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Soil nutrient input effects on seed longevity: a burial experiment with fen-meadow species

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Summary

1. Effects of high nutrient input on the longevity and viability of buried seed are examined. Seeds of 17 fen-meadow species were buried in nylon mesh bags at four sites in the Netherlands and one site in Great Britain in plots to which N, P, K fertilizers are applied. Prior to burial germination tests were conducted on the seeds of each species. This paper describes the results of the viability tests on the seeds that were exhumed after one and 2 years of burial.

2. The percentage of seeds that germinated after 1 year of burial was significantly lower than the pre-burial percentage for the majority of the species. After 2 years of burial the germination percentage further decreased. A few species, such as the *Carex* species, did, however, show an increase in germination percentage indicating that the burial conditions allowed dormancy controls to be broken.

3. Differences in the edaphic conditions between the sites appeared to affect germination percentages after 1 year of burial. A difference in germination response between sites was observed for *Carex acutiformis*, *Filipendula ulmaria* and *Lychnis flos-cuculi*.

4. A significantly higher germination percentage was found at the Great Britain site for *F. ulmaria* in the phosphate treatment compared with the potassium treatment and the control after 1 year of burial. In contrast to many literature assessments no significant effects of fertilizer application was found after 2 years.

5. For all sites, except one in the Netherlands, the total number of seeds that germinated was lower in 1996 than in 1995.

Key-words: Fertilization, nitrogen, seed bank, seed persistence, species-rich grassland

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Introduction

Intensification of grassland exploitation throughout western Europe has resulted in a dramatic reduction of biological diversity within the agricultural landscape. The decrease in floristic diversity in temperate grasslands is mainly influenced by soil nutrient availability (Grubb 1987; Bakker 1989; Hodgson & Grime 1990). The enhanced nutrient availability as a result of high fertilizer inputs is a major cause of low plant species diversity and a key factor limiting the restoration of biological resources when extensification takes place (Bakker 1989). However, experiments have shown that de-intensification of agricultural practices by a reduction in fertilizer input and the adoption of traditional management regimes, such as hay making, often fail to achieve a recovery in floristic diversity (Tallowin 1996; Oomes, Kuikman & Jacobs 1997).

Where plant species have disappeared from the established vegetation but possibly survive in the seed bank, buried seeds can play an important role in conservation and restoration management. Research has shown that species of fen-meadow and hay-meadow systems often appear to be poorly represented in the soil seed bank (Chippindale & Milton 1934; Bakker 1989; Bekker *et al*. 1997; Thompson, Bakker & Bekker 1997). This could be the result of a reduced survival of buried seeds in the soil or a reduced seed rain owing to a species-poor vegetation, both of which could be either directly or indirectly influenced by enhanced soil nutrient availability derived from former intensive agricultural exploitation. For example, the presence of nitrate ions is known to stimulate germination in several plant species (Cavers & Benoit 1989; Pons 1989, 1991, 1992; Karssen & Hilhorst 1992) and this may be a factor causing depletion of the seed bank under intensive management. Also, differences in temperature, light and/or moisture content of the soil, i.e. the storage conditions that the seed are exposed to, can have major impacts on seed viability

(Roberts 1972; Baskin & Baskin 1992; Jansen & Ison 1995). Murdoch & Ellis (1992) found that under oxic storage conditions a high moisture content of lettuce seeds can increase viability but under anoxic conditions longevity declines rapidly with an increasing moisture content. Much of this research is carried out on species of commercial value. With the focus on difficulties in restoration of species-rich communities it is therefore important to identify the persistent components of species-rich grassland seed banks to understand the mechanisms by which they are lost or longevity of seeds declines. The aim of the present study is to determine the effects of the application of different fertilizers to seeds buried under species-rich communities at different sites and to estimate the effects of these treatments on the longevity of the seeds of a wide range of wet grassland species in a long-term experiment.

Materials and methods

SITES DESCRIPTION

Two sites, (NL1 and NL2), are located in species-rich *Calthion palustris* communities situated in the brook valley system of the Drentse Aa (53° 5' N, 6° 42' E) in the Netherlands. Both sites are on organic soil overlying a sandy subsoil within a deep seepage system with calcium-rich groundwater. Site NL1 is undrained and relatively wet. Site NL2 is situated near the edge of the brook valley and has been degraded owing to drainage in the past and is still disconnected from the deep seepage flow.

A third site, NL3, is located on a *Caricion curto-nigrae* community situated in the same brook valley as NL1 and 2. NL3 is on an organic soil overlying a sandy subsoil in an infiltration system where the groundwater is poor in minerals.

A fourth site (NL4) is located close to the River Reest near Meppel (52° 40' N, 6° 13' E) in the Netherlands in a *Magnocaricion* community situated on an organic soil overlying sandy subsoil in a nutrient-rich inundation system. The site is inundated for at least 6 months of the year. None of the sites, NL1, NL2, NL3 nor NL4, has received fertilizer in the past 20 years.

