Effect of Nitrate on the Germination of a Soil Seed Bank in a Norway Spruce Forest in Relation to Liming and Clear-felling

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Thesis for Master of Science in Biology

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Abstract

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Three different kinds of humus were tested in order to examine the effect of nitrate on the germination of the seed bank of a Norway spruce experimental forest at Farabol in the south-west of Sweden: 15 years old stand without soil treatment, 15 years old stand with lime treatment (in total 6000 kg/ha limestone distributed over 12 years), and 40 years old stand without soil treatment.

Using a split-plot design, three solutions were applied to the soil (0.01 M KCl, 0.01 M KNO_3, and 0.0001 M KNO_3) during five days. Thus, nitrate concentration in the humus samples reached values that are considered as very high and moderately high in forest soils, respectively. The samples were placed in greenhouse conditions. Seedling emergence was counted, and species were identified. Germination was contrasted with cover values of preceeding research conducted at the same experimental site.

Nitrate applications had no stimulating effect on the germination of the seed bank in the greenhouse experiment, and for *Calluna vulgaris* germination decreased with high nitrate concentrations. Previous lime application increased the number of germinated species and the number of seedlings. It was also concluded that the effect of lime was maintained for a long time after the application, and that others unknown factors than nitrate in limed soil may stimulate germination, or that liming had increased the seed viability of a large number of species.

Introduction

Many plant species that are established on forest sites following clear-felling are recruited from the soil seed bank (Granström, 1986). Furthermore, recruitment from seeds is determined by both the availability of seeds and the availability of sites suitable for germination. There are many factors that can influence the availability of good sites. Some studies indicate that both acidification and forest liming (as a countermeasure against acidification) affect the recruitment of plants from seeds. Hallbäcken and Zhang (1998) found that liming of a Norway spruce forest at Farabol in south-east Sweden resulted in a few more vascular species. Olsson and Kellner (2002), who studied recruitment of plants at the same site following clear-felling, observed that recruitment from seeds was significantly greater in the lime treatment than in control or acidified treatments. One principal hypothesis is that different treatments and managements involve chemical changes in the soil environment that affect the establishment of plants on clear-fellings, in particular changes that influence the breaking of seed dormancy. According to Olsson and Kellner (2002), pH, Ca, Mg, nitrate, or the nitrate-to-ammonium ratio are candidate factors that could explain the higher germination on limed plots at Farabol. However, there is little evidence from other studies that pH or the levels of metal cations in the environment affect germination (Roberts & Benjamin, 1979; Hendricks & Taylorson, 1972), whereas nitrate is known to be the most common soil chemical that promotes germination. In addition, application of lime on forest soils tends to promote nitrification (Bengtsson, 1996; Bäckman, 2003).

Influence of nitrate on germination

Seed germination depends on the presence of appropriate environmental conditions. Each species has its own characteristic set of germination requirements. In most plant communities, regeneration from seed is dependent upon the occurrence of gaps in the vegetation (Fenner, 1985).

The most common soil chemical that is known to promote germination is the nitrate ion. In the same way that germination responds to other environmental factors, the response to nitrate may be attributed to an adaptative value that may indicate gaps in the vegetation (Fenner, 1985; Pons ,1989; Baskin & Baskin, 1998).

In laboratory experiments, potassium nitrate in moderate concentrations (about 10 mM) has been used in order to check the effect on germination. The effect of nitrate as a promoter of dormancy breakage and germination has been investigated in several laboratory experiments (Steinbauer & Grigsby, 1957; Popay & Roberts, 1970; Hendricks & Taylorson, 1974; Slade & Causton, 1979; Schimpf & Palmblad, 1980; Singh & Amritphale, 1992; Gul & Weber, 1998; Henig-Sever *et al.*, 2000). Although the physiological action is not perfectly known still today, the results show that nitrate has a positive effect on breaking dormancy and germination in several species.

However, stimulation by nitrate has been found to occur in combinations with other environmental conditions such as light and/or fluctuating temperatures (Fenner, 1985), showing complex interactions between different environmental factors (Henson, 1970; Vincent & Roberts, 1977, 1979; Bostock, 1978; Roberts & Benjamin, 1979; Saini *et al*., 1987; Karssen *et al*., 1988; Boewmeester & Karssen, 1989).

Laboratory experiments showing promotion of weed seed germination and emergence after application of nitrate to soil are very rare. In Håkansson's (1979) experiments, *Trifolium arvense* was the only species that showed a tendency of increased emergence as a result of nitrogen application, either if nitrogen was applied as a nitrate salt or in a complete fertilizer, and this promotive influence was only observed in an organic soil. In a loamy soil used, the final emergence was both delayed and decreased where fertilizer had been applied.

The aim of the present study was to test the hypothesis that nitrate stimulates germination of the soil seed bank at Farabol, and to compare germination of the seed bank with previous observations of vegetation cover in control and limed plots.

Material and methods

Study site

The experimental site was located in Farabol, southern Sweden (56 26’N, 14 35’E, 150 m a. s. l. , Table 1) in a experimental forest managed by SkogForsk (Swedish Forest Research Institute).

The soil type is orthic podsol and the humus is mor. The original experiment started in 1976, when five treatments were applied in a randomized block design (three blocks, plot size 30m x30m or 35m x 35m): (1) control, (2) lime (6000 kg limestone ha-1), (3) urea (600 kg N ha-1), (4) elementary sulphur (600 and 1200 kg S ha-1), and (5) urea with lower sulphur dose. The total doses for all treatments were applied over a 12-year period (1976-1984). Dolomite and sulphur were applied annually and urea on three occasions (1976, 1980, 1984).

