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Nutrient limitation in species-rich Calthion grasslands in relation to opportunities for restoration in a peat meadow landscape

B.P. Van de Riet, A. Barendregt, K. Brouns, M.M. Hefting & J.T.A. Verhoeven

Abstract

Questions: Which nutrient(s) limit(s) vegetation productivity in Calthion grasslands? Is phosphorus release a bottleneck for restoration of species-rich Calthion grasslands on rewetted dairy meadows?

Location: Three species-rich Calthion grasslands in the Western Peat District in the Netherlands.

Methods: We conducted a field fertilization experiment with nitrogen (N), phosphorus (P) and potassium (K) in three existing Calthion grasslands to evaluate the potential for restoration on rewetted dairy meadows. Responses of above-ground biomass, tissue nutrient concentrations and nutrient ratios were determined after 2 yr of fertilization.

Results: Biomass increased with fertilization with N-only and K-only but did not react to P-only additions. Comparisons of tissue nutrient concentrations and nutrient ratios also gave indications of N and K limitation.

Conclusions: The strong P release expected after rewetting should not necessarily interfere with restoration of Calthion communities on rewetted dairy meadows. It is concluded that for successful restoration management measures should focus on reducing N and/or K availability. Potassium might be an overlooked bottleneck in the restoration of species-rich grasslands.

Keywords: Field fertilization; Nitrogen; Peat meadow restoration; Phosphorus; Potassium.

Nomenclature: Van der Meijden (2005), Schaminée et al. (1996).

Van de Riet, B. P. (corresponding author; B.P.vandeRiet@uu.nl), **Brouns, K.** (K.Brouns@uu.nl), **Hefting, M. M.** (M.M.Hefting@uu.nl) & **Verhoeven, J. T. A.** (J.T.A.Verhoeven@uu.nl): Utrecht University, Landscape Ecology, P.O. Box 80 084, NL–3508 TB Utrecht, the Netherlands.

Barendregt, A. (A.Barendregt@geo.uu.nl): Utrecht University, Environmental Sciences, P.O. Box 80 115, NL–3508 TC Utrecht, the Netherlands.

Introduction

In Europe, species-rich grasslands have become restricted in area since intensive agriculture started to expand at the beginning of the twentieth century (Fuller 1987; Ellenberg 1996; Grootjans et al. 1996; Prach 1996; Schrautzer et al. 1996). Improvement of agricultural production has gone together with the extinction of rare plant species and great losses of biodiversity (Ellenberg 1996). Small relicts of species-rich grassland communities survive in nature reserves. Nevertheless, species composition continues to be negatively affected by high nutrient concentrations in surface water and by atmospheric nitrogen (N) deposition (Ellenberg 1996; Bobbink et al. 1998).

The Western Peat District in the Netherlands is an example of an area where drainage and excessive fertilizer application have transformed large complexes of wet species-rich grasslands into highly productive species-poor grasslands (Scheygrond 1931; Zuidhoff et al. 1996). As a consequence of long-term drainage and the associated land subsidence, most peat polders (areas with controlled water levels) are now situated 1-2 m below sea level. The combination of subsidence rates of 1-2 cm yr^{-1} (Van den Akker et al. 2008) and a total predicted sea-level rise of 35-85 cm by the end of this century (KNMI 2006; IPCC 2007) require adaptation of current land-use and water-management policies.

Rewetting meadows to prevent or slow down soil subsidence caused by peat oxidation has been proposed by authorities in the Netherlands. The Dutch government currently takes initiatives in major land-use transitions; in many of these projects large areas are designated to be restored as wet species-rich grasslands.

However, raising water tables often has not led to successful restoration of species richness owing to high soil fertility (Klimkowska et al. 2007; Van Dijk et al. 2007). It is likely that the long history of intensive dairy farming is a major constraint for restoring species richness because dairy meadows contain far more nutrients than the targeted semi-natural grassland communities. Moreover, rewetting itself strongly influences the nutrient availability and stoichiometry in soils. In particular, phosphorus (P) release is considered to be a major constraint because its availability is dependent on soil redox potential and strongly increases after rewetting, particularly in soils rich in iron (Young & Ross 2001; Van Dijk et al. 2004; Zak & Gelbrecht 2007). In addition, Zak & Gelbrecht (2007) found increased ammonium concentrations in the initial phases after rewetting. It is clear that under these nutrient-rich circumstances the restoration of relatively eutrophic fen meadow communities belonging to the Calthion palustris alliance will be more feasible than plant communities from nutrient-poor ecosystems. The Calthion palustris alliance has been common in the Western Peat District till the 1960s and occurs on peat or peaty clay soils that, compared with other species-rich grasslands, have a relatively high nutrient status (Kayl 1965; Ellenberg 1996). For successful restoration of species-rich Calthion grasslands on rewetted former agricultural land it is important to create conditions such that vegetation productivity is limited by the nutrient that will be most easily reduced after rewetting. Knowledge about which nutrient controls plant growth in existing Calthion stands in the Western Peat District can provide us with valuable information. If Calthion stands would be limited by P, the potential for restoration of these communities on dairy grasslands would be minimal as phosphorus is often released in large quantities after rewetting. If they were N- or potassium (K)-limited, there would be better opportunities. Knowledge of nutrient limitation would also be valuable for choosing the appropriate restoration measures, as reducing the availability of, for example N, requires other measures than for P or K (Koerselman & Meuleman 1996; Van Duren & Pegtel 2000).

