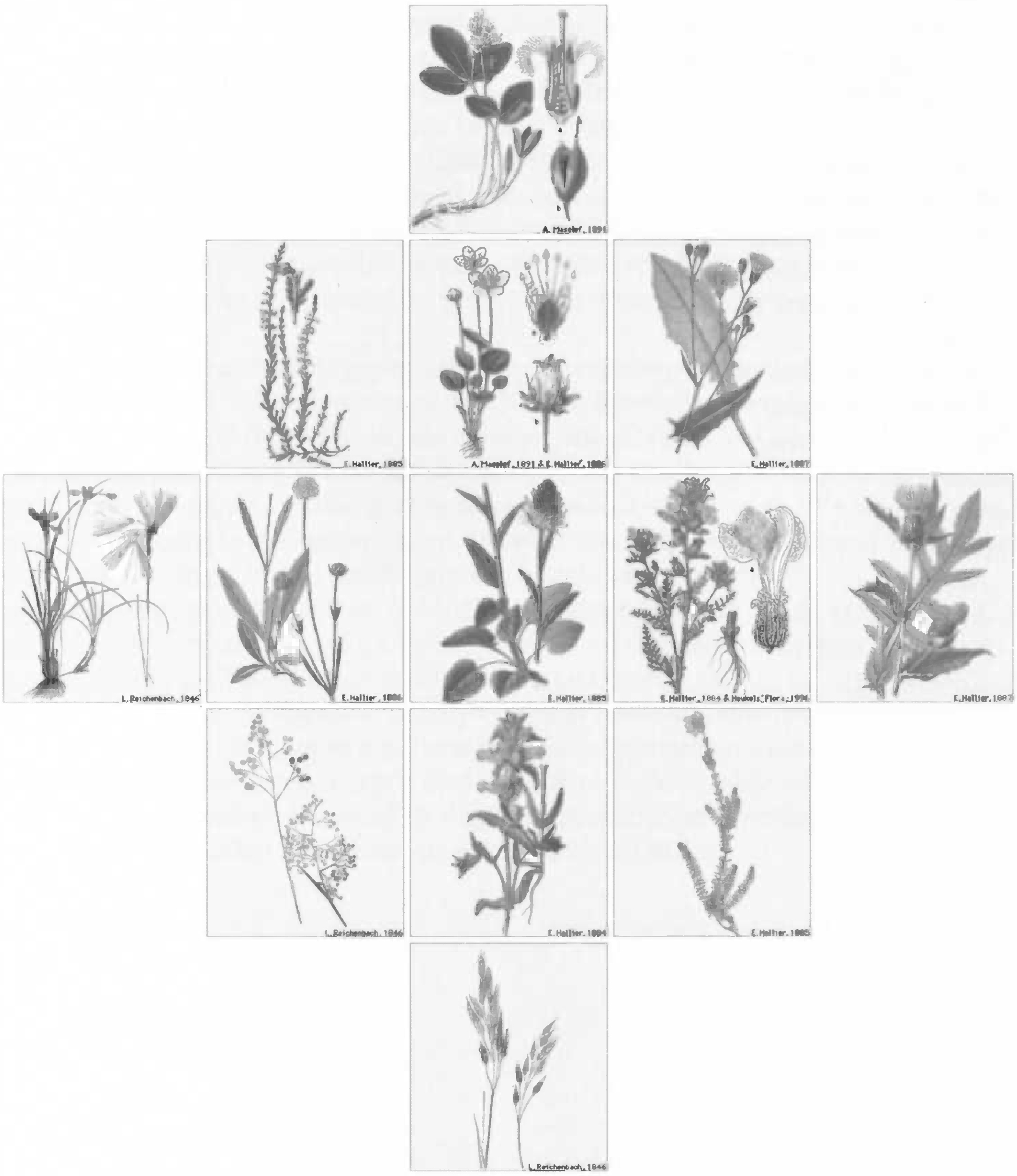
Recruitment of Target Species in the Drentsche Aa Reserve



By: L. Kochen
Supervision: V. Cordlandwehr and J.P Bakker

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Bibliotheek Biologisch Centrum
Kerklaan 30 — Postbus 14
9750 AA HAREN

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Summary:

It is a well known problem in restoration efforts that one or more important species are missing in the area. In this study three possible reasons for the absence of 13 target species are investigated. First, it could be that the abiotic ranges that are present in the fields are still not suitable for these species. Also, dispersal limitation could be a problem since most of the dispersal vectors of these plants are missing. Lastly, there could be an insufficient amount of gaps or microsites in the area, a feature necessary for some species to establish or remain present in their vegetation community.
To find the reason(s) for these species, fieldwork has been done on a running sowing experiment in the area Anlooer Diepje located in the Drentsche Aa Reserve. Seeds of target species were sown in three different treatments (bare ground, moss removed, control). Treatments were applied in 15 plots, divided over two fields containing the same plant community. In total four plant communities were used in the experiment. Soil samples, germination conditions and vegetation recordings have been taken of all 60 plots. Soil samples were analysed for moisture content, pH and organic matter content.
Out of the 13 species *Succisa pratensis, Crepis paludosa, Pedicularis palustris, Cirsium oleraceum, Rhinanthus angustifolius* and *Bromus racemosus* germinated in the field and the first five survived the summer, all of which had a high seed weight. Between treatments only *Crepis* had statistically less seedlings in the control than in bare ground and raked. On the other species, especially *Succisa* and *Pedicularis*, the measures had no effect on germination. Between the fields the differences in seedling number of *Succisa*, *Cirsium* and *Crepis* were statistically significant.
Succisa was negatively affected by a high percentage of living plants in its vicinity, but not by moss, so in sites where living plants were abundant, bare ground became more important for *Succisa* than in sites with more moss.
In four out of the six species it is mainly dispersal limitation that prevents them from colonizing an area, while in two of them microsite limitation may also play a role. *Bromus* could not be tested, since it died out due to unfavourable weather, but as it did germinate it may also be inhibited by dispersal limitation and, perhaps, microsite limitation. For the other species no answers have been found.

Introduction:

For centuries people have used the land for agriculture. They grew their crops close by their homes, let their livestock feed in pastures, and mowed once a year to collect hay. Up to a certain point in time this did not put such a large strain on the fields they were using and the vegetation composition stayed more or less the same.
When artificial fertilizer became available, the farmers were able to greatly increase the production of their fields. To harvest their crop they lowered the groundwatertable so the soil would become drier, which allowed heavier farm equipment to access the field.
These changes had a strong effect on the vegetation as it shifted towards species-poor, high-production grassland or weeds only in case of arable fields. A lot of fields were swallowed up like this and the vegetation communities that once occupied them have become sparse. Nowadays the focus has been shifted from agriculture to the restoration of these rare communities, which have a high value for biodiversity.
In the Netherlands there are various areas which are now declared nature reserve. These places, like the Brunsummerheide in Limburg(south of the Netherlands) or the Veluwe in Gelderland(middle-Netherlands), are managed by the state to preserve and develop vegetation communities of a high nature conservation interest, in these cases forest and heathland. In the north of the Netherlands there is another reserve, which is more oriented towards grasslands, with fen meadows and wet meadows. This reserve is the Drentsche Aa Nature Reserve, which will be the focus of this study.

