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Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland

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Summary

1 Nitrogen mineralization was studied in four grasslands (fields A–D), which had not been fertilized for 2, 6, 19 and 45 years, respectively, thereby forming a chrono-sequence. Fertilizer application was stopped in these fields in order to restore former species-rich communities characteristic of nutrient-poor conditions.

2 The annual nitrogen mineralization rate was unexpectedly low in field A (124 kg ha^{-1} year^{-1}) because of the absence of net mineralization during the winter period. This may have been due to high nitrification and subsequent denitrification rates. Winter mineralization was observed in fields B–D where annual mineralization rates were 176, 140 and 61 kg ha^{-1} year^{-1}. The soil total N and C pool sizes (0–10 cm) decreased over the chronosequence (from 5110 to 2460 kg ha^{-1} and from 71 800 to 29 400 kg ha^{-1} for N and C, respectively). The relative nitrification rate (nitrate as a fraction of total mineral N) strongly decreased during the chronosequence (88, 75, 54 and 51%, respectively).

3 The seasonal dynamics of the compartmentation of biomass and of tissue nitrogen, phosphorus and potassium concentrations were studied in order to obtain insight into some of the causes and consequences of these changes in nitrogen mineralization. Field B had a high shoot and root production, with high turnover of both fractions. Field C had a low shoot production, but a high root production with high turnover. Field D had a high shoot and rhizome biomass, but within-season turnover was probably low. The peak standing crop in field A was unexpectedly low, but was consistent with the low annual mineralization rate measured in this field.

4 Fine root turnover is assumed to be the main source of the organic material which plants add to the soil in these grasslands. The N concentration of the fine roots generally decreased over the chronosequence, suggesting a decreasing quality of the dead organic matter which the plants added to the soil. There were also clear decreases in shoot phosphorus and potassium concentration during the chronosequence.

5 The amount of regrowth immediately after cutting showed a good correspondence with the nitrogen mineralization rate over the same period, with highest regrowth and mineralization rates observed in the most recently fertilized fields. However, peak standing crop prior to cutting did not correspond to the annual nitrogen mineralization rate. It is suggested that the dominant plant species in field D had more below-ground storage and remobilization and had longer-lived tissues (especially below-ground). This enables the species dominating this field to form a high biomass, despite a very low N mineralization rate.

Keywords: allocation, nitrogen mineralization, nutrient cycling, grassland productivity, succession

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Introduction

Until the 1950s, marginal agricultural use of grass-lands in the Netherlands and other parts of Western Europe resulted in species-rich, but relative unproductive communities with many characteristic and/or rare species. The intensification of agricultural practices (much higher fertilizer inputs, lower ground-water tables, higher cutting frequencies and grazing densities) during the last 40 years has led to the disappearance of these communities in most areas. Currently, nature management practice often aims to restore these communities by stopping fertilizer application and re-introducing the annual hay-making (Bakker 1989). Previous studies have found that this indeed may lead to decreasing productivity and re-appearance of species characteristic for nutrient poor conditions (Olff & Bakker 1991). The present study was set up to investigate changes in nutrient availability and nutrient use by the vegetation, after the application of fertilizer was stopped. We would like to understand more of the causes of the observed species changes, and wanted to evaluate the effects of restoration management in terms of nutrient budgets. The study focused on nitrogen, but also investigated several aspects of potassium and phosphorus dynamics.

The availability of nitrogen to plants is determined both by external inputs (atmospheric deposition, fertilizer application) and by internal recycling within the system (mineralization). Nitrogen mineralization, the microbial conversion of organic matter to ammonium is strongly dependent on temperature, moisture and substrate (Swift *et al.* 1979). The dependence on substrate makes the mineralization rate not only a determinant of the species composition of the vegetation, but also dependent on this factor, since large differences between plant species exist in the amount and chemical composition of the flow of plant organic matter to the soil (Vitousek & Walker 1987; Berendse *et al.* 1989; Wedin & Tilman 1990). Because high N addition rates can decrease tissue C/N ratios leading to lower decomposition rates (Pastor *et al.* 1987; Berendse & Elberse 1989) the N mineralization rate probably increased during the period of intensive agricultural use. Stopping the application of fertilizers would then lead to an only partially reduced N availability, since the high mineralization rates would be expected to continue until the 'high quality' organic matter was used up. We therefore hypothesized that the annual N mineralization rate would eventually decrease, but not immediately. Lower total mineralization rates would lead to less NH_4^+ accumulation in the soil, and therefore to less proportional nitrification. This may have consequences for denitrification losses (Berendse *et al.* 1994).