A fifth site (GB1) is located on a *Centaureo–Cynosuretum* community on the Somerset Levels in the south-west of England (51° 21' N, 2° 49' W). The soil consists of deep peat overlying silty clay, fed with calcareous infiltration water from limestone hills surrounding the area (Kirkham, Mountford & Wilkins 1996).

Soil sampling was carried out within each site, and the following chemical analyses were preformed: pH (H_2O), volumetric moisture content, organic matter, total nitrogen (N), total phosphate (P), total potassium (K) and exchangeable potassium (exchangeable K). The soil chemical analyses of the NL sites were carried out on soil dried at 95 °C (stove dry) with exception of the moisture content analyses, which were performed on soil dried at 30 °C (air dry). The soil chemical analyses of the GB1 site were all carried out on soil dried at 30 °C in a force draft oven to constant mass. Results of soil chemical analyses for the different sites are summarized in Table 1.

Table 1. Soil chemical analyses for each of the seed burial sites. The soil characteristics of NL1–NL4 are given per 100 g stove-dried soil (95 °C), except moisture content [100 g air-dried soil (30 °C)]. The chemical analyses of the GB1 site are preformed on 100 g oven-dried soil (30 °C). For the units of the parameters see subscript table

Parameters	NL1[1]	NL2[1]	NL3[1]	NL4[1]	GB1[1]
pH-H_2O[2]	5·7	5·6	4·9	5·8	5·9
Moisture content[3]	5·5	6·5	2·8	8·1	
Organic matter[4]	34·8	49·9	23·9	44·3	62·8
Total N[5]	1·49	2·25	0·92	1·58	1771
Total P[6]	872	353	228	844	79
Total K[7]	0·06	0·03	0·05	0·05	
Exchangeable K[8]					1·7

[1]Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo–Cynosuretum.* [2] pH-H_2O: – log (H^+) in filtrate. [3] Moisture content: g H_2O/100 g air-dry soil. [4] Organic matter: g/100 g stove-dry soil; GB1 g/100 air-dry soil. [5] Total N: g N/100 g stove-dry soil. [6] Total P: mg P_2O_5/100 g stove-dry soil. [7] Total K: g K_2O/100 g stove-dry soil. [8] Exchangeable K: mg/100 g air-dry soil.

EXPERIMENTAL DETAILS

The experimental design is a randomized block with four replicates. At each site an area of 225 m^2 was subdivided into 2 m × 2 m plots which were spatially separated by untreated buffer strips 0·5-m wide to avoid interference. At sites NL1–4 fertilizer treatments consist of applying either 200 kg nitrogen (as ureum, 39% w/w N) ha^{-1} annually (N), 80 kg phosphate (as calciumbiphosphate, 17% w/w P) ha^{-1} annually (P), 200 kg potassium (as potassiumsulphate, 37% w/w K) ha^{-1} annually (K) or a combination of all three N, P and K treatments (NPK) and an unfertilized control (Ctrl). The above fertilizer amounts were applied in the form of a 'slow release' fertilizer 'Osmocote pellets' at a single dressing in the spring of 1993, 1994, 1995, 1996 and 1997.

At site GB1 the treatments consist of applying either 200 kg nitrogen (as soluble granular ammonium nitrate fertilizer) ha^{-1} annually (N), 75 kg phosphate (as soluble granular triple superphosphate fertilizer) ha^{-1} annually (P), 200 kg potassium (as soluble granular muriate of potash) ha^{-1} annually (K) or a combination of all three N, P and K treatments (NPK) and an unfertilized control (Ctrl). The fertilizer is applied in four equal amounts at weekly intervals in the spring to avoid the risk of such high doses scorching the foliage of the established vegetation.

SPECIES

The species used in this study (Table 2) were chosen as characteristic elements of the site flora and their seeds known to have a longevity of at least more than

1 year in the soil seed bank (Chippindale & Milton 1934; Roberts & Neilson 1981; Stieperaere & Timmerman 1983; Meredith 1985; Fix & Poschlod 1993; Thompson *et al*. 1997).

The majority of the seeds was collected at the burial sites in the summer of 1993. The seeds of *Lychnis flos-cuculi*, buried in the sites NL3 and NL4, originated from a comparable community at 'Lange Sâne' *c*. 50 km from the sites. The seeds of *Potentilla erecta* and *L. flos-cuculi* buried at GB1 were purchased commercially, whereas the seeds of *Filipendula ulmaria*, buried at GB1, were collected at the sites NL1 and NL2 in the Netherlands.

Prior to burial the collected seeds were stored dark and cold (4 °C) with a relatively low air-humidity of 40–50%. Fifty seeds per species were mixed with 20 cm^3 sterilized potting compost or sterilized soil of the wet NL1 site and put into colour-marked nylon bags.

Each experimental plot was subdivided into 16 subplots and five seed bags, each containing 50 seeds of one of the five species that were used per site (Table 2), were buried in each of 10 randomly selected subplots. The burial procedure involved lifting the turf of a subplot with a spade and inserting the five bags in a fixed pattern, at ± 5 cm depth. The burial of the seed bags took place in February and March 1994 at the five sites.

