylic acid, and a mixture of sodium sulfate, copper sulfate and selenium (97 : 1.5 : 1.5). The diluted digestions were analyzed colorimetrically with a continuous-

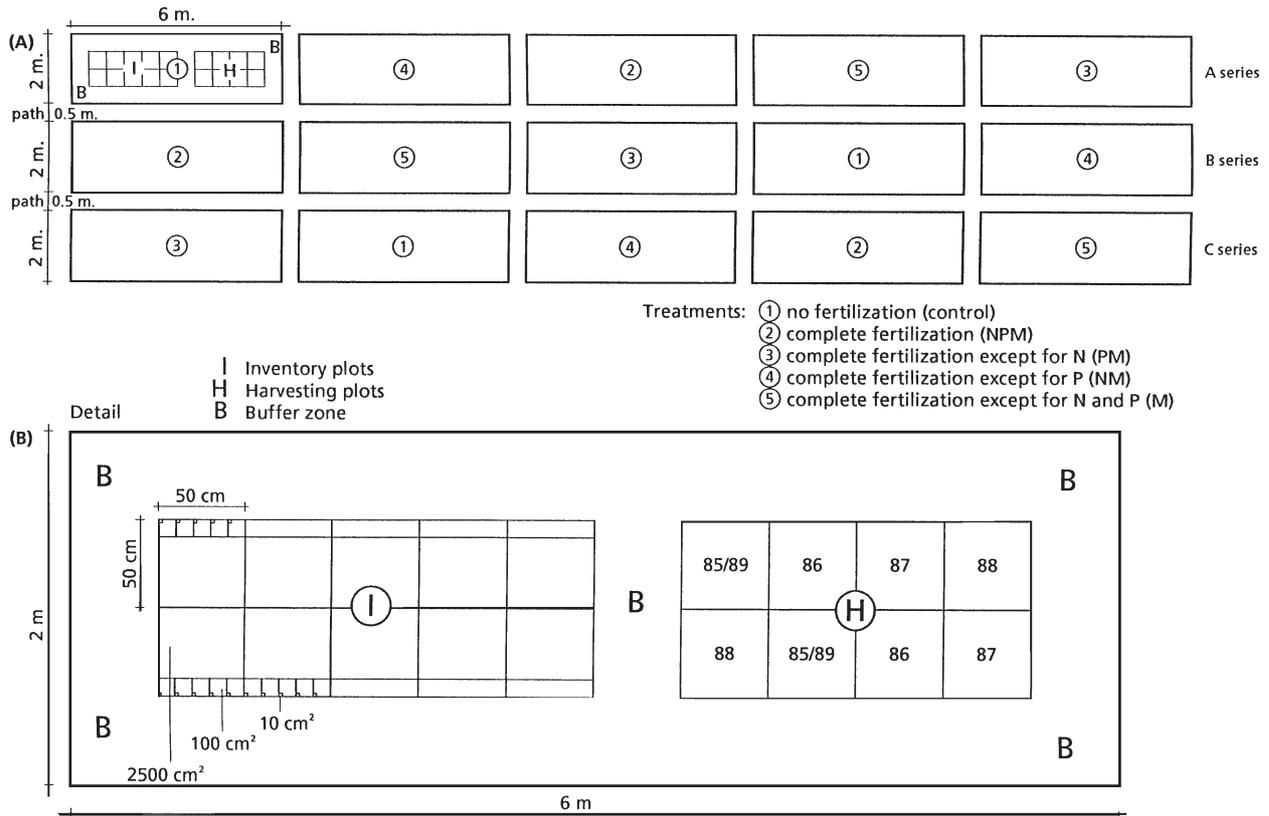


Fig. 1. The study area (A) included three replicates of five experimental treatments. In each plot (B), a central area was divided into subplots, inventory (I) and harvest plots (H), harvested as indicated. Two plots for soil sampling are situated in between these series.

flow auto-analyzer (SKALAR - SA-40) using the ammoniummolybdate method for phosphorus, the indophenolblue method for ammonium and flame emission for potassium. For soil samples ca. 10 g of dried soil was mixed with 10 ml distilled water and shaken. The filtrate was analysed colorimetrically using the auto-analyzer.