Since the mid 1960s the Dutch State Forestry Commission started to purchase fields in the northern part of Drenthe from farmers who used them as pastures for their livestock. This part of the Netherlands stood out because its small system of streams had remained unchanged, still following its original meandering route throughout the landscape. The goal was to restore the areas former species-rich grasslands. After the State Forestry Commission bought the fields, they applied a management regime consisting of several techniques. The drainage ditches that had been dug to create drier soil for the heavy mowing equipment were filled up again and the water table was raised. This restored the hydrological conditions of the site. Furthermore in some places they removed the nutrient-rich top soil to speed up the impoverishment of the land. They also mow the field once every year between July and September, after the plants have flowered, or let the farmers graze their cattle there in low densities. Over time this has had a very encouraging effect: the soil has become wetter and the amount of nutrients has decreased, bringing about the return of many species that were gone. Most of the area now has the plant communities that were there before the farmers started the intensification of agriculture.

To draw a mental line which determines whether the effort was successful or not the focus is placed on so-called target species. These are species who need particular circumstances to grow and are therefore seen as good indicators for the success of restoration measures. If they appear and establish themselves, the measures were successful. If not, the area still needs work (Rosenthal, 2003). The author uses four selection criteria to choose target species:

1. Species are chosen if they have a significantly positive deviation from expectation according to their distribution over vegetation community relevant to the situation.

2. They have to occur in the specific vegetation community that is the aim of the restoration area.
3. If the species is showing an overall decline in the landscape.
4. If it is listed as a Red List species, it is chosen regardless of the criteria mentioned above.

According to these criteria a list of target species can be constructed that suits a particular area or restoration goal.

This is the theory, but in practice it is a common problem that several target species are still missing, even after several years of management. Rosenthal (2000) found results from several restored plots indicating that target species who were deemed vulnerable, had a low invasion or immigration in general.
Quite some research has been done on the possible reasons why these species have such difficulty to reintroduce and establish themselves.
One of the first things that springs to mind is that the abiotic conditions are still not good enough for the species to occur. Fertilizer application from the past entered a very high amount of nutrients in the system and the decrease of these nutrients is very slow, giving the restoration of the target site a timescale of 8 to 10 years before the desired nutrient availability has been reached (Oomes 1990, Bakker and Olff 1993). The site may simply need more years under management to recreate the nutrient niche that target species need. According to Walker et al. (2004), it turns out that several of these species are so-called habitat specialists. They do well under less suitable conditions, but when there are plenty of nutrients available they are outcompeted by the generalists that already occupy the area. Once limitation of one or more nutrients is reached, the competition pressure is reduced and target species will be able to reestablish themselves.
Apart from the nutrients there are other abiotic factors that need to be restored. In close relation with the nutrient status are the moisture and acidity of the soil. A lowered groundwater table not only decreases the amount of water in the soil which prevents moisture-loving species to settle, it also has a disturbing effect on the chemical balance of cations and anions in the soil. This will often lead to a decrease in pH, especially when the influence of precipitation is increased. Raising the groundwater table in such cases may not be enough and extra measures will be needed to restore the former conditions(Van der Hoek and Heijmans, 2007).
The three abiotic factors: nutrients, moisture and acidity are closely related to eachother. They are also subject to many underlying causes that could affect their behaviour. For example a diminished upward seepage of base-rich groundwater through abstraction for drinking water, a brook whose path has been altered so the land around it is less flooded, a peaty soiltype which will mineralize and cause an increase in nutrients instead of a decreased availability, or leakage of nutrients from neighbouring fertilized arable fields. With a soil system that relies on balance and buffering it may be possible that some factors are not yet in order to create a suitable habitat for target species to occur.

If the abiotic conditions are right, then there could be a problem with the dispersal of the species. There are several ways in which a plant can disperse its seeds: anemochory (dispersal by wind), hydrochory(dispersal by water), zoochory (dispersal by animals, internally through the digestive system or externally by clinging to fur), myrmecochory (dispersal by ants) and ballistochory(dispersal by ballistic or 'explosive' means). Each of these ways have their own effectiveness, but most do not

disperse further then a few meters at best (Howe and Smallwood, 1982).This is illustrated in a study by Coulson et al. (2001), who measured the distance of several dispersal ways in a managed grassland. They did this for the species *Rhinanthus minor*, a wind-dispersed plant, and *Leucanthemum vulgare*, a plant without dispersal adaptations. *Rhinanthus minor* achieved the largest distance of around 4 meters, when it was helped along by hay-cutting. *Leucanthemum vulgare* however never came further than 1 meter. This is similar to Donath et al. (2003), who measured the dispersal distance of three other species that live in meadows: *Cirsium tuberosum* (wind-dispersed), *Carex tomentosa* (water-dispersed) and *Peucedanum officinale* (wind-dispersed). All were able to cover a maximum distance of over 10 metres (54, 19 and 12 metres respectively), but the median distance they managed was only 6 metres for *Cirsium tuberosum* and 1-4 metres for the other species.
Another possibility could be dispersal through time since resident plant communities build up seed banks in the soil. But in most cases this is not possible because the seeds do not remain viable for a long enough period.
This shows that, whether a plant produces alot of seeds or just a few, in most cases they are not likely to get very far from the parent. In case of restoration this can be a problem because populations that can act as a seed source are often far away or not present at all. There are some records that longer distances can be overcome, like *Cirsium tuberosum* mentioned above. Cain et al. plus citations therein (2000) report that zoochory, hydrochory and in some cases (like updrafts and storms) anemochory are best at dispersing seeds for long distances, and that maximum dispersal distances have been found of 1-20 km. But they also mention that not much is known about it, only that it happens once in a while. Adding to that is that dispersal can be hampered by strips of trees and shrubs that often separate fields because of their isolating effect (Donath et al., 2003).
All in all it could very well be that even though the abiotics are right, target species are failing to establish themselves simply because their seeds can not get there.