PRE-BURIAL GERMINATION TEST

In autumn 1993 a pre-burial germination test was carried out on three replicates of 50 seeds of each species. The seeds were stratified at 4 °C in the dark for 4 weeks prior to the test. The germination test was carried out in a controlled environment cabinet providing 16 h light at 25 °C and 8 h darkness 15 °C (Thompson & Grime 1979; Bekker & Zandvoort 1993).

Table 2. Origin of seeds, burial site and persistence in the soil of the 17 buried species. The persistence classes of the species are based on ≥ 10 records of the seed bank database of Thompson *et al*. (1997). The persistence classes: transient, persist < 1 year in the soil; short-term persistent, persist > 1 but < 5 years in the soil; long-term persistent, persist ≥ 5 years in the soil; –, < 10 records available (Thompson *et al*. 1997)

Species	Site[1]	Persistence
Anthoxanthum odoratum	NL2	transient/short-term persistent
Carex acutiformis	NL1 NL2	–
Carex curta	NL3	–
Carex echinata	NL3	–
Carex flacca	GB1	short-term persistent
Carex hostiana	GB1	–
Carex nigra	NL4	transient/short-term persistent
Crepis paludosa	NL1	–
Filipendula ulmaria[2]	NL1 NL2 GB1	short-term persistent
Lychnis flos-cuculi	NL1 NL2	long-term persistent
Lychnis flos-cuculi[3]	NL3 NL4	
Lychnis flos-cuculi[4]	GB1	
Pedicularis palustris	NL4	–
Potentilla erecta[4]	GB1	long-term persistent
Potentilla palustris	NL3	short-term persistent
Ranunculus flammula	NL4	long-term persistent
Scirpus sylvaticus	NL1 NL2	–
Senecio aquaticus	NL4	–
Viola palustris	NL3	–

[1]Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo–Cynosuretum*. [2] Seed from the Netherlands (NL1 and NL2). [3] Seed from the Netherlands (Lange Sâne). [4] Seed purchased commercially.

EXHUMATION, WASHING AND GERMINATION

The seed bags were exhumed by lifting the ± 5 cm deep turf of two randomly selected subplots in March (NL) and May (GB) in 1995 and 1996. The bags were stored in the dark at 4 °C in a refrigerator at a relative air-humidity of 40–50% for a maximum of 3 weeks prior to germination testing. The outside of the seed bags was washed and cleaned of any soil or seeds. They were then cut open and the content was washed onto a 1-mm sieve that was placed over a 0·212-mm sieve (Ter Heerdt *et al*. 1996). This procedure washed the seeds free of sand and soil particles. The seeds were then placed on moistened filter paper in Petri dishes (9-cm diameter). The number of seeds was checked and if appropriate the remains of seed coats, indicating possible early germination or decay, were recorded. The Petri dishes were placed in an illuminated controlled environment cabinet providing the same day and night conditions as used in the pre-burial germination test, which, according to Bekker & Zandvoort (1993), was suitable for the germination of all the species used in this study. The Petri dishes were checked for germination every 2–3 days. The filter paper was kept moist throughout the germination test period. Seeds were counted and removed when at least one leaf had developed. After 30 days the germination test was terminated and the remaining seeds were tested for viability by squeezing the seed onto a hard surface. The seeds which were soft, i.e. easy to squash, and brown in colour were considered not viable/dead.

STANDING CROP

The standing crop on each experimental plot was measured in July 1994 by cutting a 60 cm × 60 cm area (n = 5) at 5 cm above ground level and recording the dry mass yield.

DATA ANALYSIS

The mean number of germinated seeds in the pre-burial test (**B**) was determined and used to test changes in germination success owing to burial and treatment effects.

The total number of seeds per species that were retrieved (**TR**) from each treatment at each exhumation was divided into the number of seeds that subsequently germinated (**TG**), the number not germinated but still had a hard seed coat and was not infected by fungi (**viable**), and the number not germinated but had a soft seed coat and/or was strongly infected by fungi (**dead**).

The difference between the 50 seeds in each bag and **TR** represents seeds **lost** owing to processes such as deterioration, early germination and predation. The **B** and **TR** data were tested for homogeneity of variance and where necessary arcsin or square root transformations were carried out to homogenize the variances before conducting statistical analyses. One-way analysis of variance ANOVA was used to examine the differences between 1995 and 1996 for **TG**, **viable** and **dead**. A two-way ANOVA for the detection of fertilizer effects was carried out with the factors treatment and species with year as covariable. For site effects per species a two-way ANOVA was carried out with the factors treatment and site with year as covariable. The contrasts were determined with a one-way ANOVA, and a Tukey's Pairwise Comparison of Means test was used to indicate differences between group means. All ANOVAs were carried out using **TG** with a rejection level of $P < 0{\cdot}05$ with the program SPSS + version 5·0.