Canopy light profiles were constructed in late June 1989 for six 0.5 m × 0.5 m subplots in each treatment type. Photosynthetically active radiation (PAR, 400 - 700 nm) was measured using a line sensor with a sensitive surface of 8 cm × 0.8 cm (TFDL, Wageningen). The sensor was shaped like a narrow rod and could be inserted horizontally into the vegetation without disturbing the foliage distribution. Light was recorded on sunny days at 5 cm vertical intervals for the first 30 cm above the ground, and then at 40, 50, 60, 80 and 100 cm (see Bobbink, Bik & Willems 1988).

Statistical analyses were conducted using SAS version 6.04. Where an ANOVA indicated significant heterogeneity within a set of treatments, additional Tukey's studentized range tests were carried out. Means which share the same letter are not significantly different ($p <$

0.005).

Results

Soil nutrient content

Differences in soil nutrient content between the control plots and the complete fertilization plots were observed after three months (July 1985) and after 5 yr (September 1989) (Fig. 2). Soil nitrogen content increased in both the control and the fertilized plots ($p < 0.05$), and the increase in the controls was greater than the difference between the controls and the complete fertilization plots.

The overall increase in nitrogen content is typical of the Dutch landscape where atmospheric inputs of nitrogen now average around 5 g·m⁻²·yr⁻¹ (Bobbink & Willems 1987; van Breemen & van Dijk 1988). The shift over the 5-yr period toward a higher nitrate-nitrogen percentage and a lower ammonium-nitrogen percentage likely reflects the high rates of nitrification that occur in calcareous soils and the increased pool of

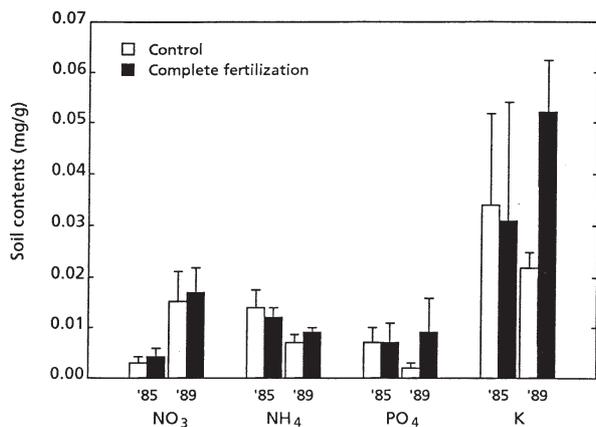


Fig. 2. Soil nutrient content of the control and complete fertilization plots at the start of the experiment and after 5 yr of treatment (error bars indicate S.E.).

available soil nitrogen (van Breemen et al. 1982; van Dam et al. 1990). Nonetheless, the nitrogen concentrations observed in the fertilized plots were higher than those from the control plots, although the difference was not statistically significant.

Phosphorus content decreased significantly ($p < 0.01$) in the control plots to extremely low values, whereas a slight increase occurred in the soil of the complete fertilization plots. The decrease in soil phosphorus in the control plots might be attributable to summer mowing and harvesting (before the autumn retranslocation), or to increased phosphorus demand by the plants owing to enhanced nitrogen supply.

Potassium was present in the soil at relatively high concentrations. The data suggest a substantial increase in potassium following 5 yr of fertilization, and a decrease in the control plots (perhaps owing to the same mechanisms responsible for the phosphorus decline), but the differences are not sufficiently large as to be statistically significant.

Above-ground biomass

The year after the initial nutrient application, the biomass on the complete fertilization plots (NPM) was approximately 1.85 times that on the controls (Fig. 3). Production on the NM plots was also high, followed by that on the PM plots and finally by the M plots which barely exceeded the controls. Yield dropped significantly ($p < 0.05$) in 1988 for all five treatments (Fig. 3), most likely in response to precipitation being nearly 35% below normal for the three-month period preceding the July 1988 harvest.