If the two factors mentioned above do not play a role, there could be a third point, which is the offer of so-called microsites or “gaps” in the vegetation. These are small patches of bare soil in otherwise closed vegetation and several species depend on them to successfully colonize an area (Poschlod & Biewer, citation therein, 2005). Gaps can serve a variety of purposes, of which the most important one might be to provide an increased chance of successful germination for seeds.
Goldberg and Werner (1983) investigated the effects of several gap sizes on the germination and establishment of two *Solidago* species. They found that germination of one species increased with gap size, while survival of seedlings increased with gap size for both species. This indicates that a dense sward of vegetation can negatively affect germination of seeds and survival of seedlings. This has been found by Gross and Werner (1982), where the colonizing abilities of four monocarpic plant species were related to ground cover. It turned out that relatively more seeds had germinated in gaps than in areas that were already occupied by other plants. One of the investigated species did not even emerge in the vegetation, all the germinated seeds were in areas with bare soil. Also the seedlings of all species survived better in open areas, two even required them since they did not survive in the vegetation. This shows how important gaps are for successful germination of seeds.
This is further illustrated when an area becomes limited in these open areas of soil. Poschlod and Biewer (2005) investigated whether restoration success of a fen was seed- and gap-limited. They found that harrowing the soil before application of seed

increased establishment of the species that were sown. The findings of Donath et al., (2007) are in agreement with this, as their experiment on species-poor grassland gave the same results: The amount of both species number and species cover increased significantly when the grassland was disturbed before application of plant material. Next to increasing the chance for establishment gaps can also be necessary for the survival of a species in an area. This goes mainly for species that have an annual life cycle, like *Bromus racemosus*. Since they do not build a persistent seed bank they need gaps for their seeds to germinate succesfully, else they will disappear from the area (Rosenthal, 2003). The same holds for the perennial *Lychnis flos-cuculi* (Biere, 1991).
Gaps also play a role in protection from predation. It appears that seedlings in meadows suffer quite severely of predation by slugs and snails (Overbeck et al., 2003). Rodents also play an important role, but they have a bigger influence in grasslands where the vegetation is taller and thus provides more cover (Hulme, 1994). According to Hitchmough (2003), who studied emergence and establishment of *Trollius europaeus*, losses to slug herbivory were significantly less in the mown treatment. Seeing as a mown piece of vegetation shares several characteristics with gaps; open, light and exposed, it indicates that gaps may protect seedlings from slug predation. The findings of the author point into this direction, as most seedlings survived in a gap of 100 mm diameter, but no significant differences were found between gap sizes. Also Hanley et al. (1996) reported no differences in herbivory between gap sizes. Unfortunately the authors did not test their gaps against controls, so the effect of gap size is unknown. All in all open areas may offer somewhat protection against herbivores, but whether it is indeed the case is still unclear.
Everything combined there are several reasons that could cause the lack of target species in an area: abiotic conditions that do not suit the niche of the species, dispersal limitation, or microsite limitation.

So a number of answers exist to the question of why they could be missing, but they aren't the same for each area. What I want to try and answer in this research project is why the target species are missing in the Anlooer Diepje area and how the situation can be handled to solve the problem. To answer my research question I will investigate three factors within this experiment. First I will check if the abiotic conditions of the site are at the desired amounts. Parameters are soil moisture content, pH, organic matter content, and availability of the nutrients nitrogen, phosphate and potassium. Second I will measure several seed characteristics of the target species used in the experiment and carry out a germination test to look at the dispersal potential. Lastly I will look at microsite limitation by studying the germination conditions and vegetation composition of the sites, which will show if the species have preferences for a certain environment.

Material and Methods:

Study site

The experiment is running in a part of the Drentsche Aa Nature Reserve, on several fields that lie between Gasteren and Schipborg(53'°N, 6.6'°E). It has a mean annual temperature of 8.5 – 9°C and a mean precipitation of 750-800 mm. A map of the area is included in Appendix I.

It is composed of 60 plots with a size of 0.81 m^2 divided evenly over four different vegetation communities , with three treatments in each plot. These treatments are: bare soil (a), removal of moss (b) and control (c). Each has five subplots of 0.01 m^2 per plot, making a total of 15 subplots per plot. Per vegetation community, three to five target species were sown in these plots in the autumn of 2007 (Table 1).

The four vegetation communities are spread over six fields: two fields each for vegetation communities A, B/C and D. The fields have been acquired by the State Forestry Commission in the time period 1968-1972. Since then they have been mown once a year between August and October.

Name:	A	B	C	D
Vegetation Community:	Calthion palustris	Calthion palustris	Juncion acutiflori	Caricetalia nigrae
Characteristic species:	*Caltha palustris* *Lotus pedunculatus* *Carex nigra*	*Rhinanthus angustifolium* *Anthoxanthum odoratum*	*Rhinanthus angustifolius* *Anthoxanthum odoratum* *Juncus acutiflorus*	*Carex nigra* *Luzula sp.* *Galium saxatile*
Fields:	A1 & A2	B/C1 & B/C2	B/C1 & B/C2	D1 & D2
Plots:	15	15	15	15
Sown Species:	*Cirsium oleraceum* *Menyanthes trifoliata* *Pedicularis palustris* *Eriophorum angustifolium* *Crepis paludosa*	*Briza media* *Parnassia palustris* *Bromus racemosus* *Phyteuma nigrum*	*Briza media* *Parnassia palustris* *Bromus racemosus* *Phyteuma nigrum* *Rhinanthus angustifolium*	*Calluna vulgaris* *Erica tetralix* *Succisa pratensis*

Table 1: Vegetation communities and their respective names. Note that there are two fields per community.

Abiotic conditions

Fieldwork was conducted between March and July 2008. Fifteen soil cores were taken along the edge of each plot using a soil corer with a 3 cm diameter. Each core was 15 cm deep, and split into parts of 0-5 cm and 5-15 cm without removing the vegetation. The upper and lower parts were pooled and stored separately in plastic bags to form two samples. Bags were kept in a 4°C climate chamber until analysis.

Green parts and roots of the soil samples were removed by hand, after which the soil was mixed. Subsamples were taken to determine moisture content and pH and the rest was dried in an aluminum bowl in a stove of 40°C. After drying they were ground into fine dust with a soil grinder (type MFC, KIKA Labortechnik, Janke & Kunkel, Germany) and stored in plastic bottles at room temperature.

Soil moisture content was determined using the protocol in Appendix IIa. Because of the amount of samples and the often high water content drying was extended to two days. Samples were weighed on a PG403-S deltarange® of Mettler Toledo.

pH was measured in pH H_2O and pH KCl according to the protocol in Appendix IIb. Instead of boiled aqua destillata standard demineralised water was used. pH was measured with a Sentron pH-meter, type Titan made by Argus. It was calibrated with the meters two-point calibration procedure using the indicated buffers. The tup of the

meter was rinsed with demineralised water and gently dried off with a paper towel after every measurement.
Organic matter content was determined with the protocol in AppendixIIc.

In addition to the standard procedures a new approach was used to determine the amount of moisture and nutrients in the soil. The soil samples were fed through a MPA Multi Purpose FT-NIR Analyzer of Bruker, which measures the reflection of the sample in near infra red. A sample container called 'rotation cell' was used together with the NIR protocols 'wet soil' and 'dried soil-105°C'.
Soil was placed in the cell, after the sample had been read the soil was carefully stirred. This was repeated twice to obtain a measurement in triplo. After each measurement the cell was cleaned and dried.

Seed traits
Of each of the 13 species two seed traits were measured according to the method listed in the LEDA traitbase Collecting and Measuring Standards. One hundred seeds of each species were counted and then weighed with a AT21 Comparator of Mettler Toledo. For the biggest seeds a cup of aluminum foil was made to serve as a container and was used for the species *Briza media, Bromus racemosus, Cirsium oleraceum, Crepis paludosa, Eriophorum angustifolius, Menyanthes trifoliata, Pedicularis palustris, Rhinanthus angustifolius* and *Succisa pratensis*. For the smaller seeds of *Erica tetralix, Calluna vulgaris, Parnassia palustris* and *Phyteuma nigrum* a smaller cup was made.
Next to seed weight, seed dimensions (length, width and height) were measured with a dissection microscope of Wild/Heerbrugg using an objective micrometer of Olympus with size 0.01 mm. For the bigger seeds listed above a magnification of 16x was used, for the smaller seeds this was 30x.