Cessation of fertilizer application also strongly reduced the P and K supply to the vegetation. Atmospheric inputs of these nutrients are much lower than for N, and therefore a sudden cessation of external supply of P and K might also contribute to the decreased productivity and re-establishment of nutrient-poor species. We investigated changes in tissue nutrient concentrations of N, P and K as an indication of changes in their availability. Since tissue nutrient concentrations are highly variable within a year (e.g. Dickinson 1984) we also studied their seasonal dynamics. The compartmentation of biomass and nutrients over plant parts of several groups of species (grasses, dicots, rushes) was studied in order to obtain insight in some of the causes of the changes in the relative abundance of these species groups. In particular, the seasonal pattern of aboveground litter biomass and of root biomass may yield some insight into the longevity of plant parts of the dominant species of the various successional stages. It was expected that dominant species groups under lower nutrient conditions would be characterized by a more efficient internal recirculation (storage and retranslocation of nutrients), and less fine root turnover (Shaver and Mellilo 1984; Berendse & Jonasson 1992)

STUDY AREA

Four fields, which had not been fertilized for 2 (field A), 6 (field B), 19 (field C) and 45 years (field D) were taken to represent different stages in a successional series which starts after the application of fertilizers is stopped. The hay is cut once a year in July. Vegetation succession in field C has been followed for 18 years (field 1376 in Bakker 1989); the vegetation after 2 and 6 years closely resembled the vegetation now found in fields A and B, respectively. Field D has also been studied for 18 years (field 1371 in Bakker 1989) and showed at the start of the measurements a reasonable resemblance to the vegetation now found in field C. Before fertilizer application was stopped, fields A, B and C were subject to normal agricultural use as permanent grassland, which implied various combinations of cutting followed by light grazing by heifers, with various levels of fertilizer application (100–250 kg N ha^{-1} year^{-1}, with sufficient P and K). Although management and fertilizer application varied, the long-term average use of the three fields has been similar (personal communication from the management authorities). The vegetation composition of field D before 1945 (its period of agricultural use) is not known; it is however, likely that agricultural exploitation of this field was less intense than the agricultural use of the other three fields, prior to the start of the nature management. With some caution for field D, we can therefore state that the spatial comparison of these four fields in one year (i.e. a chronosequence) can be used to analyse the changes occurring during 45 years of grassland succession.

The fields are located along a small stream on a glacial sand plain (Anloëbrdiepje, 53°05′N, 6°40′E). Each field contains a topographic gradient ranging

from the sandy plateau to the brooklet, all measurements were done in the lower, wetter part of the gradient. This part of field A was dominated by *Agrostis stolonifera* and *Poa trivialis*, field B by *Holcus lanatus*, field C by *Holcus lanatus, Anthoxanthum odoratum* and *Deschampsia cespitosa* and field D by *Juncus acutiflorus* (Olff 1992b). Groundwater levels were recorded biweekly in 1-m tubes (2 cm in diameter) from May 1990 to June 1990, and fluctuated between –30 cm and –80 cm. Differences between fields were small (<20 cm) and not consistent (Bakker, unpublished.)

Methods

SOIL CHARACTERISTICS

In March 1990 soil samples (10 cm × 8 cm diameter) were taken at 10 randomly selected locations in each field using a soil corer. The pH of the fresh soil was determined by adding 50 mL of either water or 1 M KCl to a volume of soil, equivalent to 20 g dry mass, and shaking for 2 h. The remaining part of the soil samples was dried at 70 °C for 48 h and weighed. The soil samples were ground and N concentrations were determined by digesting 200 mg of ground material with 30 M sulphuric acid, containing 0.006% phenol, and a mixture of sodium sulphate and copper sulphate. The dilute digest was analysed colorimetrically using indophenol blue. Phosphorus concentrations were determined by digesting 200 mg of ground material in a 1:1 mixture of sulphuric acid and nitric acid. The dilute digest was analysed colorimetrically using ammonium molybdate, antimony and ascorbic acid. Total soil carbon concentrations were determined by burning a ground sample in a stream of oxygen at 950 °C, after which the resulting carbon dioxide was purified and captured in NaOH. CO_2 production was measured as the change in conductivity. The organic matter content was determined by reweighing 1 g of the material ignited at 850 °C for 1.5 h.

BIOMASS COMPARTMENTATION AND TISSUE NUTRIENT CONCENTRATIONS

In spring 1990, 35 plots (0.1 m × 0.4 m, separated by 1 m) were laid out in a homogeneous part of the wetter part of each field, located such that groundwater levels were similar between fields (but of course variable in time). The above-ground vegetation was clipped in 5 replicate plots per field to 2 cm height, on 13 April, 8 May, 9 June, 10 July, 11 September and 18 December 1990 and 20 March 1991. Samples were separated into grasses, forbs, rushes (*Juncus* species) and standing dead material. A soil core (20 cm × 7 cm in diameter) was taken with every vegetation sample ($n = 5$) and the roots were extracted from this under a fine water spray on a 0.5-mm-mesh screen. Rhizomes of *Juncus acutiflorus* were separated from roots, which could

not be separated to species. Prior to the nutrient concentration analysis, all samples were pooled per date, field and plant fraction (rhizomes, roots, and shoots of grasses, forbs, rushes, standing dead). N and P concentrations in the plant material were determined as in the soil samples. K concentrations were determined by atomic absorption spectrometry.

NITROGEN MINERALIZATION

The mineralization of nitrogen was measured in each field from April 1990 to April 1991. On each sampling date ten pairs of soil samples were taken in ten blocks per field. The samples were taken with a 4 cm diameter polyvinyl chloride tube so that the sample was a relatively undisturbed column of the first 10 cm of the soil.