Over the five-year course of the experiment, biomass yield on the complete fertilization treatments

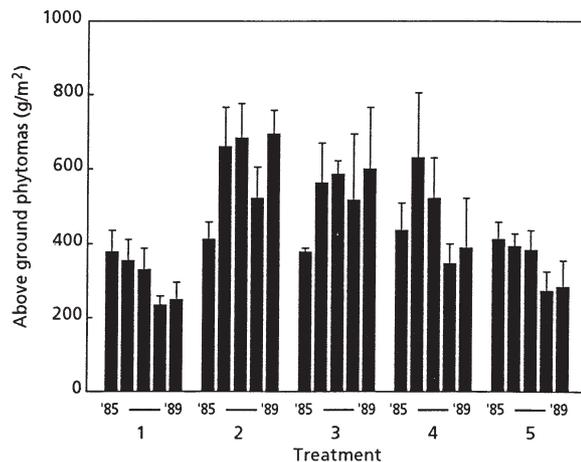


Fig. 3. Mean ($n = 6$) annual above-ground biomass from 1985 to 1989 on the five treatments: 1. control; 2. complete fertilization (NPM); 3. complete fertilization except nitrogen (PM); 4. complete fertilization except phosphorus (NM); 5. complete fertilization except nitrogen and phosphorus (M).

Table 2. Average annual number of species in plots of different size, 1985-1989. Means for 1989 that are not significantly different share the same letter.

Treatment	Plot size n m ²	1985	1986	1987	1988	1989	
1	150	0.001	2.86	3.44	2.77	2.57	3.30 A
			3.69	3.55	2.26	2.01	2.37 C
			3.57	3.27	2.61	2.39	2.74 B
			3.76	3.01	2.57	2.25	2.27 C
			2.84	3.05	2.33	2.49	2.75 B
2	150	0.01	10.91	10.87	8.61	7.71	9.67 A
			12.28	10.93	7.15	6.31	6.28 C
			11.70	10.61	8.91	8.01	8.97 AB
			12.87	10.27	6.69	5.97	5.85 D
			9.77	10.49	7.98	8.35	8.61 B
3	30	0.25	28.30	28.87	27.07	26.50	26.67 A
			28.30	28.33	23.07	20.17	19.47 C
			27.66	28.60	26.00	23.20	23.77 B
			28.96	28.60	23.03	20.13	19.67 C
			26.60	29.33	28.23	25.90	24.93 AB
4	6	1.25	38.00	40.00	36.67	34.50	35.00 A
			42.00	41.00	32.00	28.67	29.00 C
			40.00	39.00	34.50	33.83	33.17 AB
			39.00	41.00	33.00	30.67	30.67 BC
			42.00	44.00	39.83	36.50	35.67 A
5	3	2.5	46.33	missing	42.33	39.00	38.33 A
			46.00	missing	38.67	33.00	31.67 B
			44.00	missing	39.00	40.00	38.00 A
			44.00	missing	39.00	37.00	37.00 A
			47.00	missing	45.67	40.67	40.33 A

(NPM) remained roughly constant, as it did on the PM treatments. Meanwhile, production on the control, the NM and the M plots all declined significantly ($p < 0.05$). As a result, after five years the yield on the complete fertilization plots (NPM) exceeded that on the controls by nearly a factor of three ($p < 0.005$). The NM plots, after showing a large initial increase in yield, declined dramatically by the end of the experiment to nearly the level of the control and M plots (Fig. 3). This decline probably reflects an even greater decline in soil phosphorus than on the control plots resulting from export via hay removal of the initially higher amounts of phytomass.

Species number

By the end of the experiment, the average numbers of species per subplot for the different treatments had diverged at all subplot sizes from 0.001 to 2.5 m² (Table 2). At all scales there was a conspicuous decline in species number with complete fertilization (NPM). Further, an almost equal decline in species number occurred on the NM treatments at all scales except 2.5 m². The PM treatments also exhibited a decrease in species number, though not nearly as large a decrease as can be seen in the NM treatments (Table 2). Declines in species number following fertilization have typically been explained as resulting directly from the increase in above-ground production (e.g. Al Mufti et al. 1977; Grime 1979). However, our results are not consistent with such an interpretation. In particular, after 5 yr the PM treatments produced substantially more biomass than the NM treatments, just contrary to expectation, and the more productive PM plots retained significantly more species than the less productive NM plots (Table 2).

The low species numbers in the plots receiving NPM (complete) and NM treatments resulted from both higher species loss rates and lower arrival rates for new species. The decrease in species number on the plots with the PM treatment is largely compensated for by new species appearing in the plots. This number is approximately as high as in the control plots and the M plots (Fig. 4).