Germination test
To test the viability of the collected seeds a germination test was conducted in 2007. Fifty seeds of each species were divided evenly over five petridishes containing a bed of glass pearls or sand. These petridishes were placed in a growth chamber with a light regime of 12l:12d and temperatures of 25°C (light period) and 15°C(dark period). Seeds were watered regularly with demineralised water. The number of seedlings were counted and divided by the total to calculate the germination percentage.

Microsite limitation
From March to early May 2008 germination conditions were recorded. For each subplot the total cover, percentage bare ground, percentage moss and percentage springy turf moss (*Rhytidiadelphus squarrosus*) was taken. Also possible germinated seedlings of target species were noted.
In June and July 2008 vegetation recordings were taken of each subplot and in addition two to four extra subplots, each containing a seedling of a species, and a control. Parameters taken were total cover, percentage green, percentage bare ground, percentage litter and litter height, percentage moss and moss height and height of the vegetation. All recordings were done with a frame containing a grid of 10cm x 10cm squares, the same size as the subplots. Litter and moss height were measured with a ruler, vegetation height with a calibrated plastic pipe and a styrofoam circle. The circle was dropped over the pipe until it hit the vegetation, and the value it landed on was marked as the height.

Statistics
An ANOVA test was used to check for differences between treatments and T-tests were used to look between treatments as well as differences in data between fields. Results were analysed with the statistical program Statistica version 8.0.

Results:

Abiotic conditions

The parameters moisture and pH largely overlap between fields (Table 2), with the lowest values in the D fields and the highest in the A fields. Organic matter content also largely overlaps, but here the highest value is found in the D fields and the lowest in the A fields. When the amount of seedlings were plotted against the parameter values (graphs not shown), three of the four species seemed to prefer a smaller range of abiotic factors than was present in the fields. *Crepis* and *Pedicularis* had the most narrow range, followed by *Cirsium*, which distinguished itself from the other two by germinating and growing in a wider range of moisture. The other two factors are quite similar. *Succisa* had the widest range of them all, making full use of the field instead of only a narrow band. As it grows in the D-type vegetation, its requirements are different from the other three species, who grow in the A-type vegetation. In all four species, there were a few exceptions (graphs not shown), so the range given in Table 2 does not represent the boundary layers of these species, but only the most successful area found in this study.
The nutrient availability could not be tested as the results of the analysis were not available at the time of writing.

	A	B	C	D
vocht(%)	46.68 - 79.06	44.76 - 76.66	51.42 - 74.04	34.12 - 72.94
pH	5.74 - 6.81	4.44 - 6.6	4.51 - 6.5	3.88 - 4.32
humus(%)	6.68 - 28.61	13.56 - 34.19	13.64 - 28.74	10.30 - 52.63

	Crepis	Pedicularis	Cirsium
vocht(%)	70 - 80	70 - 80	45 - 80
pH	6.2 - 6.8	6.2 - 6.8	6.2 - 6.8
humus(%)	20 - 30	20 - 30	15 - 30

Succisa
35 - 73
3.9 - 4.3
10 - 52

Table 2: The abiotic ranges in which the most seedlings per species germinated together with the maximum and minimum values found for the four vegetation communities.

Dispersal potential

All species had high germination percentages except for *Phyteuma*, whose seed viability was moderate (Table 3). The high percentage of *Menyanthes* seems to contradict its poor performance in 2008, where its germination percentage was 10-15% at most (seeds were grown under the same conditions to produce seedlings for a later stage of the experiment, data not shown).

Species	% Total	Species	% Total
Menyanthes t.	96	*Cirsium o.*	80
Pedicularis p.	95	*Calluna v.*	78
Eriophorum a.	92	*Rhinanthus a.*	73
Bromus r.	89	*Parnassia p.*	67
Erica t.	87	*Crepis p.*	62
Succisa p.	86	*Phyteuma n.*	47
Briza m.	80		

Table 3: Germination percentages of the target species used in the sowing experiment.

Only six out of the 13 species germinated in the experiment (Figure 1). *Rhinanthus angustifolius* was the most succesfull, with 606 seedlings spread over different plots. *Succisa pratensis* and *Pedicularis palustris* were the second most succesfull, with 289 and 66 seedlings, respectively, third were *Cirsium oleraceum* and *Crepis paludosa* with 21 and 29 seedlings respectively. *Bromus racemosus* also germinated, but only three seedlings were counted. The species are distributed over three vegetation communities. *Succisa* was the only one in the D-type, *Rhinanthus* and *Bromus* in the B/C-type, and the remaining three emerged in the A-type vegetation (Table 1).

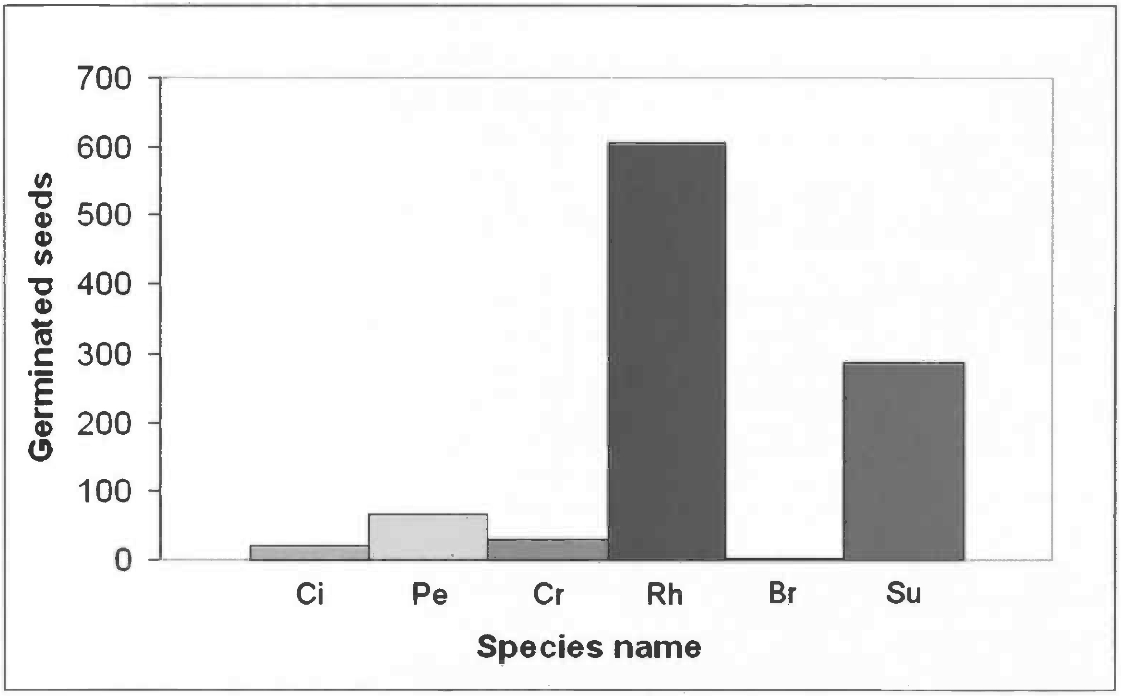

Figure 1: Amount of germinated seeds per species. From left to right: Ci: *Cirsium o.*, Pe: *Pedicularis p.*, Cr: *Crepis p.*, Rh: *Rhinanthus a.*, Br: *Bromus r.* and Su: *Succisa p.*. Species with zero emergence have been left out.