One of each pair of samples was transported to the laboratory in a cooled box and mineral N was extracted within 24 h after being collected. The other tube was put back in the soil after being closed with plastic lids in order to measure the accumulation of mineral N during the subsequent incubation period. The lids prevented water moving through the tube, but air was allowed to enter through four holes that remained above the soil surface during incubation. After 8 weeks (12 weeks for the winter period) the sampling procedure was repeated within the same 10 blocks.

Soil moisture contents were determined gravimetrically at each collection. The soil bulk density for each field was calculated from the average soil dry mass in all mineralization tubes. The NH_4^+ and the NO_3^- contents of the soil were determined by extraction of 20 g of fresh soil with 50 mL of 1 mol L^{-1} KCl within 24 h of being collected. The concentrations of NH_4^+ and NO_3^- in the extracts were measured colorimetrically using a continuous flow analyser. The rate of soil nitrogen turnover (or relative mineralization) was calculated by dividing the annual mineralization rate by the soil total N pool. The annual off take of N by cutting was expressed as a percentage of the sum of the annual mineralization rate and estimated atmospheric deposition. Nitrification was expressed as the fraction of the total mineral N pool (ammonium + nitrate) which accumulated as nitrate in the tubes. The annual net mineralization (mineral N accumulation in the *in situ* incubated soil cores) and nitrification rates were calculated separately for each block, and therefore differences between fields could be tested with a one-way analysis of variance, with Student–Newman–Keuls contrasts among means.

Results

SOIL CHARACTERISTICS

pH decreased during succession in both extractions, while total P concentration showed a four-fold

Table 1 Overview of various soil characteristics in four successional grasslands, which had not been fertilized for 2, 6, 19 and 45 years. Means ($n = 5$) with the same superscript letter are not significantly different (Student–Newman–Keuls test after one-way ANOVA)

	Field A	Field B	Field C	Field D
Years not fertilized	2	6	19	45
Soil moisture (g 0. 100 g^{-1} DW)	78.9^a	61.3^b	74.2^a	70.9^a
Organic matter (g 0. 100 g^{-1} DW)	15.4^a	10.3^b	12.6^c	7.3^d
Bulk density* (g cm–3)	1.08	0.88	0.81	0.94
pH (H$_2$O)	5.9^a	5.9^a	5.7^b	5.4^c
pH (KCl)	4.9^a	4.8^b	4.6^c	4.2^d
Total C concentration (g 100 g^{-1} DW)	6.65^a	5.09^a	6.12^a	3.13^b
Total N concentration (g 100 g^{-1} DW)	0.471^a	0.392^b	0.465^a	0.266^c
Total P concentration (g 100 g^{-1} DW)	0.124^a	0.093^b	0.055^c	0.030^d
C/N ratio	14.1^a	13.0^a	13.2^a	11.8^a
C/P ratio	53.6^a	55.9^{ab}	111.0^c	104.8^b
C pool size (g m^{-2}, 0–10 cm)	7180^a	4950^b	4470^b	2940^c
N pool size (g m–2, 0–10 cm)	511^a	362^b	365^b	246^c
P pool size (g m–2, 0–10 cm)	134^a	84^b	44^c	28^d
Clay (<2 μm)* (% of DW)	6.4	10.2	5.4	3.1

*Bulk samples were used, therefore differences between fields could not be tested.

decrease (Table 1). Total C and N concentrations were lower only in field D. The C/N ratio did not change significantly, while the C/P ratio was lowest in field A and highest in fields C and D. The bulk density of the soil was highest in field A. The pool sizes of organic matter, C, N and P in the upper 10 cm of the soil (product of concentration and bulk density) decreased from field A to D. The soil moisture content was significantly lower in field B (Table 1).

NITROGEN MINERALIZATION

Field D had a significantly lower total N mineralization rate (61 kg ha^{-1} year^{-1}) than the other three fields. Mineralization during the growing season (mid April to mid July, periods 2 and 3) was highest in fields A and B (Fig. 1). Almost no seasonal variation in mineralization rate was found in field D indicating that in this field an important fraction of the already low total mineralization occurred in winter. Relative nitrification rates (proportion of total mineralization) were significantly higher in fields A and B than in fields C and D (Fig. 1, Table 2). The absolute nitrification rate was highest in field B, and then decreased towards fields C and D. Nitrification rates were highest in summer; during the winter periods only ammonification was found (Fig. 1).