Biomass distribution

The failure of the magnitude of the decline in species number following fertilization to mirror the magnitude of the biomass increase was not anticipated when this study was initiated. One obvious difference in response between the PM and NM treatments was that *Brachypodium pinnatum* increased dramatically when only phosphorus was omitted, whereas the nitrogen-fixing legumes increased to assume dominance when only nitrogen was omitted. The possibility that these differ-

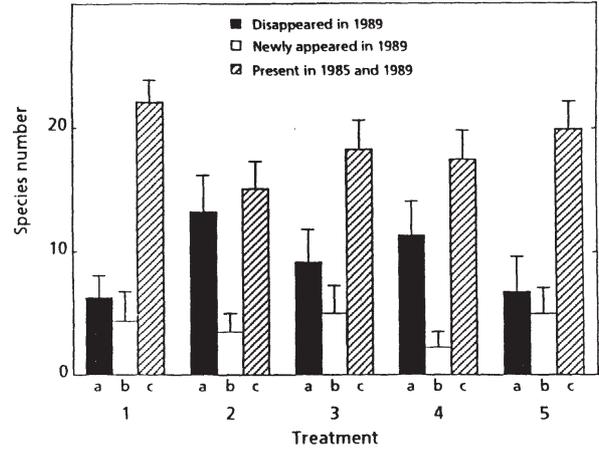


Fig. 4. Community dynamics in 0.25 m² subplots on the five treatments (see Fig. 1; $n = 30$ per treatment) as evidenced by (a) the number of species lost, (b) the number of species gained, and (c) the number of species present at both the start and the end.

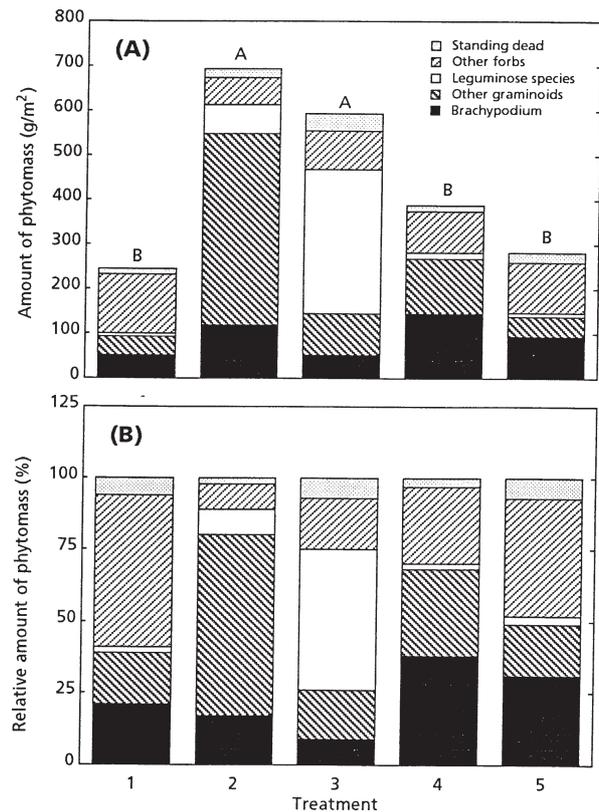


Fig. 5. Absolute (A) and relative (B) amounts of biomass in each of five fractions: *Brachypodium pinnatum*; other graminoids, including *Carex*; legumes; other forbs; and litter. See Fig. 1 for treatments.