In 1992 the Norway spruce *Picea abies* (L.) Karst. stand was clearfelled (70 years old), and all logging residues were manually removed to reduce mechanical disturbance. Four years old Norway spruce were planted.

In the present study, three original treatments were considered for the germination experiment: Clear-cutting control (CC) (without original treatment), Liming (L), and Forest control (FC) with 40 years old Norway spruce (no soil treatment). The FC areas were selected in adjacent, undisturbed mature stands of Norway spruce, one per block, where mosses and *Deschampsia flexuosa* dominated the ground vegetation.

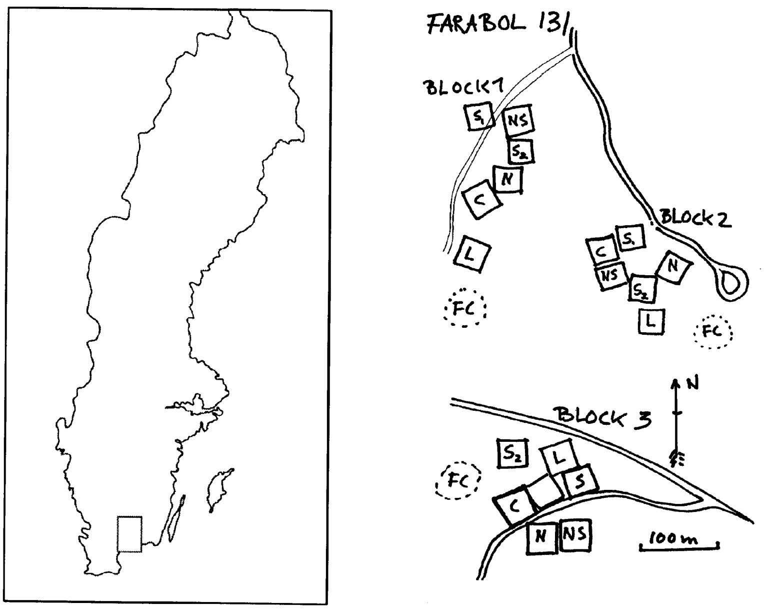

Figure 1. Location of the experimental site Farabol 131 and the original treatments. L = lime, C = control, FC = Forest control (not clear-felled).

Soil sampling

On 24th and 25th September 2003, ten soil samples were taken in each plot by a steel core (10 cm diameter) along transects in the plot. Soil cores were maintained in plastic bags in the dark and at 5°C until the germination experiment was conducted.

The depth of the humus layer was measured. In FC samples, two layers of the organic horizon were distinguished and separated: 1) the F layer, located the top and composed basically of *Dechampsia flexuosa* litters and rhizomes, and 2) humus layer in the bottom.

The ground vegetation and litter were discarded and the humus samples were sifted through a 5 mm mesh net and pooled plot-wise.

The average depths of the humus layers are shown in Table 1.

Soil chemical and physical analyses

Soil pH (H_2O) was determined in mixtures of distilled water (50 ml) and approximately 10 g fresh soil in plastic bottles. The mixtures were shaken for 2 hours, allowed to air-exchange overnight at room temperature and pH was analyzed by pH detector.

The exchangeable nitrate content of the humus was determined at the start of the experiment and after 4 weeks. Approximately 10 g fresh soil was weighed and 100 ml 1M KCl solution was added. After shaking for 1 hour, the solution was filtered (Munktell Filter AB type IF) and kept at -20° C until the determination of nitrate by the FIA system.

Table 1. Plot mean values of humus layer thickness (and SD), pH and extractable nitrate nitrogen concentrations in the humus of Farabol 2003. C = control, L = lime, FT = Forest Top, FB = Forest Bottom. Numbers assigned to samples indicate block number.

Sample	Humus thickness (mm)	SD	pH	NO3-N µg/gDM
C1	27.0	3.28	3.64	0.613
C2	29.6	6.24	3.44	0.446
C3	20.9	4.97	3.65	0.711
L1	30.2	6.28	5.14	0.614
L2	25.6	6.46	5.16	0.602
L3	24.4	8.38	5.05	0.633
F1T	25.1	9.88	3.78	0.532
F1B	39.3	16.53	3.79	0.798
F2T	20.7	5.29	4.45	0.739
F2B	26.3	6.72	3.67	0.363
F3T	17.3	6.03	3.65	0.629
F3B	24.5	5.57	3.97	0.580

Table 2. The range of nitrate concentrations in runoff and soil waters in various environments, and concentrations used for experimental breaking of seed dormancy. Extracted from: (I) Sing & Amritphale, 1992; (II) Bouwmeester & Karssen, 1992; (III) Bouwmeester & Karssen, 1993; (IV) Alonso & Peretti, 1995; (V) Gul & Weber, 1997; (VI) Henig-Server, 2000; (VII) Lieffering *et al*., 1996; (VIII) Bäckman, 2003.

M	mg NO3-N/l	Soil type	Surface waters	References
0.01	140		Extremely high levels	I, II, III, V, VII
0.001	14	Forest (high levels) soils	Very high levels	I, IV
0.0001	1.4	Forest (moderate levels) soils	High levels	VIII
0.00001	0.14	Forest (low levels) soils	Low levels	VI

Water content and dry matter was determined using approximately 10 g of fresh humus and weight determination after 24 hours at 105ºC.

The pH values, and nitrate contents before solution addition are shown in Table 1. Only in the Lime plots are thr pH value higher than 5. The lowest pH values were observed for the control plots, however control and forest plots have similar values.