BIOMASS AND NUTRIENT
COMPARTMENTATION

During the first part of the growing season (March until July, i.e. prior to cutting) the aboveground biomass of monocots in fields A and B strongly increased. This increase was less in field C, while in field D it was again higher. This last field was characterized by a high biomass of rushes which were almost absent from the other three fields (Fig. 2). Root biomass increased during spring in fields A and B, but then decreased, even before cutting, while in field C it
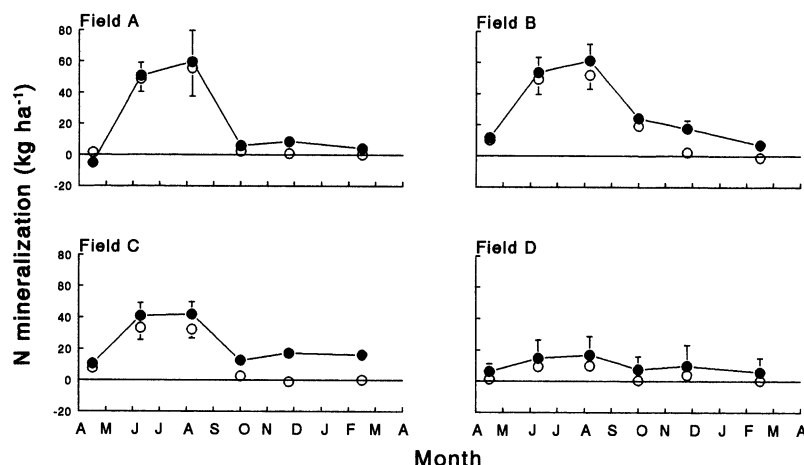

Fig. 1 Seasonal dynamics of nitrogen mineralization (kg ha^{-1} period^{-1}) in four hay-fields which were not fertilized for 2 years (field A), 6 years (field B), 19 years (field C) and 45 years (field D). Mean values ($n = 5$, ± SE) are given for each period, for nitrate (open symbols) and nitrate + ammonium (closed circles).

Table 2 Pool sizes, fluxes and some proportional features of the nitrogen cycle in four successional grasslands. Means ($n = 5$) with the same superscript letter are not significantly different (Student–Newman–Keuls test after one-way ANOVA)

	Field A	Field B	Field C	Field D
Primary measurements				
Soil organic N (kg ha^{-1}, 0–10 cm)	5110[a]	3620[b]	3650[b]	2460[c]
Shoot N at cutting (kg ha^{-1})*	92	91	72	106
Root N at cutting (kg ha^{-1})*	85	134	228	107
Total N Mineralization (kg ha^{-1} year^{-1})	124[a]	176[a]	140[a]	61[b]
Nitrification (kg ha^{-1} year^{-1})	109[ab]	132[a]	76[b]	25[c]
Relative nitrification (% of total mineralization)	88[a]	75[a]	54[b]	41[b]
Calculated features				
Soil N turnover (g g^{-1} year^{-1})*	0.024	0.050	0.040	0.026
N offtake by cutting (% of total N in system)‡	1.7	2.4	1.8	4.0
N offtake by cutting (% of N in the vegetation)*	50	39	36	88
N offtake by cutting (% of available N)*†	54	41	39	100

*These variables were calculated on the basis of several other variables; therefore their true variance is not known and differences between fields could not be tested

†Total N in plant and soil (0–10 cm)

‡An estimated atmospheric N deposition rate of 60 kg ha^{-1} year^{-1} was added to the annual mineralization rate to calculate available N

increased until cutting in July. In field D there was little seasonal variation in root biomass, but the biomass of rhizomes decreased towards the summer. The increase in aboveground biomass of *Juncus* in field D was accompanied by a similar decrease in rhizome biomass, suggesting a reallocation of biomass during this period. After cutting there was considerable shoot regrowth field A, less in field B, and hardly any in fields C and D. Regrowth in fields A and B was followed by accumulation of standing dead matter during the autumn and winter (Fig. 2).

Prior to cutting (10 July 1990), the standing dead biomass and grass shoot biomass of the four fields

were similar (Table 3), while field C had higher root biomass, and field D had higher rush shoot and rhizome biomass than the other fields. Because of the root and rhizome biomass in fields C and D, their total biomass was higher than field A (Table 3). The lowest mineralization rate was found in the field with the highest shoot biomass (Tables 2 and 3).

Peak biomass prior to harvesting is a poor measure of productivity, since it integrates autumn and spring production and winter mortality. The biomass regrowth after cutting (from 10 July to 9 September 1990) is a better measure of productivity, since little mortality occurs during this period. On 9 September
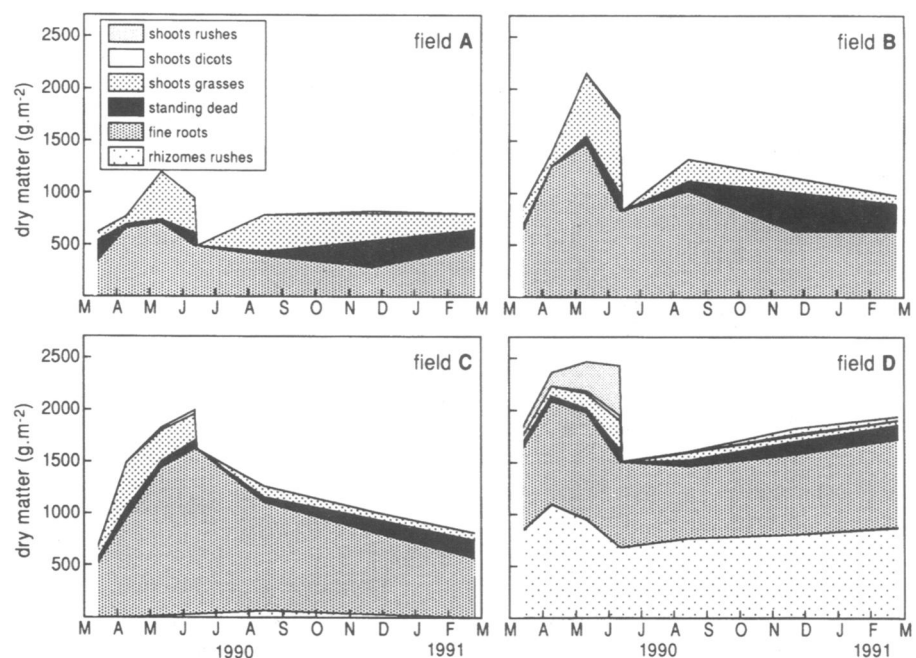