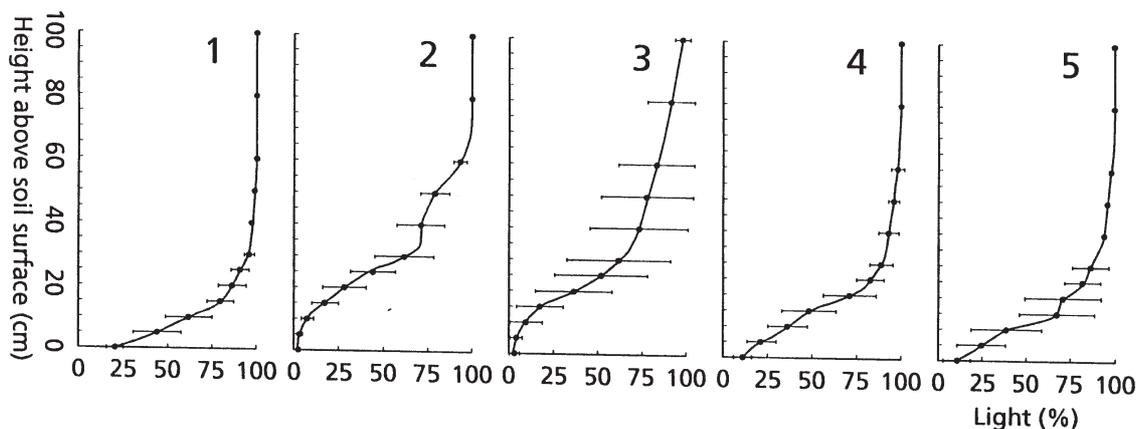


Fig. 6. Percent light penetration as a function of height above the ground surface for each of the five experimental treatments (see Fig. 1). The horizontal bars indicate the standard error of six observations.

ences in dominance might, in part, be responsible for the inconsistent relationship between biomass and above-ground production led us to document more carefully the change in relative biomass of the major plant groups. After five years, major between-treatment differences were evident in the relative importance of the plant groups (Fig. 5). Complete fertilization especially stimulated the growth of grasses (e.g. *Trisetum flavescens*, *Festuca rubra*, *Avena pubescens*, *Dactylis glomerata* and *Poa angustifolia*), compared to the control treatment ($p < 0.005$), except for *Brachypodium pinnatum*. In contrast, growth of *Brachypodium* was particularly stimulated by fertilization with all nutrients except phosphorus (NM) (different from complete fertilization; $p < 0.05$). The increase in biomass in the plots with the PM treatment is due in large part to the enormous expansion ($p < 0.005$) of legumes which were originally present throughout the area, but only in small quantities (e.g. *Melilotus officinalis*, *Genista tinctoria*, *Ononis repens*, *Medicago lupulina*, *Lotus corniculatus*). In these plots, the average amount of *Brachypodium pinnatum* is both absolutely and relatively the lowest among the five treatments (Fig. 5 a,b).

Vertical structure of the canopy and light profiles

Field observations suggested that the *Brachypodium*-dominated NM plots have a different canopy structure from the legume-dominated PM plots. This led us to examine the vertical distribution of foliage and the canopy light attenuation. We anticipated that the change in diversity might parallel the amount of canopy light attenuation and that this, rather than phytomass, might be the biologically relevant variable for understanding the decline in species richness usually observed following grassland fertilization. The canopy light (PAR) ex-

tingtion was greatest on the complete fertilization (NPM) plots, light at the soil surface being reduced to barely 2% of that above the sward (Fig. 6).

However, contrary to expectation, the legume-dominated, PM treatments decreased canopy light much more than the *Brachypodium*-dominated, NM treatments. For example, at a height of 20 cm above the soil surface the light intensity differed significantly ($p < 0.05$) between the vegetation receiving NM and PM treatment. In short, greater light extinction could not be used to explain the disproportionate decline in species richness on the plots with NM treatments.

Plant nutrient content and export through harvest

Nutrient content in the total above-ground biomass in 1989 was significantly higher in the treatments with either N or P (Table 3). Especially the high nitrogen content in the PM and the high potassium content in the NPM treatment are remarkable. To test whether the dominance of *Brachypodium pinnatum* on the NM plots

Table 3. Total amount of nitrogen, phosphorus and potassium (g/m^2) in the above-ground biomass of the five treatments at Wrakelberg, after 5 yr of fertilization (standard error between brackets; $n = 6$; Tukey $\alpha = 0.05$). Means that are not significantly different share the same letter.

Treatment	Nitrogen	Element Phosphorus	Potassium
1 (control)	2.813 (0.397) B	0.219 (0.089) B	3.814 (0.762) C
2 (NPM)	7.432 (1.118) A	1.183 (0.156) A	9.532 (0.930) A
3 (PM)	9.492 (4.373) A	1.237 (0.551) A	7.678 (2.097) AB
4 (NM)	5.692 (1.782) A	0.274 (0.070) B	5.713 (1.713) BC
5 (M)	3.273 (0.768) B	0.215 (0.047) B	4.444 (1.236) C