Soil nitrate concentrations at the start of the experiment were low and very similar in all samples, being close to the detection limit, and concordant with the values considered representative in temperate Norway spruce forests (Johnson & Lindberg, 1992).

Germination experiments

The experiment was conducted in a greenhouse, starting on 5th November 2003. The greenhouse was of “Venlo” type and equipped with microclimate control with radiation heating system, zenith and lateral windows. Artificial light was supplied from 7 to 20 h with Osram Powerstar HQI-E 400 W/D lamps, assuring minimum light intensity of 120 mol m^{-2} s^{-1} at tray level. Temperature was dynamically controlled so that values were daily ranging between 13 and 21 ºC.

The homogenized humus was spread in 7x7x12 cm plastic pots with drainage holes. The pots were filled with (from bottom to top): 2 cm layer of “Leca” expanded clay marbles to avoid anaerobic root environment, 2-3 cm layer of vermiculite and 2 cm layer of the homogenized humus. The humus soil layer thickness followed instructions from Baskin & Baskin (1998).

The pots (10 per plot) were placed in plastic trays, one for each experimental plot, soil layer and nitrate treatment. The trays were placed in random order on the greenhouse tables. Then, three different solutions were added in the trays: 0.01 M KCl (no nitrate), 0.0001 M KNO3 (low nitrate dose), and 0.01 M KNO3 (high nitrate dose) using p.a. quality of chemicals. The low and high concentrations of nitrate were chosen based on nitrate concentrations that have demonstrated effect on dormancy breaking (Singh & Amritphale, 1992; Bouwmeester & Karssen, 1992; Bouwmeester &

Karssen, 1993; Alonso & Peretti, 1995; Gul & Weber, 1997; Henig-Sever *et al*., 2000, Lieffering *et al*., 1996). "High nitrate" dose is considered an extremely high level rarely observed in forests soils. The "low nitrate" dose represents levels of nitrate in soil water often found after disturbance (Table 2).

The nitrate solutions were applied in the trays for 5 days by allowing a raised water table of the solutions in the trays. The humus samples thus absorbed the solutions at field capacity. Subsequently, the solutions were withdrawn. Then, soil pots were maintained close to field capacity by adding deionised sprinkling water regularly from the top.

The nitrate content in the soil was determined again four weeks after solution addition in order to check if the concentration was on target values. For this purpose, a small amount of humus was taken carefully from each pot and pooled plot-wise (approximately 10 g).

The nitrate concentration per unit dry weight after application of the solution is shown in Table 3, indicating that the correct application of the nitrate solutions was made. According to the classification shown in Table 2, the "high nitrate" values obtained are found in the forest soils with high nitrate and the values after clear-cutting (Bäckman, 2003); "low nitrate" and "no-nitrate" values are below the values considered as low concentrations in forest soils. Furthermore, nitrification may have started in the soil, since the values had increased slightly in the "no-nitrate" pots from the start.

At least once a week, seedling emergence was counted and reported until 16th December, six weeks after the start. Then, a liming treatment was added to half of the pots in a split-plot order. To reach pH values of 6, we added 0.3 g $CaCO_3$/16g dry height soil (Tryggve Persson, SLU, pers. comm.). To avoid contamination of lime between pots in the same tray, we rearranged the pots in the trays. Observation of seedling emergence continued for another 6 weeks.

After the end of the experiment, trays were kept in greenhouse conditions, watering sporadically with nutrient solution, in order to allow seedling growth and later species identification. In some cases, seedlings were transplanted into bigger plastic plots with commercial soil substrate.

Table 3. Extractable nitrate concentration in humus four weeks after application of solutions

Sample	NO3-N µg/gDM
Control	1.95
Control low	12.59
Control high	1 748.88
Liming	1.33
Liming low	5,07
Liming high	708.34
FT	2.60
FT low	7.55
FT high	1 674.43
FB	3.07
FB low	12.22
FB high	2 040.39

Results

Total emergence of seedlings in the main plot and nitrate treatments are shown in Table 4. Complete results are given in Appendix 1.

Period for germination

The time required for germination was different for all species but was unaffected by the different field and nitrate treatments. *Agrostis capillaris*, *Cerastium fontanum*, *Moehringia trinervia*, *Schopularia nodosa* germinated mainly within the first 14 days. *Veronica officinalis* germinated between 14 and 21 days. *Calluna vulgaris* and *Juncus* spp mainly germinated after 28 days. The curves of germination time for *C. vulgaris* and *C. pilulifera* are shown in Fig. 2, indicating that neither the nitrate nor the soil treatments influenced the time required for germination.