Fig. 2 Seasonal dynamics of the biomass of shoots of rushes, dicots and grasses, and of standing dead material, fine roots and rhizomes. Results of four fields are presented, which were not fertilized for 2 years (field A), 6 years (field B), 19 years (field C) and 45 years (field D). Mean values ($n = 5$) are given for each sampling date.

Table 3 Biomass compartmentation at two dates (prior to cutting and after regrowth) in the four fields from table 1. Means within each date with the same letter are not significantly different (Student–Newman–Keuls contrasts after one-way ANOVA)

Fraction	10 July 1990				9 September 1990			
	Field A	Field B	Field C	Field D	Field A	Field B	Field C	Field D
Above-ground biomass (g m^{-2})								
Standing dead	115[a]	159[a]	74[a]	135[a]	37[a]	86[a]	48[a]	54a
Grasses	351[a]	373[a]	239[a]	268[a]	326[a]	181[b]	58[c]	38d
Dicots	3[a]	65[c]	27a[b]	51b[c]	3[a]	2[a]	22b[c]	12ab
Rushes	0[a]	5[a]	21[a]	491[b]	0	0	5	18
Total above-ground living biomass	354[a]	448[a]	287[a]	810[b]	345[a]	222[b]	128[c]	85c
Below-ground biomass (g m^{-2})								
Fine roots	488[a]	837[a]	1627[b]	823[a]	399[a]	1033[b]	1103[b]	687a
Rhizomes*	0	0	0	681	0	0	0	784
Total below-ground biomass	488[a]	837a[b]	1627b[c]	1504[c]	399[a]	1033[b]	1103ab[b]	1471b
Total biomass – standing dead	842[a]	1285a[b]	1914b[c]	2314b[c]	744[a]	1255[a]	1231[a]	1556a

*Since some cells had zero variance for this variable, contrasts were not calculated.

1990, the total aboveground living biomass (i.e. that produced during the previous 6 weeks) decreased from field A to D, and a similar pattern of N mineralization was observed (Fig. 3). In contrast, the fields were ranked in increasing total below ground biomass from field A to D, due to a higher root biomass in field B and C and a higher rhizome biomass in field D. In consequence, the total biomass (excluding standing dead) of the fields was similar.

TISSUE NUTRIENT CONCENTRATIONS

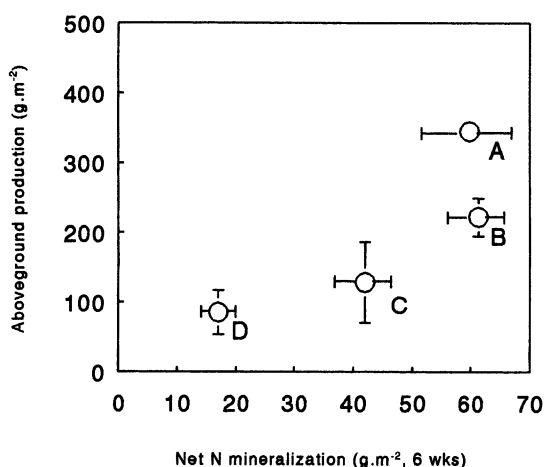The nitrogen concentration in the shoots of grasses, rushes and forbs decreased during the growing season

Fig. 3 Relationship between biomass regrowth after cutting (cutting at 10 July 1990; standing crop measured at 9 September 1990) and N mineralization rate during approximately that period in four fields which had not been fertilized for 2 years (field A), 6 years (field B), 19 years (field C) and 45 years (field D). Error bars indicate standard deviations of means ($n = 5$ for biomass, $n = 10$ for mineralization).

(Fig. 4). This decrease occurred in each field, and few consistent differences between fields in shoot N concentration were observed. The N concentration of the fine roots remained relatively constant during the year, and N concentrations were always highest in field A and lowest in field D (Fig. 4E). The N concentration in the rhizomes in field D also remained constant during the season (Fig. 4F). The standing dead N concentration was not consistently different between fields, nor did it show a seasonal pattern (Fig. 4C).