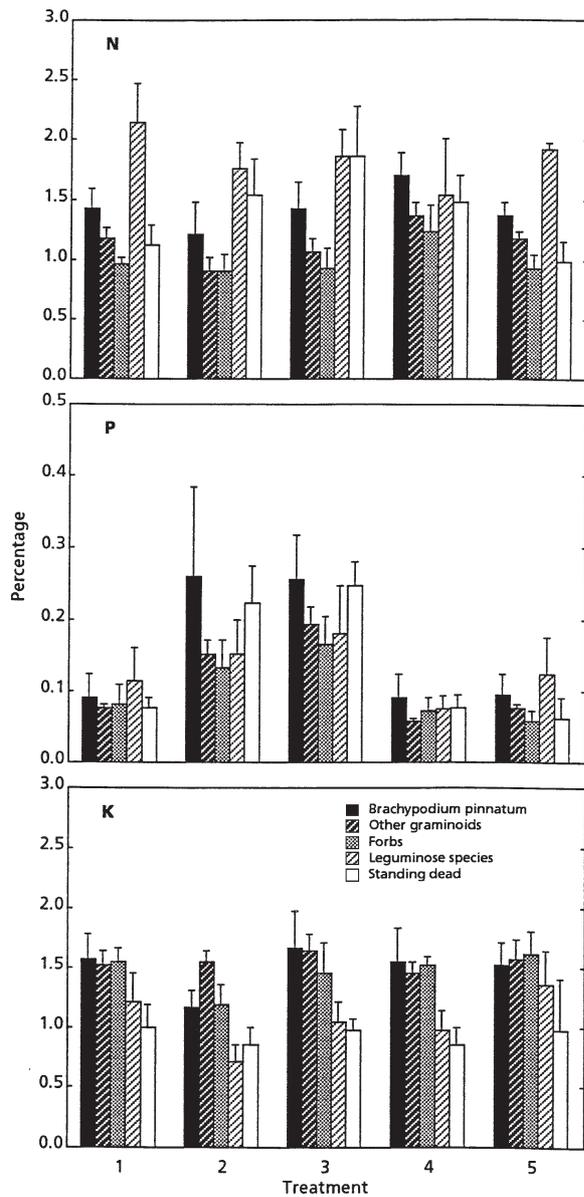


Fig. 7. Nitrogen (N), phosphorus (P) and potassium (K) content of biomass in percent dry weight. The biomass is divided into five fractions (see Fig. 5). See Fig. 1 for experimental treatments.

could be attributed to more efficient use of phosphorus, nutrient content was compared between the five recognized phytomass groups. Percentage nitrogen was especially high in legume species, whereas this percentage was lowest in the forbs (Fig. 7). Phosphorus content was highest in all fractions for those plots fertilized with phosphorus especially in the *Brachypodium* fraction. The percentage of phosphorus was also high in the standing dead fraction (mainly consisting of dead

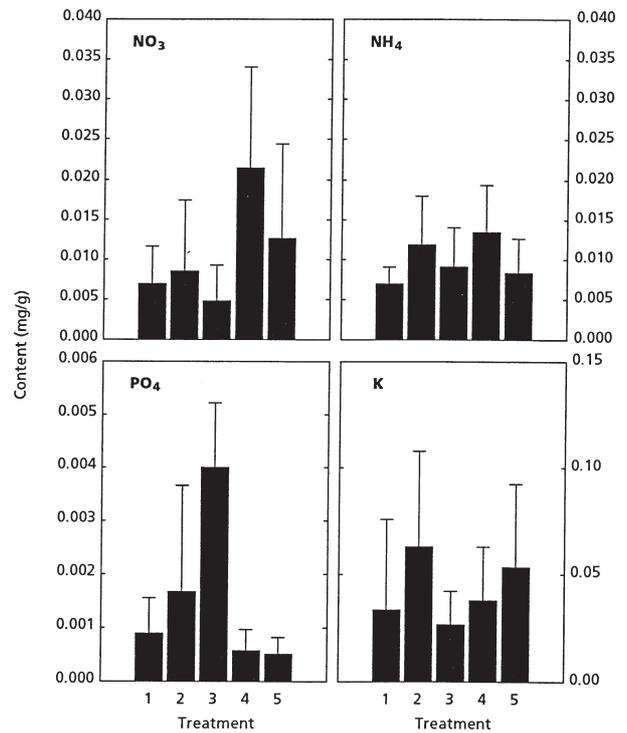


Fig. 8. Mean ($n = 6$) soil nutrient content of plots in the five treatments (see Fig. 1) at the end of the five-year experiment (1989).