Results

STANDING CROP

The supply of the combined NPK treatment had a significant effect on standing crop across all sites except NL1 and NL3 after 2 years of nutrient application (Table 3). The effects of the other nutrients on standing crop were either not significant or not consistent across the different sites. For detailed information see Van Duren *et al*. (1997).

PRE-BURIAL GERMINATION TEST AND RECOVERY OF BURIED SEED

The majority of the species showed germination percentages ranging from 31% (*Carex nigra*) to more than 95% (*Carex curta*, *L. flos-cuculi*, *Viola palustris*); the species *Anthoxanthum odoratum* (24%) and *Potentilla palustris* (1%) showed very low germination percentages although the ungerminated seeds were classified still viable (Bekker & Zandvoort 1993).

During the process of exhumation nearly all seed bags from the different sites were retrieved. The percentage of retrieval amounted to 98%, in both 1995 and 1996. In 1995 significantly less seeds were retrieved of *F. ulmaria* (Chi^2-test, $P < 0{\cdot}001$), *L. flos-cuculi* ($P < 0{\cdot}001$), *Pedicularis palustris* ($P < 0{\cdot}05$), *P. erecta* ($P < 0{\cdot}01$) and *Ranunculus flammula* ($P < 0{\cdot}001$) compared with the 50 seeds that were buried. In 1996 significant seed losses occurred in almost all species compared with the initial 50 seeds, except for *C. curta* and *Carex echinata*. From the species *A. odoratum* and *Crepis paludosa* only a few seeds per bag were retrieved in 1996.

Table 3. The mean yield of standing crop (g m^{-2}) in July 1994 for each treatment at each site. Within a site yields with different letters indicate a significant difference at $P < 0{\cdot}05$ (Van Duren *et al*. 1997)

Treatment	NL1[1]	NL2[1]	NL3[1]	NL4[1]	GB1[1]
Control	563·84 ab	270·82 ab	397·41 a	259·30 a	552·29 a
N	649·90 ab	207·83 a	608·42 a	457·90 bc	668·55 b
P	507·31 a	264·44 a	350·73 a	285·85 ab	673·95 b
K	543·68 a	418·08 bc	509·77 a	232·15 a	691·59 b
NPK	434·81 b	773·73 c	306·31 a	559·60 c	893·55 c

[1] Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo–Cynosuretum*.

PRE-BURIAL–POST-BURIAL GERMINATION

The following species showed a significantly lower germination percentage after burial compared with their germination percentages prior to burial (Fig. 1): *A. odoratum* (NL2 in 1996), *C. paludosa* (NL1 in 1995 and 1996), *F. ulmaria* (NL1 in 1995 and 1996, NL2 in 1996, GB1 in 1995 and 1996), *L. flos-cuculi* (NL3, NL4, GB1 all in 1996) and *Senecio aquaticus* (NL4 in 1996). *Carex nigra* (NL4 in 1995 and 1996) and *P. erecta* (GB1 in 1995), however, showed significantly higher germination percentages after burial compared with their initial germination percentages (Fig. 1).

FATE OF SEEDS

In 1995 most of the seeds that remained ungerminated after 30 days were still viable with the exception of *A. odoratum*, *C. paludosa*, *F. ulmaria*, *L. flos-cuculi*, *Pedicularis palustris* and *S. aquaticus*, whose ungerminated seeds were rapidly decayed and/or colonized by fungi. After 2 years of burial the germination percentages had declined significantly compared with the percentages obtained after 1 year's burial for *L. flos-cuculi* (NL1 and NL3, $P < 0{\cdot}01$, NL2 $P < 0{\cdot}05$, NL4 and GB1, $P < 0{\cdot}001$), *Pedicularis palustris* (NL4, $P < 0{\cdot}001$), *S. aquaticus* (NL4, $P < 0{\cdot}001$), *F. ulmaria* (GB1, $P < 0{\cdot}01$) and *P. erecta* (GB1, $P < 0{\cdot}001$). The germination percentage of *Carex hostiana* (GB1, $P < 0{\cdot}05$) had increased significantly between 1995 and 1996.

Focusing on the difference between the percentage of still viable seeds between 1995 and 1996, a significant decrease was found for *Carex acutiformis* (NL1 and NL2, $P < 0{\cdot}001$), *C. curta* (NL3, $P < 0{\cdot}05$), *Carex flacca* (GB1, $P < 0{\cdot}001$), *C. hostiana* (GB1, $P < 0{\cdot}001$), *L. flos-cuculi* (NL1, $P < 0{\cdot}01$), *P. erecta* (GB1, $P < 0{\cdot}01$), *Potentilla palustris* (NL3, $P < 0{\cdot}05$) and *V. palustris* (NL3, $P < 0{\cdot}001$).

In 1995 more than 50% of the ungerminated seeds of *C. paludosa* (89·6% at site NL1), *F. ulmaria* (86·7%; NL1) and *A. odoratum* (71·6%; NL2) were dead. After two years of burial (1996) all species showed a percentage of dead seeds after the termination of the germination test varying from 0·5% (*C. curta*, NL3) to 34·0% (*F. ulmaria*, NL1). In 1996 the category of lost seeds increased significantly owing to an increase in the number of already deteriorated dead seeds.