More differences between fields were observed in K concentration (Fig. 5). For the shoots of grasses, forbs and for standing dead the K concentrations were generally higher in field A and B than in fields C and D (Fig. 5A, 5B). The K concentrations were however, highly variable in time in all plant parts, except for the fine roots.

The most consistent differences between fields were found in P concentration (Fig. 6), where field D had always a much lower values than fields A, B and C. Furthermore, fine roots in fields A and B had higher P concentrations than in field C. For the other plant parts we observed less differences between fields A, B and C. The P concentration in the various plant parts showed relatively little seasonal variation (compared to N and K).

Discussion

The technique which we employed for measuring N mineralization is considered to be a good reflection of this component of N supply to the vegetation. Raison *et al.* (1987) concluded after a comparison of methods that this method provides reliable quantitative estimates of fluxes of mineral-N in field soils.

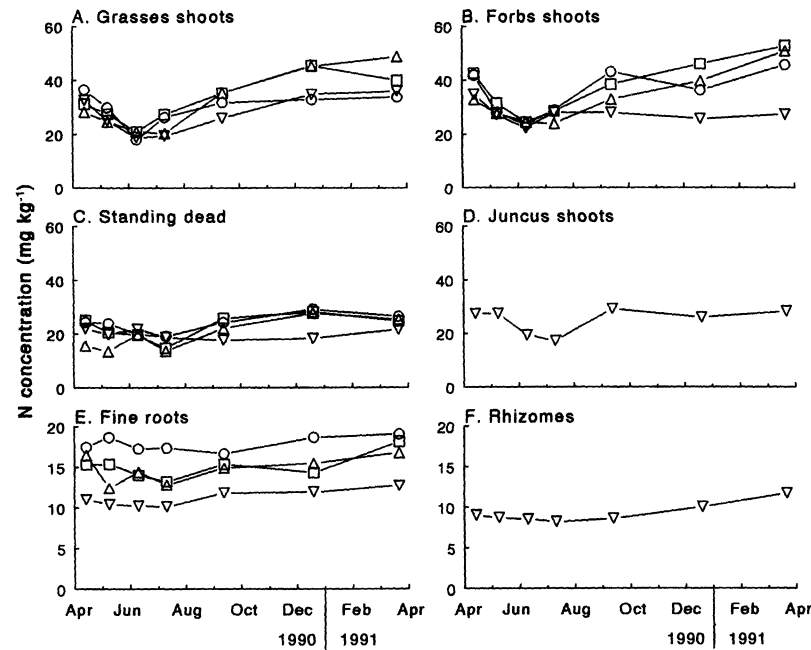Annual nitrogen mineralization rates were not sig-

Fig. 4 Seasonal changes in tissue nitrogen concentration of various plant parts (several species) in four fields which were not fertilized for 2 years (field A, ○), 6 years (field B, △), 19 years (field C, □) and 45 years (field D, ▽). Values represent the concentrations in pooled samples from five plots per field.

Fig. 5 Seasonal changes in tissue potassium concentration of various plant parts (several species) in four fields which were not fertilized for 2 years (field A, ○), 6 years (field B, △), 19 years (field C, □) and 45 years (field D, ▽). Values represent the concentrations in pooled samples from five plots per field.
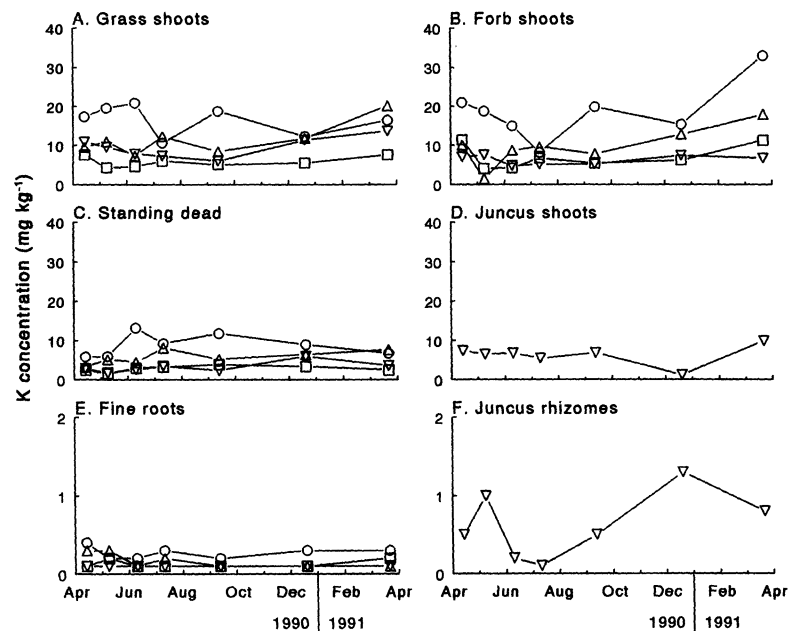
nificantly different between the three youngest fields, but lower in field D. This means that the hypothesized decrease in annual N mineralization could only be shown to have happened between 26 and 45 years. The lack of net mineralization during the autumn and winter period in field A resulted in relatively low values (Fig. 1). Since the relative nitrification rate was highest in this field but the absolute nitrification rate was lower than in field B, the relatively low total mineralization values could have been caused by high

denitrification losses of nitrate during winter and early spring. From field B to field D, a decrease in mineralization was found (Table 1).