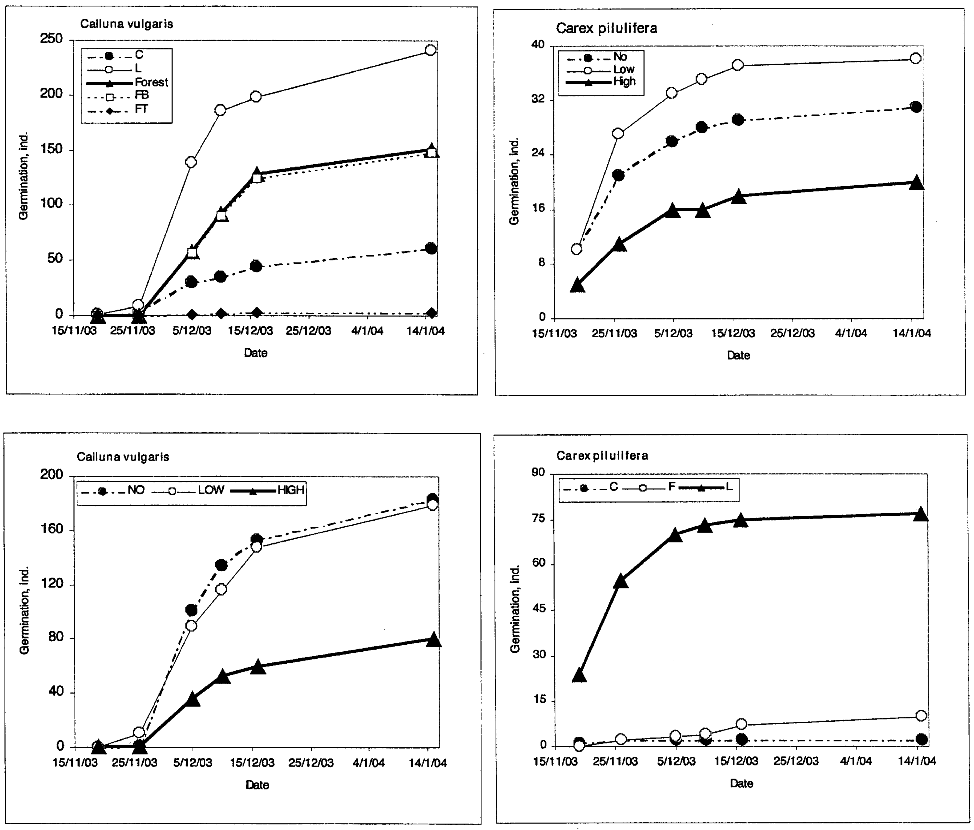

Figure 2. Germination (ind. / treatment) over time of *Calluna vulgaris* and *Carex pilulifera* in the different treatments (original soil treatment or nitrate application, respectively). C: clear-cutting control, L: lime, FB: forest control bottom, FT: forest control top, NO: no nitrate, LOW: low nitrate, HIGH: high nitrate.

Table 4. Total number of seedlings emergence by species

	Forest bottom NO	Forest bottom LOW	Forest bottom HIGH	Forest top NO	Forest top LOW	Forest top HIGH	Clear Cutting NO	Clear Cutting LOW	Clear Cutting HIGH	Liming NO	Liming LOW	Liming HIGH	Total
Agrostis capillaris										10	19	16	45
Arabidopsis thaliana										2	5	5	12
Betula spp.	1		1	4	5	9			1			1	22
Calluna vulgaris	61	67	20	1	2		27	24	10	94	96	50	452
Carex pilulifera	3	5	2				1	1		27	32	18	89
Cerastium fontanum										42	36	33	111
Cirsium sp												1	1
Deschampsia flexuosa				1				1	1	3	6	6	18
Epilobium montanum						1				1	1	1	4
Galium saxatile											1		1
Hypericum sp.										3	2	2	7
Juncus sp.				1						7	13	4	25
Luzula pilosa					1					1		2	4
Moehringia trinervia										10	9	15	34
Potentilla erecta										3	2	2	7
Rubus idaeus	1	2	2			2		1			2	2	12
Rumux acetosella										3		3	6
Schropularia nodosa										5	7	7	19
Senecio sylvaticum				1			1			18	16	15	51
Veronica officinalis										37	26	30	93

Table 5. Number of species emerged at the end of the germination experiments

Split Plot (Nitrate)	Main Plot (Soil)	Number of species
No nitrate		18
	Control	3
	Forest Bottom	4
	Forest Top	5
	Lime	16
Low nitrate		19
	Control	3
	Forest Bottom	3
	Forest Top	3
	Lime	16
High nitrate		19
	Control	2
	Forest Bottom	4
	Forest Top	3
	Lime	19

Number of species and individuals

Nitrate treatments had no effect on the number of species (Table 5). However, there was one single observation of *Cirsium palustre* in high nitrate+lime treatment (Table 4).

There were differences between main plots (field treatments) on the number of species. Lime plots had a markedly greater number of species than FC and CC.

Table 6 shows the total number of seedlings that had emerged at the end of the germination experiments. The p-values of split-plot analyses are in given in Table 7. The “high nitrate” treatment tended to result in a general reduction in the total seedling number (p=0.056). Both in control plots and in forest (top + bottom) the total number of seedlings in the high nitrate concentration was less than half of the number of seedlings compared with low and no nitrate. The same tendency can be detected in the lime plots, although it was not so pronounced (20% less). The negative effect of nitrate on *C. vulgaris* emergence was the most pronounced for all the treatments, and it was the only one that showed a significant effect of nitrate (p=0.0263). *Betula* spp., and *C. fontanum* also showed this trend (p=0.225 and p=0.273). On the other hand, the germination of *Rubus idaeus* tended to be improved by nitrate, but the effect was not significant (p=0.284). Also, *M. trinervia* tended to have more individuals in the high nitrate plots (p=0.281).

There were significant differences in the number of species and individuals between the original field treatments. Limed plots had a markedly greater total number of individuals (p=0.003). *V. officinalis* and *Betula* spp. had a significantly higher number of seedlings in limed soil (p=0.009 and 0.04). The effect on *C. pilulifera* was very close to significant. In general, limed soil had more herb (p=0.01) and graminoid (p=0.001) seedlings.

Calcium carbonate

No effect was detected after the application of calcium carbonate the 6th week on the number of species or number of individuals. Because no additional seedlings emerged, no statistical test was made.