All six species have a relatively high seed weight(Figure 2), but no significant relationship has been found between seed weight and amount of seedlings. *Bromus* had the highest weight with 705 mg/100 seeds, yet had the lowest germination. *Menyanthes* is the second highest with 292 mg/100 seeds, but it did not produce seedlings at all. However *Rhinanthus* (251 mg/100 seeds) did very well, as did the other four species that were mentioned earlier. Their weight was mostly high too (200-160 mg/100 seeds), with the exception of *Crepis*, which had a seed weight of 57 mg/100 seeds.

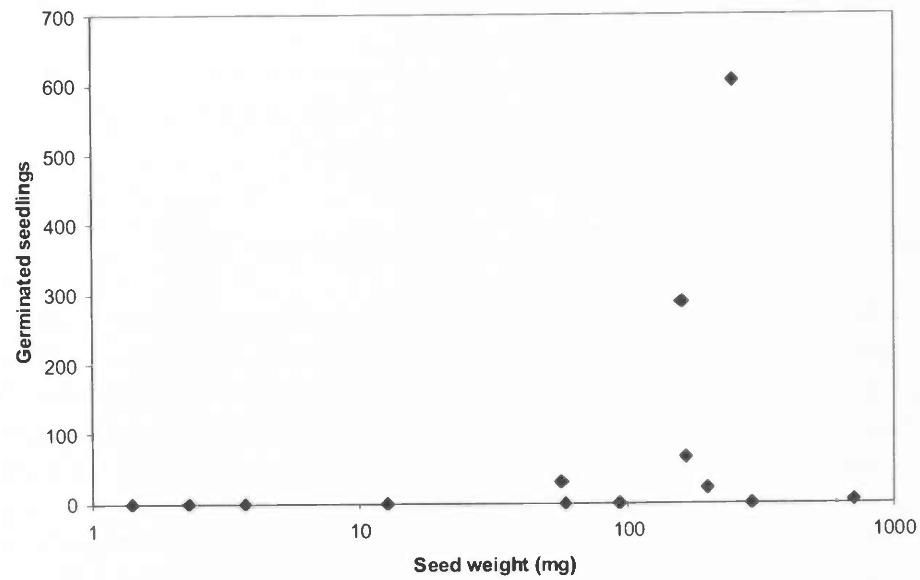

Figure 2: Seed weight in relation to seedling amount.

The seeds of the germinated species are large in size compared to the others (Table 4). This is mostly true for the width of the seed, but not so much for the length and height, were the differences are less clear.

Species	width (mm)	length (mm)	height (mm)
Briza media	2,9	2,2	2
Bromus racemosus	9,9	1,9	1,5
Calluna vulgaris	1	0,7	0,6
Cirsium oleraceum	3,7	1,4	0,8
Crepis paludosa	4,1	0,6	0,5
Erica tetralix	0,7	0,6	0,6
Menyanthes trifoliata	2,5	2	1,6
Parnassia palustris	2,2	1	0,6
Pedicularis palustris	2,7	1,3	1
Phyteuma nigrum	2,2	1,1	0,8
Rhinanthus angustifolius	4,3	3	0,6
Succisa pratensis	4,6	1,2	1,1

Table 4: Seed dimensions of the target species used in the experiment. Values are averages of five seeds chosen at random.

Of the six species that germinated only four were studied in detail. As *Bromus* died during the summer it could not be used, while *Rhinanthus* was already present at the area and has therefore been excluded from the analysis. These two species were the only ones that were found on the fields for the B/C community, so the data for these fields was left unexamined. Only the fields for the A and D community were studied in detail.

Microsite limitation: Treatments

Of the four species that survived only *Crepis* showed a significant difference in germination rate between treatments (Figure 3.2, ANOVA, P=0.039). Further t-tests proved that significantly (t-test, P=0.022) more seedlings emerged at bare soil than at the raked and control treatments, which did not differ from eachother(t-test, P=0.161). *Cirsium* almost had significantly more seedlings in the bare soil treatment (Figure 3.3, ANOVA, P=0.0501), and *Succisa* and *Pedicularis* had statistically equal seedling amounts at all treatments (Figure 3.1 and 3.4, ANOVA,P=0.957 and P=0.849).

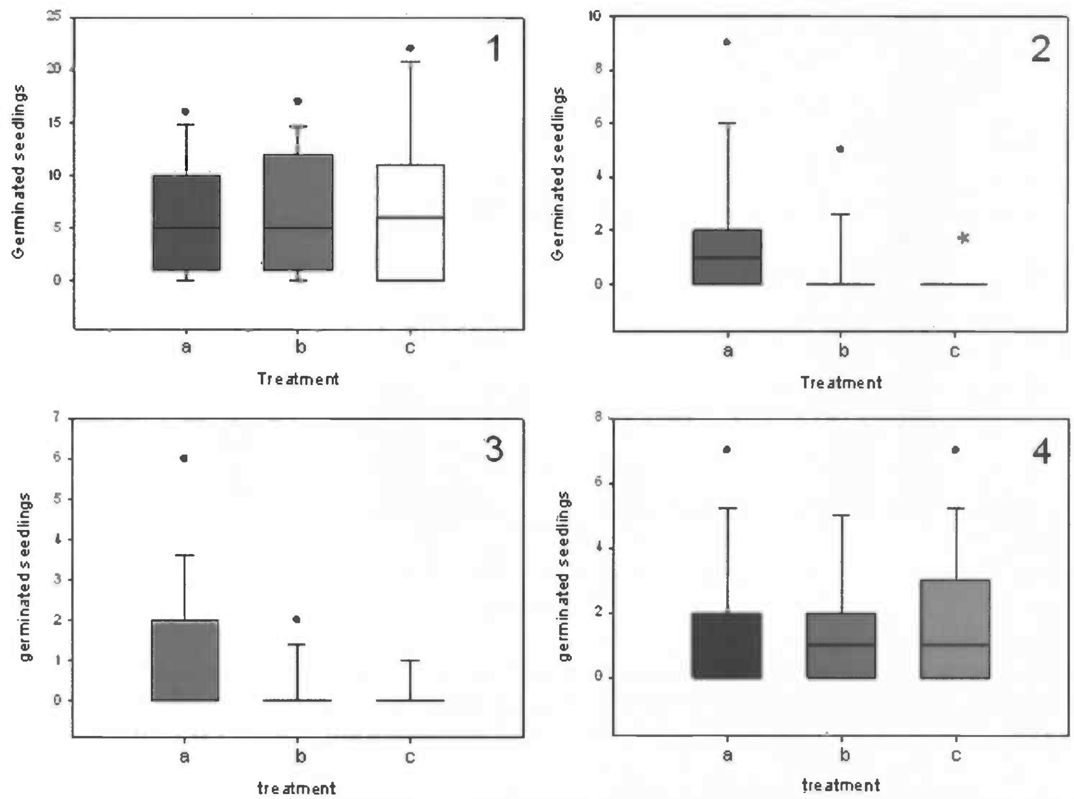

Figure 3: Seedling numbers of the four germinating species against the treatment. 1: *Succisa p.*, 2: *Crepis p.*, 3: *Cirsium o.* and 4: *Pedicularis p.*. Treatments are a: bare ground, b: raked and c: control. Dots represent outliers. The asterisk indicates that the respective column is significantly different from the others.