The percentage of seeds lost varied from 2·8% (*C. flacca*, GB1) to 24·7% (*R. flammula*, NL4) in 1995,

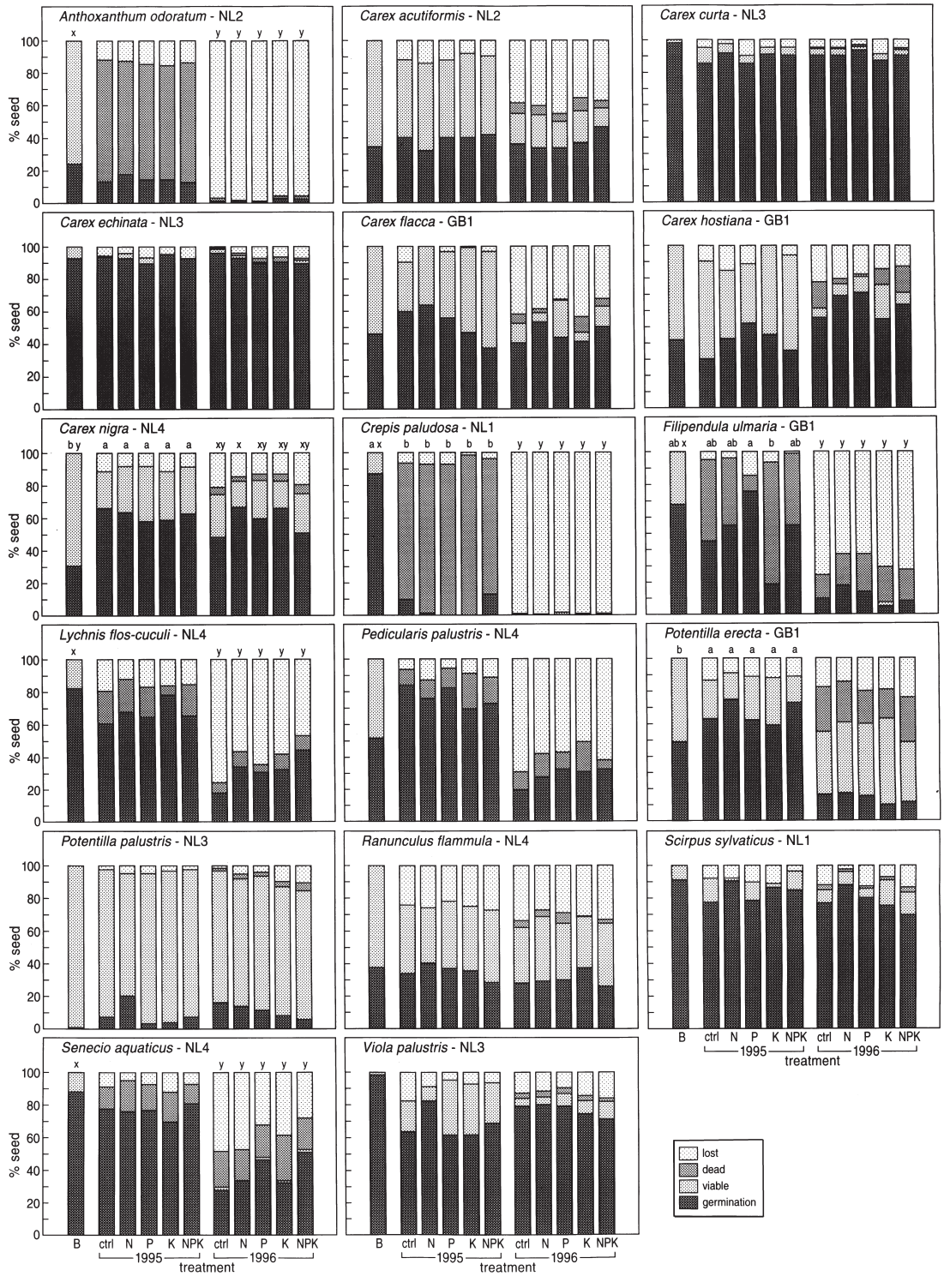

Fig. 1. Changes in the number of germinated seeds (**TG**) for 1995 and 1996 compared with **TG** of the pre-burial germination test (**B**) for all species buried. The bars represent, respectively, the amount of seed that was germinated, that was still **viable**, that was **dead** and that was **lost**, given per treatment in percentage of the initial 50 seeds. Treatments: **B,** pre-burial germination test; **Ctrl,** control, no fertilizer application; **N,** nitrogen; **P,** phosphorus; **K,** potassium; **NPK,** full treatment. Each two columns with the same letter do not differ significantly in **TG** (1995 characters *a* + *b;* 1996 *x* + *y*). Contrast obtained by one-way ANOVA. Level of significance: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Significant differences: *Anthoxanthum odoratum* 1996***; *Carex nigra* 1995***, 1996*; *Crepis paludosa* 1995***, 1996***; *Filipendula ulmaria* 1995*, 1996***; *Lychnis flos-cuculi* 1996***; *Potentilla erecta* 1995***; *Senecio aquaticus* 1996***.

whereas in 1996 the percentage of lost seeds varied from 5·9% (*Potentilla palustris*, NL3) to 99·1% (*C. paludosa*, NL1). Only for *Scirpus sylvaticus* (NL1), *C. curta* (NL2), *C. echinata* (NL2), *R. flammula* (NL4) and *P. erecta* (GB1) were no significant losses found in 1996 compared with 1995.

