

Natural versus artificial cold-stratification: which one allows better estimates of soil seed banks in a forest ecosystem?

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Seedling emergence techniques provide an estimate of viable seeds in the soil seed bank based on germination of seeds under favourable germination conditions. The present contribution is aimed at studying whether artificial stratification in a cold chamber is capable of revealing more viable seeds in soil samples compared with natural cold-stratification in the soil through a winter period in order to estimate density and composition of soil seed bank in temperate forest ecosystem. A total of 384 soil samples were collected in December and June from permanently marked plots in a forest ecosystem from central Belgium. December samples were artificially stratified (AS) by undergoing a cold-moist stratification, before laying them in the greenhouse. June samples were put in a greenhouse for direct germination of seeds which were naturally stratified (NS). Emerging seedlings were identified and counted during 12 months. A total of 6960 seedlings emerged in the AS samples and 14,928 seedlings in the NS samples, corresponding to 2719 and 5831 seeds/m² respectively. A total of 27 species emerged from the AS samples and 19 species from the NS samples. Based on our data in a forest ecosystem with temperate humid climate on loessic materials, artificial stratification allowed the emergence of a larger number of species from the soil seed bank, but natural stratification improved estimates of the seed bank density. We therefore recommend the use of both methods to optimize the estimation of both species number and density in soil seed banks.

Keywords: Cold-stratification, forest ecosystem, seedling emergence, soil seed banks.

A SOIL seed bank begins at seed dispersal and ends with germination or death of the seed¹, is a reserve of mature viable seeds located on the soil surface or buried in the soil² and provides a memory of past vegetation and repre-

sents the structure of future populations³. The soil seed banks also play an important role in annual plant populations by buffering them from temporal variation⁴ and by avoiding demographic effects of reproductive failure⁵. Many such seeds buried in the soil bank are dormant and will not germinate until the dormancy has been broken. In the wild, seed dormancy is overcome naturally by the seed spending enough time at fluctuating temperatures in the soil through a winter period. In this way the seed undergoes a natural form of cold-stratification. This process can be simulated artificially by putting soil samples in a cold and moist environment for a period of time found to be usually sufficient for the species in question. By subjecting the seeds to this kind of pre-treatment, we attempt to provide them with the effect that nature would have had on the seeds if they had been left to their natural course. However, by applying the pre-treatment in a controlled environment such as a cold chamber, the process is possibly accelerated and factors often detrimental to the survival of a seed are controlled and diminished. Therefore, cold-stratification prior to germination generally results in speedier, more complete germination over a wide range of incubation temperatures, and for a given incubation condition, cold-stratification reduces the variance in germination rate among individual seeds in a population⁶. It is however not clear whether the artificial form of cold-stratification is really capable of revealing more viable seeds in soil samples compared with natural cold-stratification. The present experiment aims at exploring this question.

The research was conducted in the Sonian Forest, south of Brussels (50°47'N; 4°26'E), which is mainly composed of beech (*Fagus sylvatica* L.), oak (*Quercus robur* L. and *Q. petraea* (Mattuschka) Lieblein) and pine (*Pinus sylvestris* L. and *P. nigra* Arnold). The prevailing soil type is a silt loam (USDA: Hapludalf; FAO: Luvisol; French classification: Sol lessivé acide). The climate of the area is temperate and humid, with a growing season of seven months. Mean annual temperature is 9.9°C and annual precipitation is 835 mm.

Soil samples were adjacently collected in early December 2001 (after seed setting of all species) and in early June 2002 (before the input of fresh seeds) from eight permanently marked plots randomly placed in eight representative stands in the Sonian forest, i.e. two pure *Fagus* stands, two pure *Quercus* stands, two pure *Pinus* stands and two logged areas (at least four years old). In both months, eight blocks of 20 × 20 cm² in three layers (0–5, 5–10 and 10–15 cm depth) were randomly taken per plot. This gave us a total of 384 soil samples (8 plots × 8 blocks × 3 layers × 2 seasons).