Microsite limitation: Fields

There were several differences between the fields of each plant community and the germinated seeds of the species (Figure 4). *Crepis* had significantly (T-test, P=0.014) more seedlings in the second field than in the first, while for *Cirsium* this was the opposite(T-test, P=0.022). *Succisa* was also different, with more seedlings in the first field than in the second (T-test, P=0.025). *Pedicularis* was the only one who was the same in both fields(T-test, P=0.176).

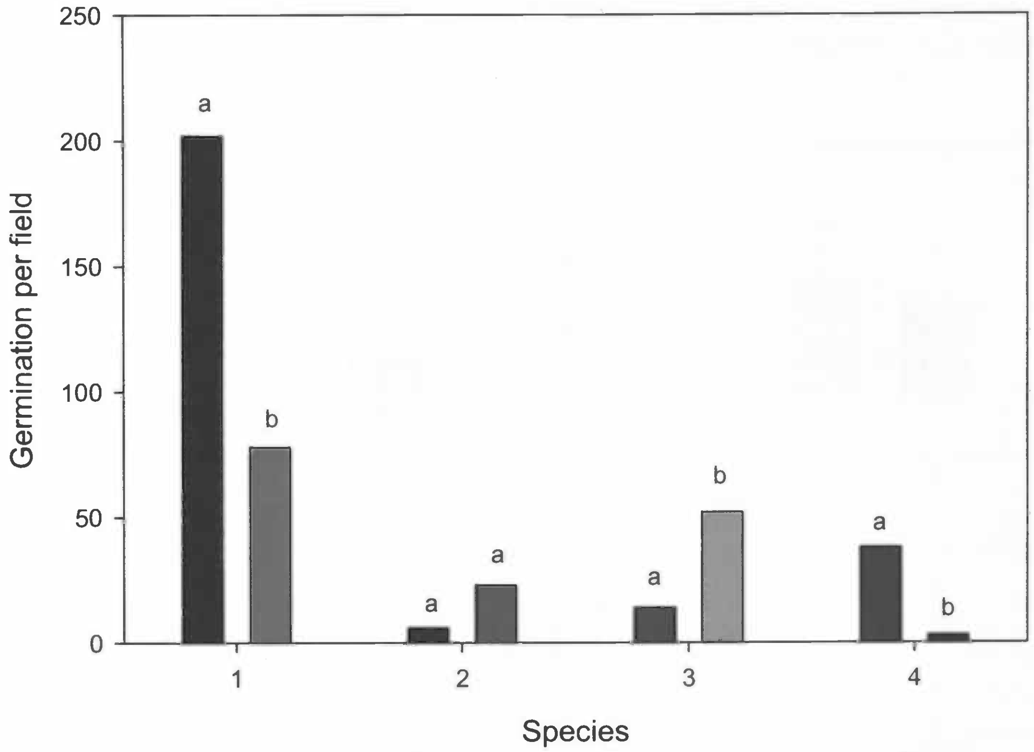

Figure 4: Germinated seedlings of the species per field. Columns indicate the two separate fields of each vegetation community. Numbers on the x-axis represent the different species: 1 = *Succisa*, 2= *Pedicularis*, 3= *Crepis*, 4= *Cirsium*. Significant differences are indicated by different characters.

The germination conditions of the first A-type field were less mossy and contained less bare soil than the second field (T-test, $P<0.001$ for the moss and $P=0.010$ for the bare ground). For the fields of the D-type vegetation only the moss differed in that the first field had less moss than the second one (T-test, $P=0.006$).

As for the vegetation recordings there is a similar pattern. The A-type fields only differed in their moss content, the first field had less moss than the second (T-test, $P=0.010$). The D-type fields were different in both moss and green, where the first field had less moss but more green plants than the second field (T-test, $P<0.001$ for both moss and green).

Individual species response

In the case of *Crepis*, because of the differing fields more t-tests were done to find out where the difference on treatment level was located. It turned out that treatment only had an effect on *Crepis* in the first field ($P=0.032$, difference between bare ground and control treatment), but not in the second field($P=0.068$ and higher). However, when focusing on the first field, there are only differences between treatments bare ground and raked/control, with no distinctions between raked and control. This is true for both the germination conditions and the vegetation recordings. Also there were no significant differences found in the abiotic factors organic matter, pH and moisture. For *Cirsium* no significant relation has been found, but the trend suggests that more seedlings are found when a low amount of moss is present (Figure 5).

Pedicularis did not differ between treatments nor between fields. Also no effects of factors in the germination conditions or vegetation recordings was seen.

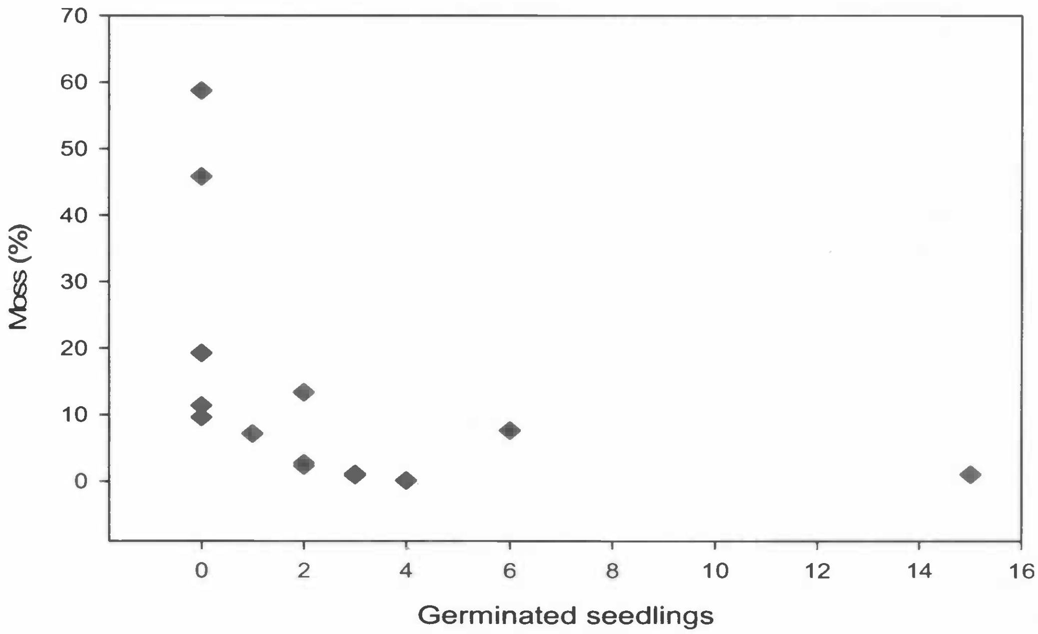

Figure 5: *Cirsium* seedlings against the percentage of moss in the plots. Moss cover is taken from the vegetation recordings. Note that most seedlings have sprouted in the areas with less than 20 percent moss.