Brachypodium leaves) for those plots with complete fertilization or PM treatment. Thus, retranslocation in *Brachypodium* is less efficient where phosphorus is most abundant and likely to be available to other species. In contrast, potassium is either retranslocated or is leached from the dead plant material in all plots. The 1989 phosphorus levels were lower in soils from the complete fertilization plots (NPM) than in those from the PM plots (Fig. 8). This probably resulted from greater plant uptake of phosphorus where growth was not restricted by lack of nitrogen (coupled with subsequent loss through harvest).

Discussion

Relationship between production and species richness

Numerous studies in grassland systems, including chalk grassland, have shown fertilization to cause an increase in above-ground production and a decrease in species richness (Willems 1980; Peet, Glenn-Lewin & Walker Wolf 1983). Grime (1979) and Huston (1979) have argued that an increase in above-ground production increases mortality rates and increases the intensity

of competition, thus leading directly to a decline in richness. However, our results are not consistent with such an interpretation. The increases in production we observed following fertilization clearly demonstrated that both phosphorus and nitrogen are limiting for plant growth in the chalk grassland at Wrakelberg. When both elements were absent from the fertilizer, there was no significant change in above-ground phytomass compared with the control plots, but either one alone could increase production, however, by different fractions in the vegetation (Fig. 5). Further, the observed level of above-ground production on the control plots was consistent with previously reported long-term observations of chalk grasslands in South Limburg (Willems 1980, 1990; During & Willems 1984).

Two surprising results were observed with respect to species richness and above-ground production. First, the plots receiving PM treatment yielded around 600 g/m² above-ground biomass, nearly as much as the complete fertilization plots. This was more than twice the production in the control plots and those receiving the M treatment. This high level of production has been shown in previous fertilization experiments to have a dramatic negative impact on species richness (Willems 1980; During & Willems 1984; Bobbink, Bik & Willems 1988). However, in the present experiment the PM treatments showed no significant decline relative to control treatments in species number per 0.01-m² plot, and very little decline was evident in the 0.25-m² plots.

The second surprising result complements the first. In plots receiving the NM treatment, above-ground biomass was about half that of NPM plots and also significantly less (Fig. 5) than that of PM plots. Yet, species richness, unlike the case in the PM plots, declined sharply. These results suggest that the mechanism(s) responsible for the decline in richness with fertilization consists of more than just increased production.

Light penetration and canopy structure

The most conspicuous change observed on plots receiving either complete fertilization (NPM) or the NM treatment was a dramatic increase in the tall grass, *Brachypodium pinnatum*. Bobbink & Willems (1987) suggested that the growth form of this species and the consequent tight, light-obstructing canopy might cause a decline in species number. However, in this study we found average light extinction to be greater in the legume-dominated, high richness, PM treatments than in the *Brachypodium*-dominated, low richness, NM treatments. A possible explanation for the observed species richness patterns might be found in the light profile data. The standard errors associated with the light measurements were far higher in the legume-dominated plots

than in any of the other plots. The legume dominance on the PM plots is very patchy and the soil surface has a mixture of areas with high and low light levels. The diversity of microhabitats could account for the maintenance of high species richness on the PM plots. This is partly due to the relatively high number of species which could newly establish in these plots. The observed number of invading plants equals the number in the control plots (Fig. 4). If this interpretation is correct, the PM plots should be patchier than the NM plots. However, there was no significant difference between the treatments in the variance in species number per 0.001 or 0.01 m² plot.

In spite of the high above-ground phytomass and high rate of light attenuation in the plots receiving PM treatment, the upright growth and densely clumped growth-forms of a number of species, coupled with the sprawling growth of many of the legume species, created a mosaic of micro-habitats with highly variable light availability. The light measurements confirmed higher spatial variation in light penetration in the PM treatments (Fig. 6). These results support the assumption of Tilman (1982, 1985) that small-scale resource heterogeneity might enhance species co-existence.