Nitrification rates (both absolute and relative) decreased during succession (Fig. 1, Table 2). This might either reflect an advantage of plants over nitrifying bacteria when competing for limiting amounts of free ammonium in the soil (Riha *et al.* 1986; Verhagen *et al.* 1994) or an increased allelopathic inhibition of nitrification (Robertson 1982).
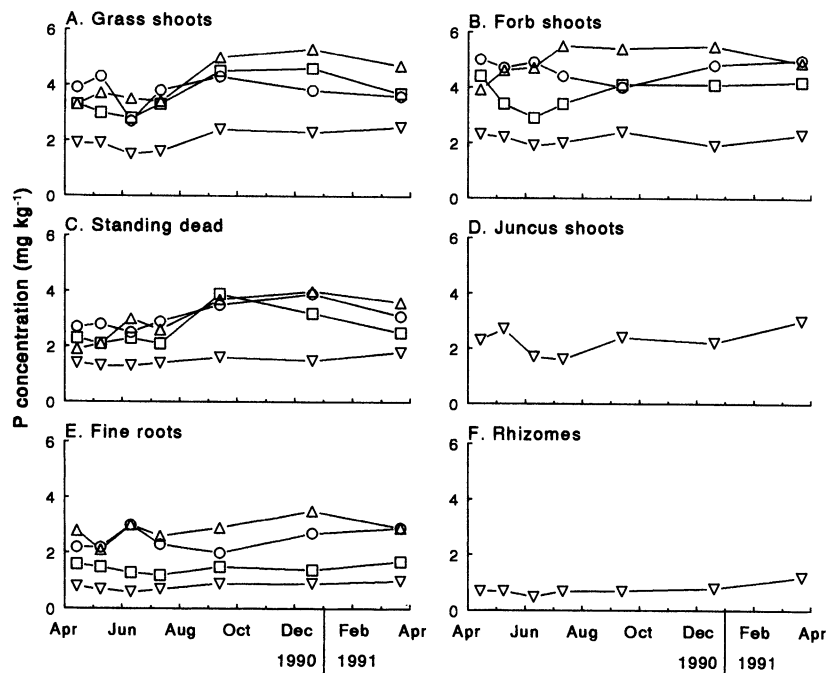
Fig. 6 Seasonal changes in tissue phosphorus concentration of various plant parts in four fields which were not fertilized for 2 years (field A, ○), 6 years (field B, △), 19 years (field C, □) and 45 years (field D, ▽). Values represent the concentrations in pooled samples from five plots per field.

Experiments on the potential nitrification activity and counts of the number of ammonium oxidizing bacteria in the root zone of different plant species in fields A–D provided evidence for the hypothesis that in these fields decreasing nitrification was related to decreasing ammonium availability (Stienstra *et al.* 1994). This corresponds to the finding that nitrifying bacteria can only build up large populations in situations of excess ammonia (Robertson 1982). This may happen especially under conditions of high N mineralization or high fertilizer application rates, where plant growth may become light limited. However, high nitrification rates may also result in high denitrification rates, leading to an underestimation of the actual mineralization rates in the recently fertilized fields. Further experiments are needed to quantify the magnitude of this effect.

The combination of soil N pool sizes (0–10 cm) and mineralization rates led to estimates of soil N turnover ranging from 24 to 50 g kg^{-1} $year^{-1}$. Again with the exception of field A, this measure decreased from field B to field D (Table 2). An average soil N turnover of 35 g kg^{-1} $year^{-1}$ would lead to an expected decrease over 45 years in soil total N (0–10 cm) of 4186 kg ha^{-1} (assuming exponential decrease). Since the observed decrease was lower, both atmospheric N deposition and the return of N by dying plant material (especially roots) are also likely to be important for the dynamics of the soil N pool (Bakker & Olff 1994). But the conclusion is clear that whereas fertilizer application to grasslands usually leads to accumulation of N (and organic matter) in the soil (Hoogerkamp 1973; Cuttle & Bourne 1992), the opposite

pattern is found under conditions of hay making without fertilizer application.

We observed that N in roots (85–228 kg N ha^{-1}) formed 4–6% of the total N pool in the soil, a range also reported by Dickinson (1984). This suggests that only a small part of the soil N pool is annually recycled by the vegetation. Cutting however, removes a major part of the available N in the system, ranging from 36% (field C) to 88% in field D (Table 2), assuming a complete uptake of mineralized N and an additional 45 kg N ha^{-1} $year^{-1}$ atmospheric deposition (Berendse *et al.* 1994).