For *Succisa* some relationships have been found. The species produced more seedlings in the first field than in the second (T-test, $P=0.025$) (Figure 6a). When set against the amount of bare soil in the plots a negative relationship was found for the first field, but not the second (Figure 6b). When comparing these results with the vegetation parameters green plants and moss, it shows that there is a larger percentage of green plants and a lower percentage of moss at the first field (Figure 6c and d).

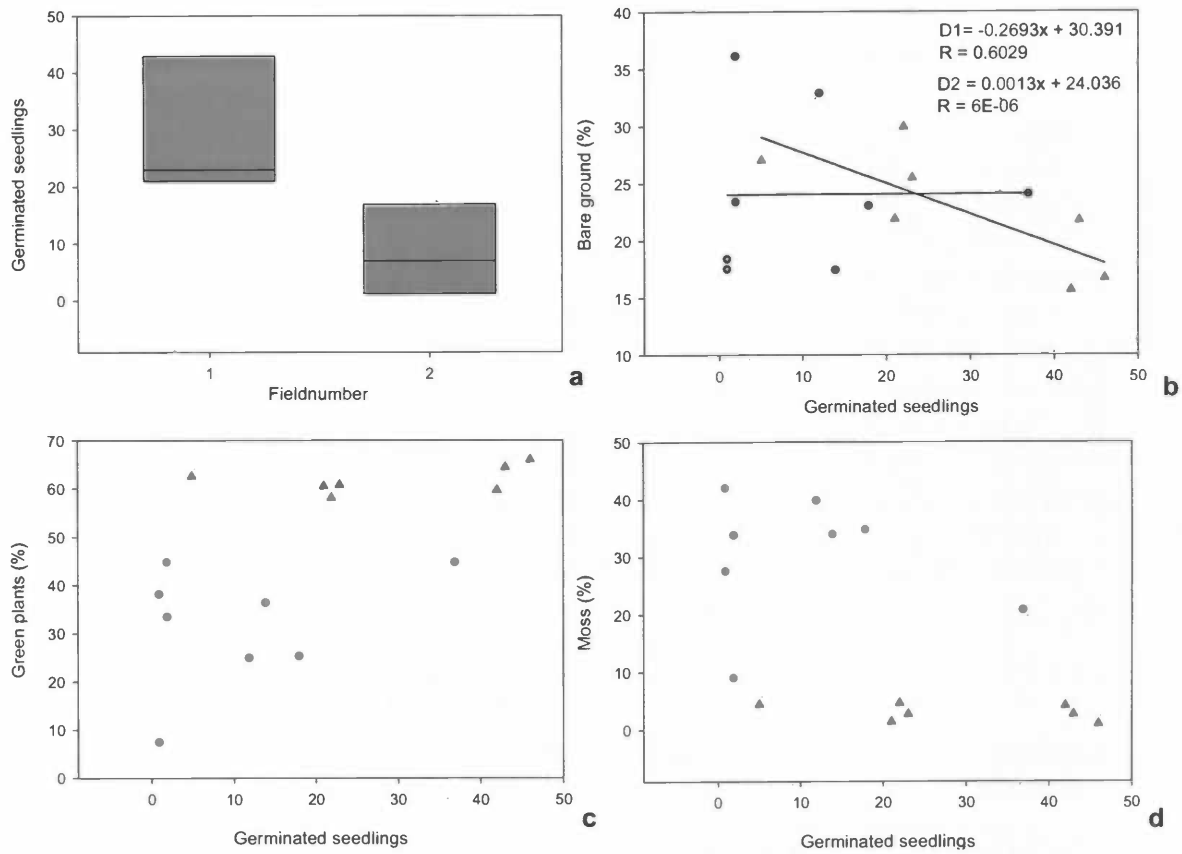

Figure 6: *Succisa* seedlings against a)field, b)bare soil, c)green plants and d)moss. Colour codes per graph: Blue triangles represent the first field (D1), red circles the second field (D2).

Discussion:

Abiotic conditions

Crepis, *Cirsium* and *Pedicularis* seemed to show a preference for the topmost values of the analyzed abiotic parameters. It could be that these responses give an indication for the abiotic quality of the A-community fields, but without the specific habitat requirements of these species to compare our dataset with, it is not possible to give any definite answers. It is possible that these three species also presented a reaction to the nutrient concentrations of the soil. However, this data was not yet available during the writing of this report so this possibility remains unexplored.

Dispersal potential

Of the 13 species that were selected to be sown only six germinated and only five survived the summer. The reasons why so many species failed to produce seedlings are unknown since there were factors present that are beyond our control. For starters the site had a dry spring which caused the soil to become dry, perhaps too dry for the other species to germinate. Another possibility is that they did germinate, but the dry weather killed the fragile embryos. Most of them prefer a moist environment (Soortenbank.nl). Also there were problems at both fields were the A-type vegetation grows. Several of the plots were submerged by water for several weeks, which could have flushed seeds away (especially those species who did not produce seedlings), who otherwise may have germinated there.

What makes it so strange is that the germination percentages of the used seeds were good and raising the seedlings afterwards in a greenhouse went well too. This shows that the problem does not lie with the seeds themselves. One species was an exception though. *Menyanthes* germinated very poorly in 2008 and the few seedlings that did come up did not grow well either. Hewett (1964) points out that it is difficult to grow an adult from a seed as it needs very distinct circumstances to do so. The author describes that a seedling will grow if water is allowed to reach inside and if enough of the seed coat is removed to allow the embryo to grow. Especially this last requirement seems to be important. He also mentions that the germinated seedlings from experiments always died. In this study only one seedling survived to grow into an adult form, and despite the lack of competition and plenty of water and nutrients, it is small. Once it has recruited an area though, vegetative reproduction takes over and it is able to establish itself.

When taking the seed weight into account it seems as if there is a threshold for successful germination. With the exception of *Crepis* only seeds with a weight above 159 mg produced seedlings(Figure 2). Heavier seeds have more reserves at their disposal to overcome small changes in their environment, which makes them more successful at surviving to a seedling. It could also be argued that, since the seeds of the others were very light, they failed to penetrate the dense vegetation canopy that was present on the sites, but this does not explain why no seedlings were found in the a treatment, where all vegetation was removed.