YEAR

For all sites, except NL3, there was a significantly lower number of germinated seeds (**TG**) in 1996 compared with 1995. At all sites a significantly higher total number of seeds was lost in 1996 than in 1995 (Table 4). This, not surprisingly, means that seeds died at all sites.

TREATMENT

No treatment effects were found on the total number of seeds that germinated within any of the sites for any species (Table 4) with one exception. In 1995, at site GB1, *F. ulmaria* showed a significantly higher germination in the P treatment compared with either the K or unfertilized control (Table 5; Fig. 1). No treatment effect was found for the total number of seeds lost (Table 4).

SPECIES

Significant differences in seed survival and total number of seeds lost were found between different species at different sites and between the sites (Tables 4 and 5). No interactions between species and treatment were found for **TG** and **lost** seed (Table 4).

SITE

For the species *C. acutiformis*, *F. ulmaria*, *L flos-cuculi* and *S. sylvaticus*, buried at more than one site, site differences were found over the control treatment (Ctrl) and over the 2 years of burial (Table 5). Only for *F. ulmaria* and *L. flos-cuculi* was a year effect found (Table 5). The mean **TG** in 1995 for *F. ulmaria* at the sites NL2 and GB1 was significantly higher compared with NL1 (Fig. 2). This trend is continued in 1996 although not significant any more owing to too much variation in the dataset. For *L. flos-cuculi* in 1996 a significantly higher mean **TG** was found for NL2 compared with NL3, NL4 and GB1 (Fig. 2). There are no significant differences in **TG** between the sites NL1 and NL2 and between NL3 and NL4 (Fig. 2).

Also for *C. acutiformis* a higher **TG** was found for NL2 compared with NL1 over 2 years of burial but the

Table 4. Results from two-way ANOVAs for changes in (1) total germination (**TG**) and (2) **lost**, conducted separately for the sites. ANOVAs tested with covariate year for effects of treatment, species and an interaction effect. Level of significance: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}005$

	Site[1] (*F*-values)				
Effect	NL1	NL2	NL3	NL4	GB1
(1) Total germination					
Year (covariate)	15·86***	43·44***	1·87	174·50***	63·64***
Treatment	0.29	0.070	1·20	1·40	3·90
Species	434·92***	403·43***	391·05***	23·47***	2·54***
Treatment × species	0.92	0.90	0.90	0.90	0.74
(2) Lost					
Year (covariate)	394·38***	279·57***	70·34***	251·67***	329·43***
Treatment	0.86	0.66	0.13	2·0	1·50
Species	41·68***	55·59***	117·98***	30·09***	40·44***
Treatment × species	0.92	0.26	0.56	0.73	0.566

[1]Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo–Cynosuretum.*

Table 5. Results from two-way ANOVAs for changes in **TG** conducted separately for the four species buried at more than one site. ANOVAs tested with covariate year for effects of treatment, site and their interaction effect. Level of significance: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}005$

			F-values			
Species	Site[1]	df[2]	Year covariate	Site	Treatment	Interaction treat. × site
L. flos-cuculi	NL1 NL2 NL3 NL4 GB1	1 4 4 16	183·76***	31·91***	1·65	0.74
F. ulmaria	NL1 NL2 GB1	1 4 2 8	86·15***	67·72***	2·42*	2·39*
C. acutiformis	NL1 NL2	1 4 1 4	0.20	5·5*	0.09	3·56**
S. sylvaticus	NL1 NL2	1 4 1 4	0.16	0.78	0.78	0.85

[1]Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo–Cynosuretum.*
[2] df, Degrees of freedom for covariate year, treatment, site and interaction.