We hypothesize that N, rather than P is limiting growth in Calthion grasslands, because N frequently has been found limiting, especially in relatively undisturbed herbaceous wetland communities (Van Duren & Pegtel 2000) such as in various wet meadow types (Vermeer 1986a, b; Van der Woude et al. 1994; Boeye et al. 1997) and fens (Verhoeven et al. 1983; Verhoeven & Schmitz 1991; Wassen et al. 1995, 1998). Phosphorus limitation seems mostly confined to low productive ecosystems that have had a long history of mowing and no fertilizer use (Koerselman et al. 1990; Verhoeven & Schmitz 1991) or to sites that are rich in iron, aluminium (De Mars et al. 1996; Boeye et al. 1997) or calcium (Boyer & Wheeler 1989). We also wanted to test the possibility of potassium limitation and included it as a treatment, because K limitation, although not frequently found in wetland vegetation (Verhoeven et al. 1996), has been demonstrated for a Calthion grassland in the Pleistocene District in the Netherlands (Van Duren et al. 1997b).

A field fertilization experiment was carried out to investigate which (combination) of the macronutrients N, P, or K increases biomass production in existing Calthion meadows in the Western Peat District. The following research questions were addressed: (1) What type of nutrient limitation controls biomass production in Calthion grasslands? (2) Is phosphorus release after rewetting a bottleneck for restoration of species-rich Calthion meadows?

Methods

Study sites

A fertilization experiment was carried out on three species-rich Calthion grasslands in the Peat District in the western part of the Netherlands (Fig. 1). The grassland Hazeleger is situated in the Nature Reserve 'De Nieuwkoopse Plassen' (52°08′25″ N, 4°48′04″ E). The grasslands Kattendijk (51°58′47″ N, 4°40′19″ E) and Veerstalblok (51°59′52″ N, 4°42′16″ E) are both small nature reserves located in the Polder Krimpenerwaard along the River Hollandse IJssel. The grassland Hazeleger has been in extensive agricultural use at least since the seventeenth century, but probably since the region was reclaimed in the Middle Ages. It is relatively poor in soil nitrogen and soil phosphorus (Table 1). The other two grasslands functioned until the end of the nineteenth century as water storage bodies in which water from the surrounding polder was collected and subsequently discharged into the river. Around 1885 the vegetation was dominated by *Phragmites australis* and *Scirpus lacustris*, but after losing their initial water storage function both sites were drained and used for haymaking (Scheygrond 1931). Both grasslands are relatively eutrophic compared with Hazeleger; the Veerstalblok soil is relatively rich in phosphorus, while the Kattendijk soil is rich in potassium (Table 1). Both grasslands are currently isolated from the river and are never flooded. The meadow Kattendijk, however, is receiving some seepage water from the river, as can be seen in the high concentrations of sulphate, bicarbonate and potassium in pore water (Table 1).