Table 6. Number of seedlings emerged at the end of the germination experiments

Main Plot (Soil)	Split Plot (Nitrate)	Seedlings	
CONTROL		68	
	No nitrate		29
	Low nitrate		27
	High nitrate		12
FOREST BOTTOM+TOP		193	
	No nitrate		74
	Low nitrate		82
	High nitrate		37
LIME		752	
	No nitrate		286
	Low nitrate		273
	High nitrate		213

Table 7. Results of split-plot analyses of germination, showing p-values for tests of effects on the number of seedlings for different species (only the most abundant) or plant form

	Effect of field treatment (main plot)	Effect of nitrate (split-plot)	Interaction: field treatment x nitrate
Calluna vulgaris	0.190	0.026	0.685
Agrostis capilaris	0.404	0.444	0.461
Arabidopsis thaliana	0.444	0.444	0.461
Betula spp.	0.046	0.255	0.265
Carex pilulifera	0.051	0.655	0.899
Cerastium fontanum	0.162	0.273	0.217
Deschampsia flexuosa	0.238	0.848	0.737
Epilobium montanum	0.588	0.444	0.461
Moehringia trinervia	0.418	0.281	0.228
Rubus idaeus	0.160	0.284	0.126
Veronica officinalis	0.008	0.550	0.615
All species	0.003	0.056	0.859
Herbs	0.011	0.654	0.754
Graminoids	<0.001	0.534	0.728

Table 8. Field cover data of ground vegetation in previous experiments for lime and control plots in Farabol. Extracted from Hallbäcken & Zhang (1998), Olsson & Kellner (2002) and B. Olsson (unpublished data)

	Control					**Lime**				
	Cov. 91	Freq 91	Cov. 93	Cov. 94	Cov. 95	Cov. 91	Freq. 91	Cov. 93	Cov. 94	Cov. 95
Agrostis capilaris			0,01					0,04	0,03	0,64
Arabidopsis										
Betula spp.				0,03	0,04			0,01	0,07	0,51
Calluna vulgaris	0,1	5	0,06	0,1	0,18	*0.1*	-2 to 5%	0,06	0,08	0,03
Carex pilulifera			0,08	0,19	0,01			0,06	0,24	0,01
Cerastium fontanum									0,29	2,22
Cirsium arvense									0,02	0,03
Cirsium palustre								0,01		0,04
Deschampsia flexuosa	18	62	22,9	32,23	56,88	*20*	+10%	35,13	57,71	69,38
Epilobium montanum										0,02
Galium saxatile									0,01	0,01
Hypericum maculatum								0,01		0,02
Juncus spp.			0,01	0,01	0,01				0,02	0,02
Luzula pilosa	0,2	5				*0.2*	+10%	0,37	0,54	0,88
Moehringia trinervia								0,03	0,18	0,2
Potentilla erecta								0,02	0,03	0,35
Rubus idaeus			0,01	0,01	0,02			0,16	0,63	3,53
Rumex acetosella								0,02	0,48	3,29
Schropularia nodosum									0,01	0,01
Senecio sylvatica			0,03	0,03	0,03			2,21	7	0,75
Vaccinum myrtillus	0,1	3								
Veronica officinalis								0,73	2,14	3,18