After removal of the litter layer as well as roots and plant fragments, the soil samples were spread in 192 seed trays of 15 × 25 × 5 cm³, one tray for each sampled layer. We put the June samples in a greenhouse at 25/15°C with 12/12 h light/dark condition for direct germination of

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Table 1. Species occurring in naturally (cold) stratified (NS) versus artificially stratified (AS) soil samples with mean, minimum, and maximum seed density (seedlings/m²; depth: 0–15 cm). The life form (LF) of species is specified: C, Chamaephyte; G, Geophyte; H, Hemicryptophyte; P, phanerophyte; T, Therophyte. *P* value according to a Wilcoxon matched pairs test

Species	LF	NS			AS			<i>P</i> value
		Mean	Minimum	Maximum	Mean	Minimum	Maximum	
<i>Ajuga reptans</i>	H	0	0	0	1	0	75	n.s.
<i>Betula pendula</i>	P	188	0	1800	93	0	1725	0.0009
<i>Calluna vulgaris</i>	C	34	0	950	67	0	3525	n.s.
<i>Carex remota</i>	H	5144	0	39,000	1731	0	8850	<0.0001
<i>Circaea lutetiana</i>	G	0	0	0	1	0	75	n.s.
<i>Cirsium arvense</i>	G	0	0	0	1	0	75	n.s.
<i>Conyza canadensis</i>	T	0	0	0	1	0	75	n.s.
<i>Cytisus scoparius</i>	P	1	0	50	5	0	75	n.s.
<i>Digitalis purpurea</i>	H	165	0	4050	57	0	1950	<0.0001
<i>Dryopteris dilatata</i>	H	31	0	900	381	0	5625	<0.0001
<i>Epilobium angustifolium</i>	H	18	0	450	0	0	0	<0.0001
<i>Epilobium cf. ciliatum</i>	H	9	0	200	180	0	4725	<0.0001
<i>Geum urbanum</i>	H	1	0	50	1	0	75	n.s.
<i>Hypericum humifusum</i>	T	69	0	700	39	0	750	0.0029
<i>Impatiens parviflora</i>	T	0	0	0	4	0	75	n.s.
<i>Lotus pedunculatus</i>	H	0	0	0	2	0	75	n.s.
<i>Luzula sp.</i>	H	20	0	200	5	0	150	0.0329
<i>Oxalis acetosella</i>	H	5	0	100	21	0	600	n.s.
<i>Plantago major</i>	H	0	0	0	1	0	75	n.s.
<i>Polygonum hydropiper</i>	T	87	0	4950	33	0	975	n.s.
<i>Robinia pseudoacacia</i>	P	0	0	0	1	0	75	n.s.
<i>Rubus fruticosus s.l.</i>	P	54	0	350	80	0	750	n.s.
<i>Sambucus nigra</i>	P	2	0	50	1	0	75	n.s.
<i>Scrophularia nodosa</i>	H	0	0	0	1	0	75	n.s.
<i>Sonchus oleraceus</i>	T	0	0	0	2	0	75	n.s.
<i>Stellaria media</i>	T	2	0	50	0	0	0	n.s.
<i>Teucrium scorodonia</i>	H	1	0	50	7	0	375	n.s.
<i>Urtica dioica</i>	H	1	0	50	0	0	0	n.s.
<i>Veronica montana</i>	C	3	0	100	1	0	75	n.s.
<i>Viola sp.</i>	H	0	0	0	1	0	75	n.s.

n.s., Not significant at the 0.05 level.

seeds, which were naturally stratified (NS) the previous winter. December samples were artificially stratified (AS), undergoing a cold–moist stratification period at 5°C in an unlit cold chamber for three months, before laying them in the greenhouse in the same environmental conditions as the NS samples. To provide a reliable estimate of the number of individuals (density) and the number of species (composition), the seedling emergence technique was used⁷. Seedlings that were identified at the species level were counted and removed, and those that could not be identified were individually transplanted to pots and grown, where necessary, until flowering. After six months, seedling emergence in most trays ceased, partly due to colonization of the trays by mosses and liverworts. At this stage, both the NS and AS soil samples were stirred in order to expose to the light seeds that may have been covered by relatively deeper layer of soil, which might have hampered germination. After 12 months, no seeds germinated further even immediately after stirring the soil, and the experiment was concluded. Very few seedlings had died before they could be identified.