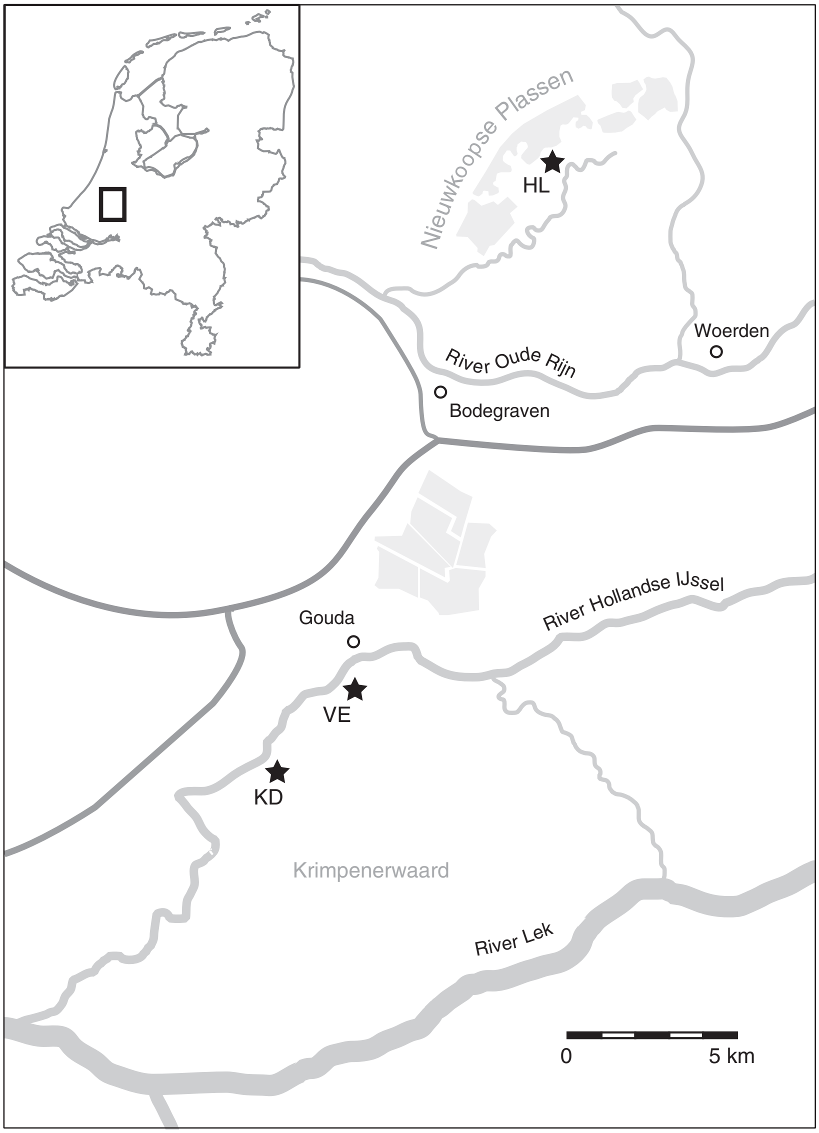

Fig. 1. Location the Calthion grasslands (★) that were included in the field fertilization experiment. HL = Hazeleger, KD = Kattendijk and VE = Veerstalblok.

All three meadows are annually mown in June-July. A second mowing takes place in the grasslands Veerstalblok and Kattendijk in case of strong regrowth of the vegetation. Occasionally, in Kattendijk low-intensity sheep grazing is introduced in autumn. Water tables vary from close to the ground surface in winter to 15-30 cm below surface in summer. However, the grassland Kattendijk is hydrologically connected to the surrounding polder and groundwater levels may decrease significantly in dry summers. All three meadows have high conservation value, because they harbour plant communities that belong to the most valuable in the Western Peat District in terms of species richness of higher plants. Syntaxonomically, the vegetation belongs to the Calthion palustris alliance and is characterized by the presence of, for example, *Caltha palustris*, *Lychnis flos-cuculi* and *Rhinanthus angustifolius* (Zuidhoff et al. 1996). Dominant graminoids are *Holcus lanatus*, *Anthoxanthum odoratum* and *Carex acuta*. Species richness is high in all three meadows with approximately 19 species per square metre (Table 2).

Field fertilization

At each grassland five replicate blocks of homogeneous vegetation were selected. Each block

Table 1. Soil nitrogen (N), phosphorus (P) and potassium (K) pools in the 0-20 cm soil layer ($g\,m^{-2}$; $n = 5$), in the above-ground biomass ($g\,m^{-2}$; $n = 5$), and the nutrient concentrations in pore water ($mg\,l^{-1}$; $n = 30$) (average values $\pm$ 1 SE) in the three Calthion grasslands Hazeleger (HL), Kattendijk (KD) and Veerstalblok (VE).

	HL	KD	VE
Soil and vegetation			
Soil $N_{0.4\,M\,KCl}$	0.30 (± 0.04)	0.47 (± 0.07)	1.42 (± 0.34)
N in standing stock	6.16 (± 0.59)	8.63 (± 0.51)	5.64 (± 0.64)
Soil $P_{lactate}$	2.17 (± 0.19)	4.06 (± 0.80)	12.38 (± 2.17)
P in standing stock	0.60 (± 0.09)	1.16 (± 0.06)	1.30 (± 0.21)
Soil K_{demi}	0.45 (± 0.28)	2.12 (± 1.26)	0.21 (± 0.18)
K in standing stock	1.83 (± 0.13)	5.60 (± 0.98)	1.53 (± 0.16)
Pore water			
NO_3	0.15 (± 0.04)	0.28 (± 0.07)	0.13 (± 0.03)
NH_4	0.15 (± 0.04)	0.32 (± 0.05)	0.24 (± 0.05)
PO_4	0.11 (± 0.03)	0.21 (± 0.04)	0.21 (± 0.07)
K	0.66 (± 0.14)	1.62 (± 0.59)	0.81 (± 0.10)
Fe	1.47 (± 0.24)	0.88 (± 0.10)	1.69 (± 0.32)
Al	0.17 (± 0.02)	0.08 (± 0.01)	0.09 (± 0.01)
Ca	20.0 (± 1.8)	69.5 (± 12.6)	12.5 (± 1.5)
Mg	4.32 (± 0.34)	5.11 (± 0.97)	1.74 (± 0.25)
HCO_3	28.4 (± 4.2)	88.7 (± 17.9)	27.7 (± 4.8)
SO_4	42.6 (± 5.2)	117.3 (± 24.3)	10.7 (± 1.0)
Cl	7.17 (± 1.18)	17.2 (± 3.2)	0.67 (± 0.32)
Na	7.97 (± 0.45)	14.5 (± 2.3)	1.26 (± 0.19)