Most of the seedlings made it to the end of the summer except *Bromus*. This species did germinate but was killed when a lot of rain fell in a short time and a second cold period came over the land. The fact that it did grow indicates that the sites are suitable for *Bromus* to grow, but its dependence on gaps could not be proved due to the unfortunate weather of 2008 (Rosenthal, 2003). Since no source populations exist nearby, this species may suffer from dispersal limitation. This theory needs to be proved directly though by witnessing a *Bromus* population taking hold in the area, which unfortunately could not be done at this time.

Microsite limitation

According to the statistical tests it doesn't really matter where the seeds of *Succisa* or *Pedicularis* end up; they will germinate about everywhere. Less *Succisa* may have germinated in the first field, but the numbers where still satisfying when compared to the rest. The negative relationship found between seedling number and bare ground can be explained by *Succisa* taking advantage of the open soil to sprout seedlings, because the high amount of green plants in the plots affects its germination. Since the percentage of green plants is lower in the second field, the patches of bare ground are less important, leading to no trend line. Despite the higher amount of moss in the second field, it does not seem to be hindered by it, as the germination numbers did not decline or stay at the low level of the first field, but went up. So for *Succisa* and *Pedicularis* the circumstances were good: the abiotics were in their preferred range, and both were not microsite sensitive as they germinated in all treatments. My conclusion for them is that they were dispersal-limited. Both species produce heavy seeds and have no special adaptations. Also no source populations are located nearby and the fields are surrounded by trees and shrubs, making dispersal difficult.

For *Crepis* and *Cirsium* it's a bit more complicated. The first proved to be barely significant between treatments, while the other was on the edge of being so. There were no differences between the germination conditions nor the vegetation recordings on treatment level to explain this result. It is because of the small sample size (not many germinations) that the results are dubious. If the sample size would have been bigger, it might have gone both ways: If they would become insignificant, it would point into the direction that they were dispersal-limited, like *Succisa* and *Pedicularis*, since they would germinate equally well in all treatments. However, if they were to become significant, it could indicate that next to being dispersal-limited, they are sensitive for microsite-limitation, which could be the cause for their rare-ness in the area. In case of the latter, the results also point in the direction that *Cirsium* may prefer an environment that contains a low amount of moss. *Crepis* may turn out to be only dispersal-limited, as the results between fields show that it did better in the field that had the higher moss content. However, since no germination differences have been found between the raked and control treatments the theories about the last two species could not be tested.

Conclusion:

For the two species *Succisa* and *Pedicularis* the major problem is dispersal-limitation. Both species were able to germinate in their respective vegetation communities and survive the summer. The same holds true for *Crepis* and *Cirsium*, as they also germinated and survived in their community, but their results hint at microsite limitation as well. Lastly for *Bromus*, it seems to be dispersal as well, but due to unfavourable weather this remains to be tested.
Combining these finds, and taking the landscaping of the area into account (fields are surrounded by strips of trees and shrubs), it maybe necessary to reintroduce these species by hand, as it will be very difficult for them to reach the area by natural dispersion.

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Appendix I: Aerial view of the study area

Courtesy of Google Maps.

The coloured dots indicate the locations of the studied fields. Green is vegetation type A, cyan is vegetation type B/C and red is vegetation type D. Types are described in the introduction.

Appendix IIa: Soil moisture content protocol

Vocht

Veldvochtige grond

Werkwijze

Stel de balans op nul.

Weeg het aluminium doosje leeg (met deksel).

(Gebruik nummers die in Al doosje zijn geslagen)

Al doosje voor ½ vullen met veldvochtige grond en weer wegen met deksel.

Gedurende 1 nacht drogen bij 105°C (deksel onder bakje).

Na drogen Al doosjes sluiten en laten afkoelen in exsiccator tot kamertemperatuur.

Dan terug wegen (balans nul).

Grond controleren of het droog is.

Berekening

Al doosje + natte grond	= a gr	Al doosje + natte grond	= a gr
Al doosje + stoofdr. „	= b gr	Al doosje	= c gr
Vocht	=(a-b) gr	Natte grond	=(a-c) gr

$$\frac{\text{grammen vocht x 100\%}}{\text{grammen natte grond}} = \text{vocht in \%}$$

Appendix IIb: pH protocol

Zuurgraad in grond

PH H_2O PH KCL

Weeg ongeveer 15 gr. goed gemengde veldvochtige grond in pH buizen en voeg ongeveer 20 ml. uitgekookt aqua destillata toe

Buis met rubber stop afsluiten en de grond door krachtig schudden met het a.d. vermengen.

Tot de volgende dag laten staan.

Dan eerst pH meter ijken (buffer 7 en 4).

Vervolgens pH H_2O meten.

De pH buis eerst krachtig schudden en hierna direkt de pH van de suspensie meten (electrode 2-2.5 cm. in de suspensie laten zakken.)

pH KCl van grond suspensie als volgt.

Na het meten van pH water 2.5 ml. KCL 1 N toevoegen aan de pH buis . Afsluiten en de suspensie krachtig schudden. Daarna pH opnieuw meten.

De gevonden waarde is de pH KCL .

Reagentia;

Buffer oplossingen: pH 4.00 en pH 7.00 Titrisol ampullen.

Kalium chloride 1 N : 74.5 gr. KCL in 1 liter a.d. op pH brengen pH 7 met HCL of KOH 1N.

Uitgekookt water : A.D. 10 min. koken.

Afkoelen en geen lucht laten toetreden.

Slechts enkele uren houdbaar.

Appendix IIc: Organic Content protocol

Weeg gloeischaaltje vooraf in 3 decimalen (4) nauwkeurig.
Balans vooraf op nul.
Nummers gebruiken die op schaaltjes staan.
19.000 gr. Droge grond afwegen in gloeischaaltje.
Gloeischaaltjes in gloeioven, gloeien 4 uur bij 500°C.
Na afkoelen tot ± 150°C in exsiccator, afsluiten en verder tot kamertemperatuur laten afkoelen.
Wegen (balans op nul stellen).
Gloeischaaltjes terug in gloeioven, gloeien 4 uur bij 850°C.
Na afkoelen tot ± 150°C in exsiccator, afsluiten en verder tot kamertemperatuur laten afkoelen.
Wegen (balans op nul stellen).

Berekening:
Gloeischaaltje + droge grond = a gr. (105°C)
" = b gr.
droge grond = (a-b) gr.

Gloeischaaltje + droge grond = a gr. (105°C)
" + gegloeide grond = c gr. (500°C)
Humus = (a-c) gr.

%Humus = (Humus/droge grond) x 100%.
Humus% = Gloeiverlies% - CO_2%.

Gloeischaaltje + droge grond = a gr. (105°C)
" + gegloeide grond = d gr. (850°C)
Gloeiverlies in gr. = (a-d) gr.

%Gloeiverlies = (gloeiverlies/droge grond) x 100%.

%CO_2 = %Gloeiverlies - %Humus.

Opmerking: Als de pH van de grond lager is dan pH = 6 (zuurder) dan is %CO_2 = 0.