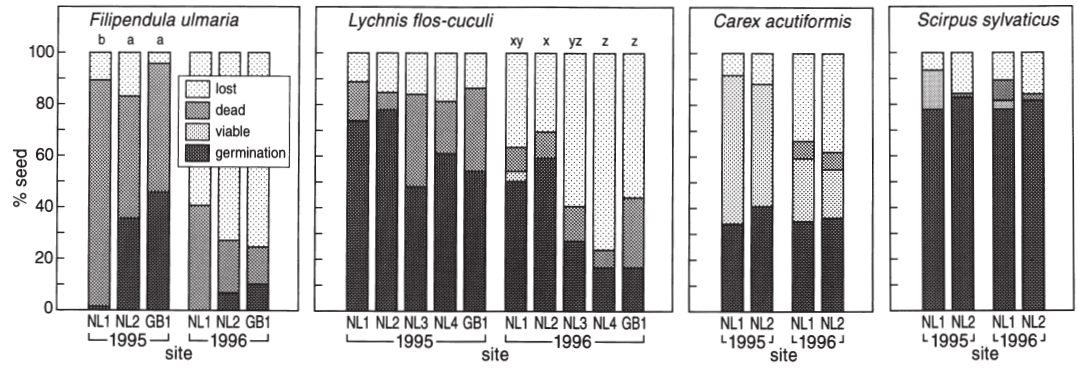

Fig. 2. Changes in mean number of germinated seeds (**TG**) in the control treatment (**Ctrl**) of different species buried at different sites buried over 1995 and 1996. Sites: NL1, *Calthion*; NL2, drained *Calthion*; NL3, *Caricion curto-nigrae*; NL4, *Magnocaricion*; GB1, *Centaureo-Cynosuretum*. The diagram bars represent, respectively, the amount of seed that germinated, that did not germinate but was still **viable**, that was **dead** and the amount of seeds **lost** given as a percentage of the initial amount of 50 buried seeds (% seed). Each two columns with the same letter do not differ significantly in **TG** (1995, characters $a + b$; 1996, $x + y$). Contrast obtained by one-way ANOVA. Significant differences: *Filipendula ulmaria* 1995, $P < 0{\cdot}01$; *Lychnis flos-cuculi* 1996, $P < 0{\cdot}001$. For *Carex acutiformis* and *Scirpus sylvaticus* no significant differences were found.

differences were not significant in either 1995 or 1996 (Fig. 2). There was no site effect found for *S. sylvaticus* (Table 5; Fig. 2). Significant interactions between treatment and site effects were found in *C. acutiformis* and *F. ulmaria*; however, the two species did not show a consistent pattern in these interactions. However, if all four species are considered, the germination response at site NL1 is always lower, although not significant, than at site NL2.

Discussion

The objective of this seed burial experiment was to establish whether enhanced availability of nitrogen, phosphorus or potassium, or a combination of all three nutrients, could affect the longevity of seeds of key species of species-rich wet grasslands. To date, the only indication of an effect was found in 1995 at site GB1 where a significantly lower total number of *F. ulmaria* seeds germinated in plots to which high amounts of potassium fertilizer had been applied compared with phosphate fertilized plots. Significant differences were found between the pre- and post-burial germination percentages in some of the different fertilizer treatments for some of the species. Some species showed a higher germination percentage after 1 year of burial compared with their pre-burial germination indicating that dormancy controls were overcome during the burial period.

FATE OF SEEDS

At the end of the 30-day period for testing the germination of the exhumed seeds most of the ungerminated seeds were still viable. It might therefore be argued that 30 days was too short to obtain a measure of the actual potential germinability of the exhumed seeds. In defence of the procedure it has to be pointed out that during the pre-burial germination tests the majority of the seeds of the selected species had in fact germinated. A possible reason for the lack of germination of apparently viable seeds after exhumation could be that they were in an induced state of dormancy. This is a mechanism that prevents germination under unfavourable circumstances, for example, insufficient moisture, light or unsuitable temperature (Fenner 1985; Baskin & Baskin 1989, 1992; Murdoch & Ellis 1992).

Germination in the soil possibly accounts for most seed losses in the seed bank (Roberts 1972; Fenner 1985; Baskin & Baskin 1989; Rice 1989; Lunt 1995) therefore an attempt was made to check the total number of seeds and seed coats before washing. This was found to be almost impossible to do owing to the presence of the sand and soil particles obscuring seeds. Counting was therefore carried out in the Petri dishes after washing. This means that no direct evidence of the loss of seed from the seed bags as a result of germination could be found. The counting by hand after washing revealed that there were lower numbers of seeds compared with the initial number placed in the seed bags. It was not possible to determine whether losses of seed were the result of lethal germination in the seed bags or solely owing to death and decay as ageing followed by decay is also a major factor of seed bank depletion (Villiers 1972). Nitrate can break dormancy in many species, either on its own or in combination with temperature or light and darkness (Bliss & Smith 1985; Cavers & Benoit 1989; Pons 1989, 1991, 1992; Hilhorst 1990; Baskin & Baskin 1992; Karssen & Hilhorst 1992; Bouwmeester *et al*. 1994). In this present experiment there was no evidence found for early germination in the soil caused by nitrate. Also the moisture content of the burial environment is mentioned to be of importance in breaking dormancy of the seeds in the soil seed bank (Roberts 1972; Cook 1980; Jansen & Ison 1995).

However, as it is difficult to identify the sources of mortality, these judgements remain highly speculative. In general, the rates of losses and depletion from the seed bank are dependent upon the different germination and dormancy characteristics of the species buried (Grime *et al*. 1981).

A possible explanation for the decrease in seed numbers in the seed bags could also be that they were removed by predation (granivory: e.g. Fenner 1985; Jansen & Ison 1995). Literature on seed predation suggests that predation can have a significant effect on the seed bank (Louda 1989). No direct evidence for this was found in the present experiment.