When the amount of N mineralization is expressed relative to the total pool size in the soil this can indicate the importance of substrate quality as a factor in controlling N mineralization rates (Nadelhoffer *et al.* 1983; Hart & Gunther 1989). If indeed field A was an exception due to high denitrification rates, our results would indicate a decreasing substrate quality (observed from field B to field D) after stopping fertilizer application. The decreasing N concentration of fine roots (Fig. 4), which have high turnover rates (Fig. 2) suggests that the quality of the main source of organic matter is decreasing. A similar conclusion was drawn by Wedin & Tilman (1990) who found that growing monocultures of grass species which differed in root N concentration led to ten fold differences in N mineralization rates after three years. However, more detailed experiments are needed which separate 'fresh' organic matter from the large pool of 'old' organic matter.

When the present results are combined with estimates of other nitrogen flows in similar grasslands as

measured by Berendse *et al.* (1994), we can calculate a provisional input/output balances for N (Table 4). It is concluded from this that all fields had a net N offtake, indicating that impoverishment of the system was continuing, even for the field which had not been fertilized for 45 years. Apparently, this field is still draining its large pool of soil organic N, which is thought to have been formed in the period before its use as grassland, when it probably had *Alnus* forest or fen vegetation.

Changes in N supply are probably not the only cause of the observed species replacement during this grassland succession. Large changes during succession in tissue concentrations of K and P were observed in the present study, where differences between fields were present throughout most of the year. As for nitrogen, this could be caused either by species changes during succession, where later species (in the less productive stages) had lower tissue nutrient concentrations, or by plasticity within species in tissue nutrient concentrations. A different study where differences between these same fields in tissue nutrient concentrations of individual species were investigated, harvested on a single occasion in the year (July), showed that this latter process was an important explanation of the differences between fields (Olff 1992b; Bakker & Olff 1994). A similar conclusion was drawn when species were compared under controlled conditions at different light and nutrient supply rates (Olff 1992a). Fertilizer trials in these four fields revealed that all macronutrients eventually become limiting in field D (Olff 1992b). This means that a decreased availability of P and K may also be an important determinant of species replacement during this succession.

The observation that the increase during the growing season in shoot biomass of rushes was accompanied by a decrease in rhizome biomass in field D, suggests that translocation of reserves from rhizomes to shoots plays an important role in this

field. The observation that very little standing dead material was found furthermore suggests that the above-ground plant parts in this field are long-lived. A similar phenomenon was observed by Defoliart *et al.* (1988) in *Eriophorum vaginatum* L, where the decline in rhizome nutrient stores during spring coincided with the increase in photosynthetic potential of the shoot. A lower turnover rate (higher longevity) of aboveground biomass furthermore means that a relatively high biomass can accumulate towards the cutting date (July), even at low productivity.

The extensive rhizome systems in field D might store nutrients. Storage in general protects against periods of sub-optimal nutrient availability by making growth relatively independent from the instantaneous nutrient supply rate (Jonasson & Chapin 1985; Berendse & Jonasson 1992). Given the low N mineralization in field D, the demand for N during the growing season (March–July) is likely to be greater than the supply of N, and N remobilization from rhizomes may permit continued growth.

The amount of standing dead material which appears and subsequently disappears can be an indication for the longevity of above-ground tissues. During autumn and winter, large amounts of standing dead material accumulated in fields A and B, which disappeared during the subsequent growing season. This temporal accumulation of standing dead material was much lower in fields C and D, suggesting that leaf mortality in these fields was lower than in fields A and B, resulting in less litter fall.

Synthesizing all the information on nitrogen dynamics, we observed that mineralization during the growing season decreased only towards the last field in the chronosequence, while proportional nitrification decreased over the whole sequence. The recently fertilized fields had a clear peak of N mineralization during the growing season, while the seasonal pattern became less distinct in fields which had not been fertilized for longer time periods. Possibly in response to this, recently fertilized fields had a high shoot and root production after cutting, with high shoot mortality. Field C seemed to have a high root production with high mortality and a low shoot production, while field D had a low root and shoot production after cutting. However, this nevertheless resulted in a high peak standing crop in field D, most likely due to low turnover rates and efficient internal remobilization of reserves. It is suggested that these latter features are important explanations for the dominance of *Juncus acutiflorus* in field D. It means that the decrease in supply rate of mineral nitrogen during succession is compensated for by species replacement during succession, leading to dominance of species with a more efficient use of nitrogen. This species replacement might in turn be the cause of a further decreasing mineralization rate, due to negative feedback through (especially below ground) litter quality. The importance of this explanation for the

Table 4 Estimated input/output N balance of the four fields for 1990. All values are given in kg N ha^{-1} year^{-1}

	Field			
	A	B	C	D
Input				
Atmospheric deposition*†	60	60	60	60
N fixation‡	<1	<1	<1	<1
Output				
Denitrification*	16	16	16	16
Offtake by cutting	92	91	72	106
Input – Output	−66	−67	−48	−77

*Not measured in the present study: values were measured by Berendse *et al.* (1994) in a system with a species composition similar to field A. †Sum of throughfall and canopy uptake. ‡Estimated small since leguminous species were very low in biomass.

decreasing N supply rate, relative to the simple disappearance of a high quality organic matter pool in the soil, should be investigated further. The effects of the various dominants of this succession on the nutrient cycle are currently being investigated, by growing these species in monocultures in a garden experiment.

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