Pore water samples were collected in Apr 2007, soil nutrients were determined in Sep 2008 following the procedures described by Houba et al. (1989).

contained five 1×1 m plots for fertilization with N, P, K, NPK-combined and a control treatment, respectively. Between the treatment plots there was a 50 cm wide buffer and within each block the treatments were randomly assigned to the plots. For the nutrient solutions NH_4NO_3 (N treatment), NaH_2PO_4 (P treatment), KCl (K treatment) were dissolved in demineralized water. Fertilization was applied in four dressings per growing season; each plot received 2 l of fertilizer solution at 3-wk intervals, starting in March. The control treatment received only demineralized water. The quantities applied amounted to $150\,kg \cdot N \cdot ha^{-1}\,yr^{-1}$, $60\,kg \cdot P \cdot ha^{-1}\,yr^{-1}$ and $150\,kg \cdot K \cdot ha^{-1}\,yr^{-1}$, respectively. The NPK treatment received a combination of all nutrients in quantities similar to the single treatments. Fertilization was carried out for two successive growing seasons, in 2007 and 2008.

Biomass

In July 2008 the above-ground biomass was harvested at peak standing crop by clipping the vegetation in subplots of 50 cm×50 cm to ground level. Vegetation samples were stored at −20°C. The harvested vegetation of Veerstalblok and Hazeleger was sorted into different species groups (Poaceae, Cyperaceae, Juncaceae, Fabaceae, Equisetaceae), standing dead and herbs. The harvested vegetation of Kattendijk was not sorted and only total dry weight was measured. For that purpose samples were dried until mass constancy (70°C, for at least 48 h) and weighed. Per plot vegetation was ground and 150 mg per sample was used to determine total N, P and K concentration of above-ground biomass with a salicylic acid thiosulphate modification of the

Table 2. Synoptic table summarizing species richness (average ± 1 SE) and both the frequency (1-5) and average cover class (I-VII) for the higher plant species in the control plots (n = 5) in the Calthion grasslands Hazeleger (HL), Kattendijk (KD) and Veerstalblok (VE) in June 2008.

	HL	KD	VE
Species richness (m^{-2})	18.8 ± 1.4	18.8 ± 1.3	19.4 ± 0.8
Species			
Holcus lanatus	5 – VI	5 – VI	5 – IV
Anthoxanthum odoratum	5 – V	5 – VI	5 – VII
Rhinanthus angustifolius	4 – I	5 – II	5 – V
Rumex acetosa	4 – I	3 – IV	5 – I
Ranunculus acris	3 – I	5 – III	4 – IV
Festuca rubra	5 – VI	2 – I	4 – V
Cardamine pratensis	5 – I	2 – I	4 – I
Plantago lanceolata	4 – II	1 – II	5 – VII
Lychnis flos-cuculi	4 – II	2 – I	4 – I
Lotus pedunculatus	5 – VI	2 – II	3 – V
Carex acuta	1 – V	3 – VII	5 – VII
Ranunculus repens	2 – II	3 – IV	3 – II
Galium palustre	3 – I	2 – I	3 – I
Juncus conglomeratus	3 – I	2 – I	2 – V
Filipendula ulmaria	3 – V	2 – IV	1 – I
Cerastium fontanum subsp. *vulgare*	1 – I	2 – I	1 – I
Trifolium pratense	2 – II	1 – I	1 – I
Cirsium palustre	4 – II	–	5 – IV
Lythrum salicaria	2 – I	–	3 – I
Valeriana officinalis	1 – I	–	2 – I
Vicia cracca	4 – I	–	–
Phragmites australis	4 – I	–	–
Glechoma hederacea	3 – I	–	–
Carex nigra	3 – VI	–	–
Luzula multiflora subsp. *multiflora*	2 – I	–	–
Carex riparia	2 – I	–	–
Carex disticha	3 – VI	5 – VII	–
Equisetum fluviatile	–	5 – I	–
Cynosurus cristatus	–	2 – VI	–
Carex hirta	–	2 – I	–
Poa pratensis	–	4 – IV	4 – II
Lolium perenne	–	4 – IV	3 – II
Festuca pratensis	–	5 – IV	2 – I
Caltha palustris subsp. ***palustris***	–	5 – VI	1 – I
Taraxacum officinale	–	4 – I	1 – I
Juncus effusus	–	–	4 – V

Character species of the Calthion alliance are shown in bold type (according to Schaminée et al., 1996). Infrequent species are not listed (i.e. frequency = 1 in complete dataset). Average cover classes: I = 0-1%; II = 1-2%; III = 2-3%; IV = 3-4%; V = 4-8%; VI = 8-18%; VII = 18-38%.

Kjeldahl digestion (Bremner & Mulvaney 1982). The N and P concentrations were measured colorimetrically on a continuous flow Auto Analyser (Skalar SA-40; Skalar Analytical, Breda, the Netherlands) and K concentrations by flame emission spectroscopy (IL 543; Instrumentation Laboratory, IJsselstein, the Netherlands).