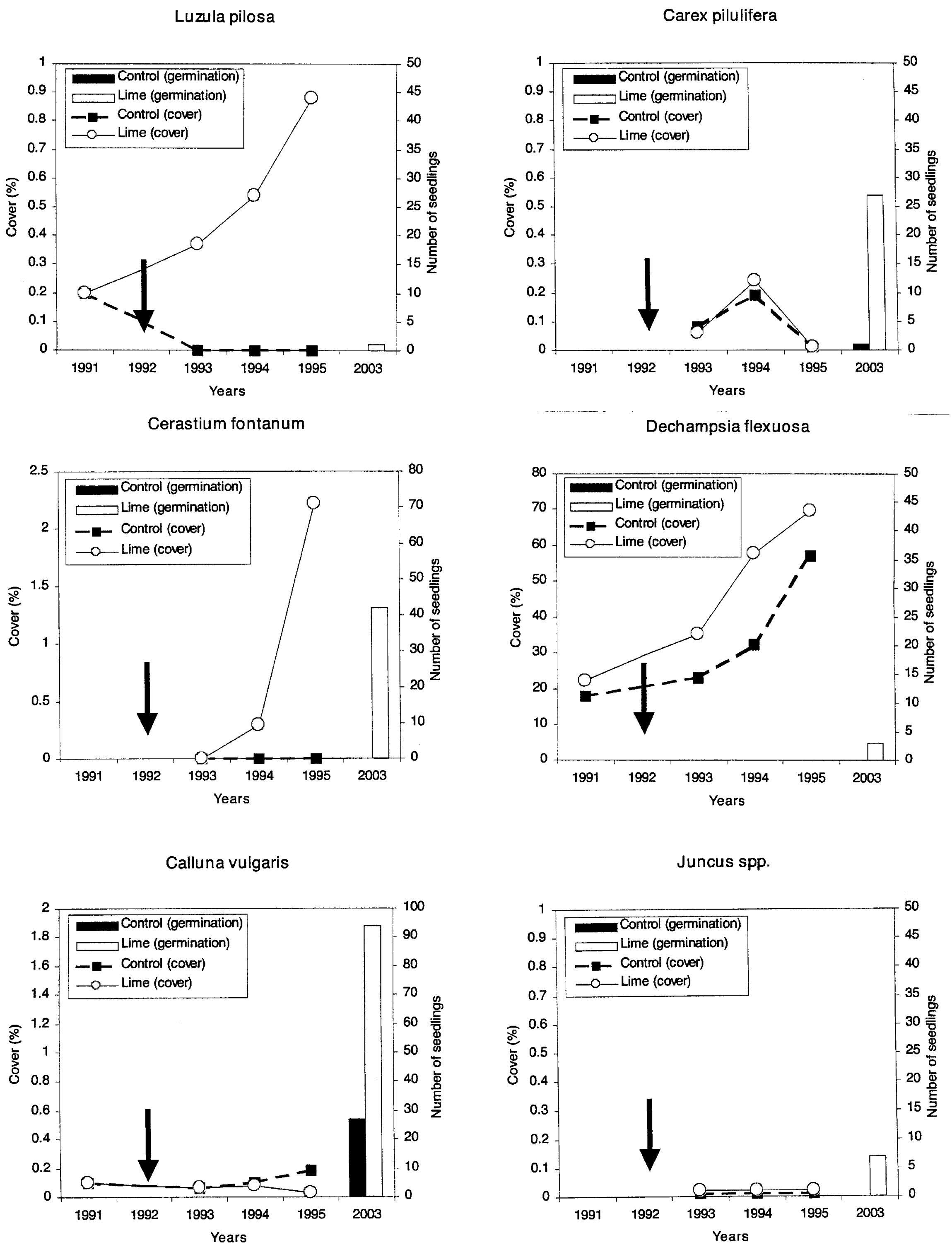

Figure 3. Field cover and germination of *Luzula pilosa, Cerastium fontanum, Calluna vulgaris, Carex pilulifera, Deschampsia flexuosa* and *Juncus* spp. for lime and control plots in Farabol. Cover data extracted from Hallbäcken & Zhang (1998), Olsson & Kellner, (2002) and B. Olsson (unpublished data). The arrow shows year of clear-cutting.

Discussion

Effect of nitrate

For many years, nitrate has been known as a key substance causing dormancy breakage and germination. But the results of the present study give no support to the hypothesis that nitrate should be important for the establishment of forest vegetation through this mechanism. High nitrate concentrations did not promote a higher number of species, and decreased the number of seedlings except for *R. idaeus*. According to Jobidon (1993), calcium nitrate fertilization causes an increased rate of germination of *R. idaeus*. The repetition of the treatment in two consecutive years intensified the effect. This fact could explain the weak effect of the nitrate trend in our experiment, where nitrate was applied only once.

Calluna vulgaris is the only species that had significantly fewer seedlings with higher nitrate levels. *C. vulgaris* is typically abundant in open low-nutrient environments where nitrate normally does not occur. In addition, our results suggest that high nitrate levels may reduce the germination of *C. vulgaris*.

However, the results of this study must be carefully interpreted for two major reasons: 1) There are few laboratory experiments with application of nitrate to soil, and they have given contradictory results. For example, Håkansson (1979) found opposite results in different kind of soils, 2) The germination process is very complicated, with multiple interrelations between different environmental factors. For example, *Veronica montana* showed maximum germination after 21 days at 18° C with potassium nitrate + light + stratification treatment, but no germination with potassium nitrate or light or stratification alone, or light + stratification, or nitrate + stratification, or nitrate + light (Slade & Causton, 1979). There are other experiments that reveal interaction of factors, environmental influences (e.g. Henson, 1970; Popay & Roberts, 1970; Schimpf & Palmblad, 1980; Hurtt & Taylorson, 1986; Singh & Amritphale, 1992; Alonso & Peretti, 1995). Thus, it cannot be excluded that nitrate will have a positive effect on germination, but only in combinations of environmental conditions that were not met in the present study.

Effect of field treatments

The differences detected in number of species and individuals between the original field treatments can be explained in two different ways: 1) the actual seed bank of the soil were different; and 2) the different germination conditions.

It is obvious that differences in the seed bank have developed in recent years because the ground vegetation has been different in the lime and the control plots after clear-felling (Kirkham & Kent, 1997) as showed by the study by Olsson & Kellner (2002). Thus, it cannot be directly concluded that lime has increased the germination of more species and individuals.

Vegetation development after clear-cutting

Table 8 summarizes field cover data results in previous experiments for lime and control plots. Species like *Epilobium montanum, Luzula pilosa, M. trinervia, Potentilla erecta, Rumex acetosella, S. nodosa, Agrostis capillaris, C. fontanum* germinated only in limed plots, and they were also established only in limed plots after clear-cutting. For some of them as *E. montanum, S. nodosa,* and *C. fontanum*, higher pH levels in the lime plots could explain it, since these species prefer less acid soils (Grime *et al.*, 1990).

Conditions

C. vulgaris showed very low cover in control and limed plots (field observations, Table 8), both before and after clear-cutting, indicating that for this species no significant difference in seed bank between clear-cut limed and clear-cut control soils had developed after clear-felling. However, the seedling emergence in our experiments is particularly high in limed soils. Although the cover is very similar along the years in the different soils, the germination is three times higher in lime plots than in control plots, although soil pH of limed plots was higher than typical sites for this species (Grime *et al.*, 1990). *C. pilulifera* followed the same trend, that was even stronger: the cover was of the same order for several years in both treatments, but the germination in limed pots was almost significantly greater. *D. flexuosa* and *Juncus* can also be included in this group.

Thus, those findings indicate that the conditions in lime plots improved the germination, or alternatively, that these conditions had improved the seed viability.

Arabidopsis thaliana and *Hypericum* germinated in limed plots whereas no presence was reported from previous field studies. In general, *Arabidopsis* is confined to soils with higher pH and the seeds are spread by the wind, which might explain why this species only occurred in limed pots.