Nutrient use and availability

Bobbink (1991; Bobbink & Willems 1987) suggested that competition for phosphorus might provide an additional mechanism, beyond canopy heterogeneity, to explain between-treatment differences in species richness. Soil nitrogen content is relatively high in all treatments owing to atmospheric inputs, and is especially high in those treatments receiving nitrogen fertilization. The high nitrogen content leads to extremely low amounts of phosphorus in these plots owing to enhanced growth and phosphorus uptake by the plants. The resulting extremely low phosphorus levels may preclude growth of many species.

Bobbink, Bik & Willems (1988) have earlier shown *Brachypodium pinnatum* to be extremely plastic in tissue phosphorus content. This grass species takes up all phosphorus that is available and efficiently retranslocates the larger part of it into the rhizome system at the end of the season: besides, it is able to survive on very low levels of phosphorus. One mechanism by which species richness may be kept low in *Brachypodium*-dominated plots is the efficient uptake and storage of phosphorus by *Brachypodium* (and export through mowing and raking at peak biomass, prior to leaf senescence). When phosphorus is added to *Brachypodium*, a large amount is taken up and stored in the underground rhizomes (Bobbink 1991). Further, *Brachypodium* is able to increase its tissue N/P ratio dramatically when presented with an increase in nitrogen supply (Bobbink 1991).

The annual addition of phosphorus to the fertilized plots was 4.4 g/m², whereas the amount of phosphorus removed by the summer harvest of above-ground biomass was around 1.2 g/m². The high concentration of phosphorus in the standing dead plant material compared to the other harvest fractions is striking (Fig. 7). However, this reflects conditions only in the leaves that die prematurely during the summer; retranslocation in senescent autumn leaves is very efficient (Bobbink 1991).

Unlike most other chalk grassland species, *Brachypodium pinnatum* is known to increase its root growth when given supplemental nitrogen (Bobbink 1991). Most temperate zone species have evolved under conditions of nitrogen limitation. The current high availability of nitrogen due to enhanced atmospheric inputs, coupled with intrinsically low levels of soil phosphorus, creates conditions not previously encountered during the evolutionary history of most chalk grassland species. This might explain why Bobbink (1991) found very little root growth in most chalk grassland species when they were fertilized with nitrogen, but much root growth when they were fertilized with phosphorus. In plants that evolved in an environment characterized by nitrogen limitation, selection would favor growth of roots until the nitrogen demand is satisfied. In short, unlike most chalk grassland species, *Brachypodium pinnatum* appears to grow very well under low phosphorus, and moderate to high nitrogen levels. This probably leads to the competitive displacement of the other species in the chalk grassland ecosystem.

Implications for conservation and management

As a semi-natural ecosystem, chalk grassland has to be managed to prevent succession to woodland. This management can take the form of grazing, mowing or burning, but it always involves a reduction in above-ground phytomass. Without management removing above-ground phytomass, species diversity declines dramatically (ca. 70 %) within a period of ca. 10 - 15 yr (Willems 1990).

In this study, the control plots and those without addition of either nitrogen and phosphorus showed a very slight decrease in richness in the 0.01 m² and 0.25 m² subplots over a five-year period. This decrease is part of an overall decline in species number in Dutch chalk grasslands which started in the early eighties as a result of soil eutrophication by atmospheric nitrogen input (Bobbink & Willems 1987; Bobbink 1991).

To counter the increase in atmospheric nitrogen input (ca. 5 g·m⁻¹·yr⁻¹), some form of nitrogen removal is needed or *Brachypodium* can be expected to dominate eventually. If management reduction in above-ground biomass involves mowing and removal of biomass (par-

ticularly in the summer before retranslocation), nitrogen levels might be partially controlled. This, in part, is the rationale for the current practice of mowing and harvesting the grass on the Wrakelberg preserve. However, the results of this study suggest that unless the removal is coupled with phosphorus replacement, a further decline in species richness can be anticipated.

Worldwide, preservation of biodiversity is one of the most challenging environmental problems confronting mankind (cf. Koshland 1991; Machlis 1992). To meet this challenge, it is necessary to prevent species-rich ecosystems from degradation, not only through habitat protection, but also by adjusting the management in the light of global environmental change. Processes responsible for the decline in species diversity often take place at small scales as shown in this paper. Insights into these small-scale mechanisms are needed to guide development of management practices that will prevent future biotic degradation.

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