Statistical analyses

All statistical analyses were carried out using SPSS 16.0 for Windows. For the overall effect of fertilization the biomass data were analysed using a two-way ANOVA with block as a random factor. For each site biomass data were analysed separately using a one-way ANOVA. If necessary, data were logarithmically transformed to stabilize variances between groups. *Post-hoc* Tukey tests were performed to test for significant differences between treatments and *P*-values were corrected for the multiple comparisons of the different treatments with the control treatment.

Differences in nutrient concentrations and nutrient ratios among sites were tested using one-way ANOVA. One paired *t*-test was used to analyse whether nutrient concentrations and nutrient ratios differed from critical nutrient concentrations and critical ratios, respectively.

Results

Above-ground biomass

Overall, total biomass production was significantly higher in the single N and single K treatments than in the control treatment ($P = 0.024$ and $P = 0.008$, respectively). The combined NPK treatment had the highest biomass production ($P \leq 0.001$) and fertilization with P had no significant effect (Fig. 2a).

When analysed separately per site, the vegetation in Hazeleger, Kattendijk and Veerstalblok increased strongly after the combined NPK treatments ($P = 0.008$, $P = 0.036$ and $P \leq 0.001$, respectively; Fig. 2b–d). Responses to single-nutrient treatments were less strong or absent, which indicated that biomass production in all three Calthion grasslands is co-limited by two or more nutrients. In Veerstalblok, K increased production significantly (Fig. 2d; $P = 0.024$), but neither N nor P addition had significant effects. In both Hazeleger and Kattendijk single-nutrient treatments did not reveal any significant effects.

Biomass increase after fertilization was mainly the effect of significantly increased production of Poaceae (data not shown). In Veerstalblok, the biomass of Poaceae increased significantly after N fertilization ($P = 0.008$) and after the combined NPK treatment ($P \leq 0.001$). Standing dead material increased in the combined NPK treatment ($P = 0.032$). Moreover, in the N treatment *C. acuta* tended to be more productive ($P = 0.084$). In Hazeleger, no significant effects on different species groups were observed; however, production of Poaceae tended to increase in the combined NPK treatment ($P = 0.060$) and standing dead tended to be increased ($P = 0.096$). In other species groups no significant effects of the different fertilizer treatments were observed.

Changes in the dominance of individual plant species

In all three grasslands N addition obviously increased the dominance and the number of inflorescences of *H. lanatus*, *A. odoratum* and *Festuca rubra*. *Carex disticha*, *Carex nigra* and *Filipendula ulmaria* also increased in dominance in response to N. In plots fertilized with K an increase in cover and number of inflorescences was observed for *H. lanatus*. The character species of the Calthion alliance (i.e. *L. flos-cuculi* and *R. angustifolius* and other wet meadow species such as *Ranunculus flammula*, *Ranunculus acris*, *Cirsium palustre* and *Juncus effusus*) showed more abundant flowering in the K-treated plots. *Lotus pedunculatus*, a legume species capable of fixing N, responded positively to potassium and in Hazeleger also to phosphorus.

Nutrients in plant tissue

In addition to measuring the biomass response after field fertilization, there are two methods that can give indications for the type of nutrient limitation that plants have experienced during growth. First, De Wit et al. (1963) showed that if the tissue concentration of a particular nutrient is lower than a certain critical value, there is a strong indication that plant growth has been limited by that particular nutrient (Verhoeven et al. 1983). Second, ratios of tissue nutrient concentrations proved to be indicative of nutrient limitation at the vegetation level (Koerselman & Meuleman 1996; Güsewell & Koerselman 2002; Olde Venterink et al. 2003; Güsewell 2004).

Figure 3a-c depict the N, P and K concentrations in plant tissue in the control plots. The dotted lines indicate the 'critical nutrient concentrations'.