The direct loss of seeds through the nylon mesh of the bags could have been another possible cause of poor recovery. If this was the case then it might be assumed that such losses would be more likely to happen with small rather than large seeds. In fact no differences in recovery were found between small and large seeded species that would have supported this contention; no significant loss of seeds of *C. curta*, *C. echinata*, *P. erecta*, *R. flammula* or *S. sylvaticus* was found during the 2 years of burial.

SITE

A significant site effect was found after one and 2 years burial for *C. acutiformis*, *F. ulmaria* and *L. flos-cuculi*. After 1 year's burial the seeds of *F. ulmaria* from the wet NL1 showed a significantly lower germination percentage compared with the seed exhumed from GB1 or the degraded NL2 site. This effect could not have been caused by inherent differences between the buried seeds, because the seeds were collected from the same site at the same time and the seed bags from all three sites contained the same medium. The only distinction is that the NL1 site has been flooded for a long time during the spring. It has been suggested that seeds may remain viable longer if the soil is waterlogged (Villiers 1972; Howe & Chancellor 1983; Cavers & Benoit 1989; Murdoch & Ellis 1992; Bewley & Black 1994). An excessive supply of water may, however, limit the availability of oxygen for seed respiration with the consequence that the longevity of seeds declines rapidly with an increase in moisture content (Murdoch & Ellis 1992). This would suggest that the site difference observed for *F. ulmaria* was probably a result of the relatively long period of anaerobiosis at NL1. Some evidence could be drawn from the fact that all four species buried at the wet NL1 site seem to germinate less than in the dryer NL2 site.

The purchased seeds of *L. flos-cuculi* buried at site GB1 showed a significantly lower germination compared with NL1 and NL2 in 1996. This obviously results from the different origin of the seeds. Between seeds buried at different sites, but derived from the same origin, no significant differences were found. As the initial pre-burial germination percentage was as high as from the NL1 and NL2 sites; we did not expect large differences between the two seed lots.

The germination success of the seeds of *L. flos-cuculi* declined significantly after 1 year and declined even further after 2 years of burial. Milberg (1994) found that after 25 months of burial there was a significant increase in the number of non-viable seeds of *L. flos-cuculi* but the percentage of dead seeds was much lower compared with the results presented in this article. Milberg (1994) buried seeds of *L. flos-cuculi* in polyester bags with no medium at 15-cm depth in wooden boxes containing peaty soil, whereas in this study the seeds were buried in the field in seed bags containing sterilized potting compost or soil from a *Calthion* community. Research from Milberg (1994) and Thompson *et al*. (1997) indicate that *L. flos-cuculi* is long lived and has the potential to accumulate a persistent seed bank but the results presented here indicate the opposite.

We have to state that a weakness of the design of the present experiment is the few species that were buried at more than one site and the use of seeds from up to three seed sources. In defence of this we put forward that for a burial experiment of this design a large quantity of seeds were required, which was not available at the time of burial. Unfortunately, owing to technical problems, some species buried at the GB1 site had to be collected at comparable sites and seed of *L. flos-cuculi* and *P. erecta* had to be purchased commercially.

Beforehand the main aim was to investigate the fate of seeds of as many species belonging to the community under study as possible because accurate data on seed longevity for many of the species are missing (see also Table 2) . From this experiment we can conclude that all species still have some viable seeds after 2 years of burial. This means that all species can be categorized as being at least short-term persistent. The species *A. odoratum* and *C. paludosa* show a very drastic decline in viable seed numbers after 2 years which might support transient behaviour as was found in the database of Thompson *et al*. 1997. The results for *C. nigra* and *S. sylvaticus* show that these species are at least short-term persistent, contrary to the majority of the records in the database.

NUTRIENT AVAILABILITY

The supply of NPK has shown a big influence on standing crop after nutrient application at three of the five sites. Analyses of NPK nutrient yield of plant tissue of two sites, NL1 and NL2, showed significantly raised concentrations of phosphorus and potassium compared with the controls in the established treatments (results not shown, see Van Duren *et al*. 1997). A significantly raised nitrogen concentration could only be detected in NL2. This means that the availability of phosphorus, potassium and nitrogen in the soil solution of these sites has remained enhanced and

that we achieved large differences in the nutrient availability of the soil environments of the buried seeds at the different sites.

After 2 years of burial there was no direct measurable effect of the different nutrient applications to the buried seeds. The decline in viability of the seeds and the increased numbers of lost seeds appeared to be the result of decomposition processes in the soil and/or premature germination or death of the buried seed. Nevertheless, the fertilization treatments had a great influence on the established vegetation, both on species composition and total phytomass. It is very probable that mineralization rates will be influenced by the treatments. Enhanced nutrient availability owing to fertilizer inputs will have a large impact on the soil biota and decomposition processes which, in turn, may have either a direct or indirect influence on the longevity of seeds in the soil. The compilation of effects could still eventually lead to soil seed-bank deterioration in the long run, which may be found during the course of this research project.

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