Other considerations

It is not easy to estimate the age of the seed bank. In the forest, most seedlings of *C. vulgaris* germinated in the bottom of the humus layer, indicating that the seed bank was fairly old, probably originating from a period when the felled spruce stand was established. Although we did not separate different layers in Lime and Control soils, the high germination with low cover values, supports the idea of an old seed bank for *C. vulgaris*. This species is known to maintain long-term seed banks (Granström, 1987).

Finally, I suggest that lime application may result in conditions that affect not only seed germination, but also the viability of seeds. Furthermore, germination response to nitrate may depend on the environmental history of the seeds, which might explain, for instance, the different intensity response of *C. vulgaris* individuals germinating in control, forest and limed plots in relation to nitrate concentration.

More experimental research should be conducted in this field. I propose, for example, 1) to make new experiments using nitrate applications on soils combined with various ambient conditions (light, temperature regime, soil pre-treatment, pH and others); 2) to test the germination of known seeds of different origin using different soil and nitrate treatments.

Conclusions

1. The hypothesis that nitrate stimulated germination of dormant seeds in forest soils was not supported.

2. High nitrate doses had rather a negative effect on germination of *Calluna vulgaris.*

3. Previous lime applications have promoted an increase in the number of species and total number of seedlings, and these effects are maintained long after the application.

4. It is possible that other unknown factors than nitrate in limed soil may have stimulated germination. Alternatively, liming may have increased seed viability for a large number of species.

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Appendix 1.

Records of germinated plants in combinations of different soil (field treatments) and nitrate treatments .

Soil type/treatmen	Nitrate treatment	Block	Date	Agrostis capillaris	Arabodopsis thaliana	Betula spp.	Calluna vulgaris	Carex spp.	Cerastium fontanum	Cirsium palustre	Deschampsia flexuosa	Epilobium montanum	Galium saxatile	Hypericum maculatum	Juncus spp.	Luzula pilosa	Moehringia trinervia	Potentilla erecta	Rubus idaeus	Rumex acetosella	Scrophularia nodosum	Senecio sylvaticus	Veronica officinalis
C	No	1	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03				3																
			10 Dec 03																				
			16 Dec 03				2																
			15 Jan 04				2																
		Sum Block 1					7																
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04				3																
		Sum Block 2					3																
		3	19 Nov 03					1														1	
			26 Nov 03																				
			5 Dec 03				9																
			10 Dec 03				2																
			16 Dec 03				3																
			15 Jan 04				3																
		Sum Block 3					17	1														1	
	No nitrate Sum						27	1														1	
	Low	1	19 Nov 03																				
			26 Nov 03								1												
			5 Dec 03				2																
			10 Dec 03																				
			16 Dec 03				1																
			15 Jan 04				3																
		Sum Block 1					6				1												
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04				1																
		Sum Block 2					1																
		3	19 Nov 03																				
			26 Nov 03				3	1											1				
			5 Dec 03				8																
			10 Dec 03				2																
			16 Dec 03				2																
			15 Jan 04				2																
		Sum Block 3					17	1											1				
	Sum low nitrate						24	1			1								1				
	high	1	19 Nov 03			1																	
			26 Nov 03																				
			5 Dec 03				2																
			10 Dec 03																				
			16 Dec 03				1																
			15 Jan 04				1																
		Sum Block 1				1	4																
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum Block 2																					
		3	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03				3				1												
			10 Dec 03				1																
			16 Dec 03																				
			15 Jan 04				2																
		Sum Block 3					6				1												
	Sum high nitrate					1	10				1												
Sum of Clear-cut control						1	61	2			2								1			1	

Soil type/treatment	Nitrate treatment	Block	Date	Agrostis capillaris	Arabodopsis thaliana	Betula spp.	Calluna vulgaris	Carex spp.	Cerastium fontanum	Cirsium palustre	Deschampsia flexuosa	Epilobium montanum	Galium saxatile	Hypericum maculatum	Juncus spp.	Luzula pilosa	Moehringia trinervia	Potentilla erecta	Rubus idaeus	Rumex acetosella	Scrophularia nodosum	Senecio sylvaticus	Veronica officinalis
FB	No	1	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03				2																
			15 Jan 04																				
		Sum of Block 1					2																
		2	19 Nov 03																				
			26 Nov 03					1															
			5 Dec 03				37																
			10 Dec 03				4																
			16 Dec 03				1																
			15 Jan 04				4																
		Sum of block 2					46	1															
		3	19 Nov 03																1				
			26 Nov 03			1		1															
			5 Dec 03				1																
			10 Dec 03				6																
			16 Dec 03				4																
			15 Jan 04				2	1															
		Sum of block 3				1	13	2											1				
	Sum of no nitrate					1	61	3											1				
	Low	1	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03				1																
			16 Dec 03				2																
			15 Jan 04				3																
		Sum of Block 1					6																
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03				16	1															
			10 Dec 03				11																
			16 Dec 03				13																
			15 Jan 04				7	1															
		Sum of block 2					47	2															
		3	19 Nov 03																				
			26 Nov 03																2				
			5 Dec 03				2																
			10 Dec 03				3	1															
			16 Dec 03				8	2															
			15 Jan 04				1																
		Sum of block 3					14	3											2				
	Sum of low nitrate						67	5											2				
	high	1	19 Nov 03																				
			26 Nov 03																1				
			5 Dec 03				1																
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04				2																
		Sum of Block 1					3												1				
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03				2																
			16 Dec 03				1	1															
			15 Jan 04				4																
		Sum of block 2					7	1															
		3	19 Nov 03																				
			26 Nov 03			1													1				
			5 Dec 03																				
			10 Dec 03				7																
			16 Dec 03				3																
			15 Jan 04					1															
		Sum of block 3				1	10	1											1				
	Sum of high nitrate					1	20	2											2				
Sum of Forest bottom humus						2	148	10											5				