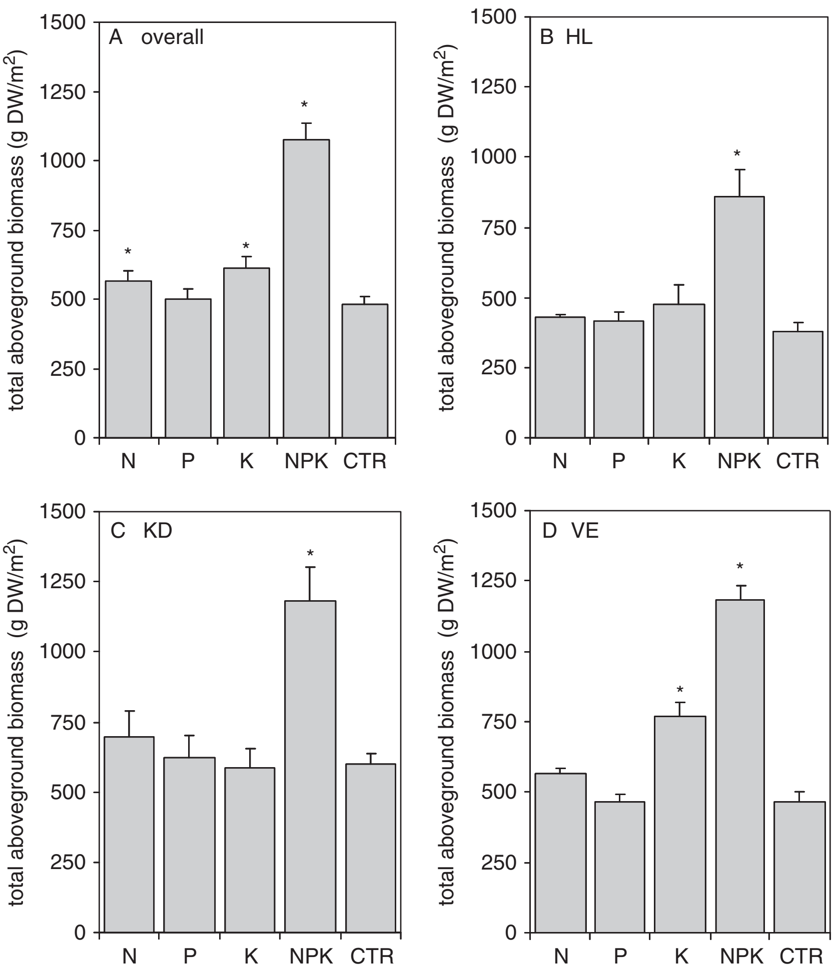

Fig. 2. Total above-ground biomass for the overall dataset and for each meadow separately. * Indicates significant differences from control treatment (CTR, $P<0.05$). overall = three meadows combined, HL = Hazeleger, KD = Kattendijk, VE = Veerstalblok. Error bars indicate +1 SE.

Data from all grasslands combined show that tissue P concentrations were significantly above, and tissue K concentrations significantly below this critical value. Tissue N concentrations were close to the critical value indicating N limitation. We found the same results for each site except for Kattendijk where K was not limiting (Fig. 3c). Nevertheless, K concentrations were significantly lower than the critical concentration in the sites Hazeleger and Veerstalblok (Fig. 3c). Figure 3b clearly shows that P was not limiting plant growth in any of the Calthion meadows investigated. Nutrient ratios in the above-ground plant tissue are shown in Fig. 3d-f. In all three grasslands the N:P ratio was significantly below the critical value of 14.5, which is a strong indication that N, rather than P is limiting. Both the N:K ratios and the K:P ratios in Hazeleger and Veerstalblok indicated that in addition to N also K was limiting. Only in Kattendijk was no indication for K limitation found.

Discussion

Nutrient limitation in Calthion grasslands

The primary goal of this research was to study the nutrient limitation in existing species-rich Calthion grasslands in order to explore possible bottlenecks in the restoration success of this plant community on abandoned dairy meadows. A field fertilization experiment is considered to be the most straightforward method for assessing the type of nutrient limitation in herbaceous vegetation (Van Duren & Pegtel 2000). In our study, high nutrient doses (comparable to the amounts used in intensive farming

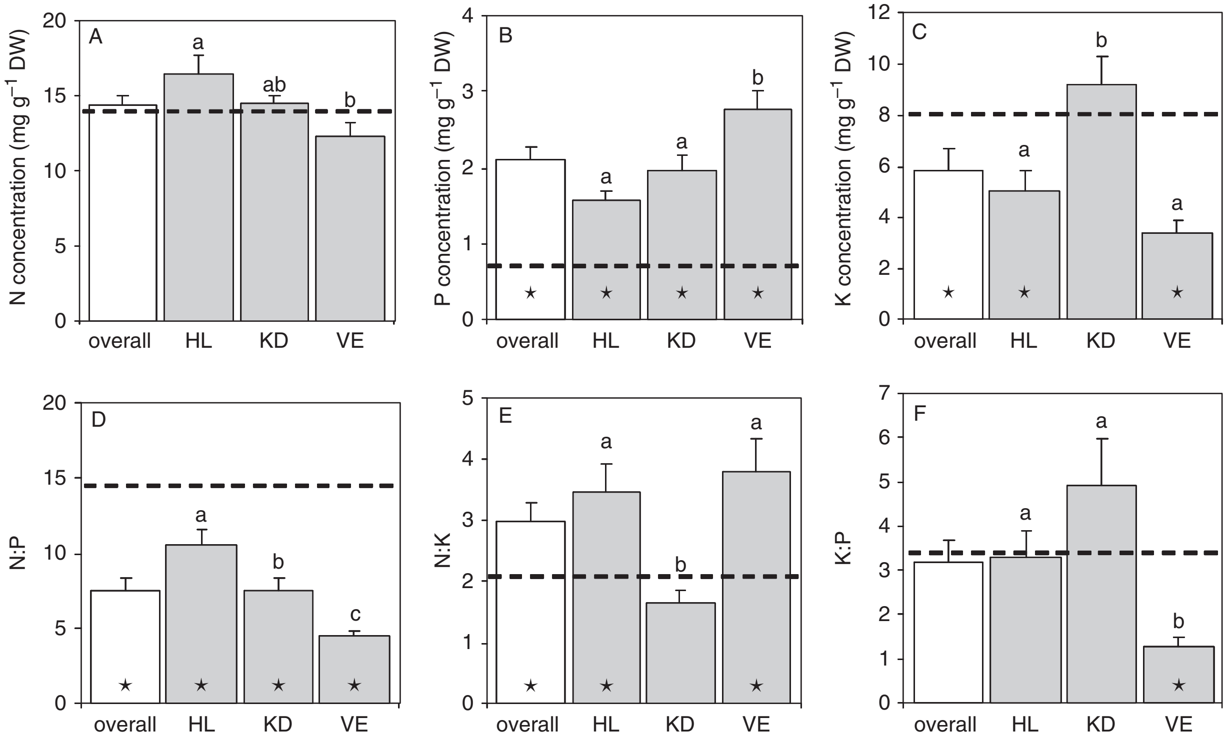

Fig. 3. (**a-c**). Concentration of nitrogen (N), phosphorus (P) and potassium (K) in above-ground biomass in the control plots. Dotted lines represent critical nutrient concentrations according to De Wit et al. (1963) and Verhoeven et al. (1983). (**d-f**) Nutrient ratios based on total nutrient concentrations in above-ground biomass. Dotted lines represent critical values for the different nutrient ratios derived from Olde Venterink et al. (2003). ★ at the base of the bars indicates that bars differ significantly from the critical values ($P \leq 0.05$). Letters above the tinted bars indicate differences between sites. Overall = all three meadows combined, HL = Hazeleger, KD = Kattendijk, VE = Veerstalblok. Error bars indicate +1 SE.

practice: $150\,kg \cdot N \cdot ha^{-1} yr^{-1}$, $60\,kg \cdot P \cdot ha^{-1} yr^{-1}$ and $150\,kg \cdot ha^{-1} yr^{-1}$) were applied during two growing seasons, which is considered a sufficiently long period to test current nutrient limitation, without the effects occurring after prolonged periods of fertilization when the nutrient dynamics of the stand will change as a result of shifts in the species composition (Güsewell 2004; Van der Hoek et al. 2004). We have also used nutrient ratios and critical nutrient concentrations for investigating indications of nutrient limitation, which are based on extensive research reviews (Koerselman & Meuleman 1996; Verhoeven et al. 1996; Güsewell & Koerselman 2002; Olde Venterink et al. 2003; Güsewell 2004).

From the overall significant growth responses to the fertilization treatments we conclude that both N and K are controlling biomass production in Calthion communities. In addition, both the nutrient concentrations and the nutrient ratios in above-ground biomass support this conclusion. Van Duren et al. (1997b) found comparable results in fertilization experiments in Calthion communities in brook valleys on mineral soils, where they occurred under quite different conditions with respect to hydrology and geomorphology.

The observed differences in responsiveness between the three sites, although not investigated in detail, might be explained by differences in local hydrology, water level and land-use history. For example, the absence of K limitation in Kattendijk might be caused by discharge of groundwater originating from the river, rich in K, contrary to the other two sites which lack these inputs (Table 1).

Nitrogen limitation is frequently found in wetlands (Van Duren & Pegtel 2000). It can occur when N inputs via fertilization, eutrophic surface water and atmospheric deposition are low and N loss is substantial: high water tables reduce N availability by slowing down mineralization (Grootjans et al. 1985; Berendse et al. 1994; Schrautzer et al. 1996) and by stimulating denitrification (Olde Venterink et al. 2002; Hefting et al. 2004).

Potassium limitation occurs only occasionally and is mostly related to drainage in combination with long-term haymaking (Verhoeven et al. 1983; Vermeer 1986b; De Mars et al. 1996; Boeye et al.

1997; Van Duren et al. 1997a). Kayser & Isselstein (2005) mention K limitation to occur mainly on sandy soil with low clay contents and it is therefore all the more surprising that it was found to be limiting in these Calthion grasslands on peaty-clayey soils in the Western Peat District. However, K is a mobile ion and several papers suggest that significant amounts can be lost by leaching after drainage (De Mars et al. 1996; Van Duren et al. 1997b; Alfaro et al. 2004).

Comparison of the soil nutrient pools in the three Calthions with the amount of nutrients annually removed by haymaking shows that the available amounts of soil N and K could gradually become depleted by annual mowing (shortages of 5–7 $g\,N\,m^{-2}$ and 1–4 $g\,K\,m^{-2}$), while the soil P pool is larger than the amount removed by mowing (surplus of 2–11 $g\,P\,m^{-2}$; Table 1). Although mineralization probably will replenish these nutrient pools partly, these data support the observation that the vegetation is not P-limited, while both N and K might be in short supply.

In many herbaceous plant communities, relatively low biomass productivity is essential for maintaining species richness (Vermeer & Berendse 1983; Oomes 1992). Eutrophication of species-rich herbaceous stands leads to increased biomass production of competitive plant species. Often, graminoid species become more dominant, which leads to monotonous stands, poor in plant species (Bobbink et al. 1998; Güsewell 2004). We indeed did find an increase in the dominance of grass species in the sites where productivity increased as a result of the addition of N or combined NPK, although the set-up of the experiment is not suitable for predicting long-term changes in species composition. These generally described shifts in species composition imply that the nutrient availability of rewetted dairy grasslands should not be too high and at least one of the major nutrients N, P or K should be limiting, preferably the same element as in the reference sites where the vegetation is still intact.