Soil type/treatmer	Nitrate treatment	Block	Date	Agrostis capillaris	Arabodopsis thaliana	Betula spp.	Calluna vulgaris	Carex spp.	Cerastium fontanum	Cirsium palustre	Deschampsia flexuosa	Epilobium montanum	Galium saxatile	Hypericum maculatum	Juncus spp.	Luzula pilosa	Moehringia trinervia	Potentilla erecta	Rubus idaeus	Rumex acetosella	Scrophularia nodosum	Senecio sylvaticus	Veronica officinalis
F1	No	1	19 Nov 03																				
			26 Nov 03				2																
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03				1																
			15 Jan 04													1							
		Sum of Block 1					3									1							
		2	19 Nov 03				1																1
			26 Nov 03									1											
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum of block 2					1					1											1
		3	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03					1															
			15 Jan 04																				
		Sum of block 3						1															
	Sum of no nitrate						4	1				1				1							1
	Low	1	19 Nov 03				2																
			26 Nov 03				1																
			5 Dec 03																				
			10 Dec 03					1															
			16 Dec 03																				
			15 Jan 04																				
		Sum of Block 1					3	1															
		2	19 Nov 03																				
			26 Nov 03																				
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum of block 2																					
		3	19 Nov 03				1																
			26 Nov 03				1										1						
			5 Dec 03					1															
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum of block 3					2	1									1						
	Sum of low nitare						5	2									1						
	high	1	19 Nov 03				2																
			26 Nov 03				1																
			5 Dec 03				2																
			10 Dec 03				1																
			16 Dec 03																				
			15 Jan 04																				
		Sum of Block 1					6																
		2	19 Nov 03				2													1			
			26 Nov 03				1																
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum of block 2					3													1			
		3	19 Nov 03										1										
			26 Nov 03																	1			
			5 Dec 03																				
			10 Dec 03																				
			16 Dec 03																				
			15 Jan 04																				
		Sum of block 3											1							1			
	Sum of high nitrate						9						1							2			
Sum of Forest top humus							18	3				1	1			1	1			2			1

Soil type/treatmer	Nitrate treatment	Block	Date	Agrostis capillaris	Arabodopsis thaliana	Betula spp.	Calluna vulgaris	Carex spp.	Cerastium fontanum	Cirsium palustre	Deschampsia flexuosa	Epilobium montanum	Galium saxatile	Hypericum maculatum	Juncus spp.	Luzula pilosa	Moehringia trinervia	Potentilla erecta	Rubus idaeus	Rumex acetosella	Scrophularia nodosum	Senecio sylvaticus	Veronica officinalis
Lime	No	1	19 Nov 03	10														1				2	2
	Sum of no nitrate			10	2		94	27	42		3	1		3	7	1	10	3		3	5	18	37
	Low	1	19 Nov 03	16	1			2	1				1									5	5
			26 Nov 03	1	1		3	3			1				1			1					1
			5 Dec 03	2			18								2								2
			10 Dec 03				1								1								
			16 Dec 03		1																		1
			15 Jan 04		2		5								1							1	
		Sum of Block 1		19	5		27	5	1		1		1		5			1				6	9
		2	19 Nov 03					3	21			1										6	3
			26 Nov 03					2	1									1					3
			5 Dec 03				12	2	2						1								1
			10 Dec 03				9				1												2
			16 Dec 03				3																
			15 Jan 04				5																
		Sum of block 2					29	7	24		1	1			1			1				6	9
		3	19 Nov 03					5	11		2			2			5				5	4	5
			26 Nov 03				4	11							1		3		2		2		2
			5 Dec 03				20	3			1				2		1						1
			10 Dec 03				9	1							1								
			16 Dec 03				2								2								
			15 Jan 04				5				1				1								
		Sum of block 3					40	20	11		4			2	7		9		2		7	4	8
	Sum of low nitrate			19	5		96	32	36		6	1	1	2	13		9	2	2		7	16	26
	high	1	19 Nov 03	11	2	1			1													1	6
			26 Nov 03	1														1					2
			5 Dec 03	2	2		6								1								
			10 Dec 03		1		2																
			16 Dec 03				1																
			15 Jan 04				4	1															1
		Sum of Block 1		14	5	1	13	1	1						1			1				1	9
		2	19 Nov 03	1				5	23	1		1		1			1	1		2		10	7
			26 Nov 03					5								2						1	10
			5 Dec 03				7	4							1								
			10 Dec 03				2																
			16 Dec 03				1	1															1
			15 Jan 04				3																
		Sum of block 2		1			13	15	23	1		1		1	1	2	1	1		2		11	18
		3	19 Nov 03				1		9								14			1	7	3	3
			26 Nov 03					1			3			1	1								
			5 Dec 03				16	1			3				1				2				
			10 Dec 03	1			3																
			16 Dec 03																				
			15 Jan 04				4																
		Sum of block 3		1			24	2	9		6			1	2		14		2	1	7	3	3
	Sum of high nitrate			16	5	1	50	18	33	1	6	1		2	4	2	15	2	2	3	7	15	30
Sum of lime / clear-felling				45	12	1	240	77	111	1	15	3	1	7	24	3	34	7	4	6	19	49	93
Total sum				45	12	22	452	89	111	1	18	4	1	7	25	4	34	7	12	6	19	51	93