Implications for restoration of Calthion vegetation on former dairy meadows

The results of this study indicate that it is important to strive for N and/or K-limited plant growth in restoration efforts to restore Calthion grasslands on rewetted dairy meadows. Rewetting will affect the availability of soil nutrients. Nitrogen availability is expected to decrease gradually in the long term because of slower N mineralization (Grootjans, 1985; Berendse et al. 1994; Olde Venterink et al. 2002) and faster denitrification (Olde Venterink et al. 2002; Hefting et al. 2004). Little is known about the effects of rewetting on K availability. In a phytometer experiment, rewetting reduced the extent of K limitation for *H. lanatus* grown on soil cores taken from a K-limited site (Van Duren & Van Andel 1997). In soil cores, Koerselman et al. (1993) showed K was released in rewetted peat soil, but others found that K was not affected by rewetting (Olde Venterink et al. 2002). Despite these inconsistent results we know that the K availability in drained dairy meadows is high and can be found to be increased after rewetting (B. P. van de Riet, unpubl. data). For P it is clear that rewetting will increase its availability, because iron-bound P becomes mobilized at low redox potentials after rewetting (Van Dijk et al. 2007; Zak & Gelbrecht 2007).

In many cases, the success of restoration after rewetting has proven to be very limited (Klimkowska et al. 2007) and P release after rewetting is often assumed to be a major constraint (Van Dijk et al. 2007). However, an important result of this research with respect to rewetting in the peat meadow area is that existing Calthion communities are not limited by P because the application of high doses ($60\,kg \cdot P \cdot ha^{-1}\,yr^{-1}$) did not cause any changes in vegetation productivity. Our results imply that the expected strong P release after rewetting, estimated to be up to $40\,kg \cdot P \cdot ha^{-1}\,yr^{-1}$ in rewetted agricultural peatlands in Germany (Zak et al. 2008), should not necessarily interfere with restoration of Calthion communities, as long as N and/or K availability can be sufficiently reduced. For the restoration of Calthion grassland on abandoned dairy meadows, management measures should be optimized to achieve N and/or K limitation. We therefore have the following recommendations for management:

(1) Extra nutrient inputs need to be prevented as much as possible. Fertilization should be ceased and restoration sites should be isolated from surrounding agricultural areas.

(2) To accomplish a net removal of N and K it is probably necessary to mow multiple times per year, as was found for abandoned peat grasslands and eutrophic Calthions in northern Germany (Schrautzer et al. 1996). The same was indicated by a nutrient budget study in fens in the Netherlands, which revealed that mowing once a year only balanced the atmospheric input of N (Koerselman et al. 1990). Oomes et al. (1996) reported that a period of 9 yr of two cuttings annually resulted in such low K content in the biomass that it was likely to be limiting plant productivity on an abandoned

agricultural field. When aiming for K limitation, grazing is not a very good option because large amounts of this element will immediately return to the soil in the form of animal excreta (Koppisch et al. 2001; Kayser & Isselstein 2005).

We expect that the efficiency of K removal is an important and probably often neglected factor that determines the restoration success of Calthion grasslands.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Marsh Marigold (*Caltha palustris*) abundantly flowering in early spring in a Calthion grassland in Nature Reserve Kattendijkblokboezem in polder Krimpenerwaard, the Netherlands.

Fig. S2. Marsh Marigold (*Caltha palustris*) abundantly flowering in early spring in a Calthion grassland in Nature Reserve Veerstalblokboezem in polder Krimpenerwaard, the Netherlands.

Fig. S3. Vegetation structure in a Calthion grassland with Marsh Marigold (*Caltha palustris*), Ragged Robin (*Lychnis flos-cuculi*), Slender-tufted Sedge (*Carex acuta*), and Sweet Vernal-grass (*Anthoxanthum odoratum*). Picture taken in April 2007 in a control plot of a fertilization experiment in Nature Reserve Veerstalblokboezem in polder Krimpenerwaard, the Netherlands.

Fig. S4. Species-rich Calthion grassland in Nature Reserve Veerstalblokboezem in polder Krimpenerwaard, the Netherlands. Early summer aspect with Slender-tufted Sedge (*Carex acuta*), Ragged Robin (*Lychnis flos-cuculi*), and Greater Yellow-rattle (*Rhinanthus angustifolius*).

Fig. S5. Species-rich Calthion grassland in Nature Reserve Veerstalblokboezem in polder Krimpenerwaard, the Netherlands. Early summer aspect with Slender-tufted Sedge (*Carex acuta*), Ragged Robin (*Lychnis flos-cuculi*), Greater Yellow-rattle (*Rhinanthus angustifolius*), Common Sorrel (*Rumex acetosa*) and Meadow Buttercup (*Ranunculus acris*).

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