Control trays containing sterilized soils were placed in the same greenhouse in order to record contamination by wind-borne seeds.

Seed density (overall, and for each species separately) and species number were compared in NS versus AS samples with Wilcoxon matched pairs test. Statistical analyses were carried out using Statistica 6.0 (Statsoft Inc.)⁸. The 0.05 level of probability was accepted as significant throughout the work. In this study, seeds were considered as diaspores, i.e. fern spores included.

More than 90% of the seedlings emerged during the first four months from the NS samples and five months from the AS samples. A total of 14,928 seedlings emerged in the NS samples and 6960 seedlings in the AS samples, corresponding to 5831 and 2719 seeds/m² respectively. On an average, we found more seedlings per tray in the NS than the AS samples (78 versus 36; *P* < 0.0001, Wilcoxon matched pairs test). The same trend was followed by each layer taken separately. Among the 30 species which were detected, 16 were common to both germination methods (Table 1). A total of 19 species emerged

from the NS samples and 27 from the AS samples. Species found in the AS samples but not detected in the NS samples were *Ajuga reptans* L., *Circaea lutetiana* L., *Cirsium arvense* (L.) Scop., *Conyza canadensis* (L.) Cronq., *Impatiens parviflora* DC, *Lotus pedunculatus* Cav., *Plantago major* L., *Robinia pseudoacacia* L., *Scrophularia nodosa* L., *Sonchus* sp. and *Viola* sp. In the AS samples, only three species (that were found in the NS samples) failed to be detected: *Epilobium angustifolium* L., *Stellaria media* (L.) Vill. and *Urtica dioica* L. The species which was by far the most abundant in both treatments was *Carex remota* Jusl. ex L. It had significantly larger seed banks in the NS samples ($P < 0.0001$). So did *Betula pendula* Roth. ($P = 0.0009$), *Digitalis purpurea* L. ($P < 0.0001$), *Hypericum humifusum* L. ($P = 0.0029$) and *Luzula* sp. ($P = 0.0329$). Two species, *Dryopteris dilatata* (Hoffm.) A. Gray and *Epilobium* cf. *ciliatum* Rafin. had significantly larger diaspore densities in the AS samples ($P < 0.0001$).

This study compares seed germination patterns in a temperate forest ecosystem after natural versus artificial cold-stratification to make a reliable estimate of seed density and species composition in buried soil seed bank through the seedling emergence technique. Similar experiments to compare germination patterns have been carried out by The Angelgrove Tree Seed Company⁹, which suggest that natural stratification will naturally satisfy a seed's requirement for cold-stratification, provided the natural conditions in question are such that the needs of the dormant seed are actually met. For tree species, the germination results were found to be as good (and sometimes better) as those resulting from seeds which have undergone an artificial cold-stratification⁹. However, in regions where there is little homogeneity in winter conditions, artificial stratification for deeply dormant seeds was recommended⁹. Esen *et al.*^{10,11} also compared germination performance of *Prunus* species after artificial versus natural stratification, and they found similar results for both treatments.

The larger number of species found in the AS samples might be due to the fact that stratification in the cold chamber makes it possible to keep seeds at constant ideal cold-moist conditions for a longer period than under field conditions where soil temperature and moisture fluctuate (for the study area, winter temperatures normally vary between 0°C and 6°C, but extreme values between -10°C and 12°C have also been observed; data from the Royal Meteorological Institute of Belgium). Seeds that exhibit a deeper dormancy require longer periods of stratification, and seedlings may not emerge after three months of natural stratification since temperatures may temporarily be too high and moisture conditions too low to allow for effective dormancy break. These circumstances may however be favourable for the emergence of other species, which may explain the larger seed density obtained with the NS samples. Indeed, for numerous species, seed germination cannot occur or is severely reduced at constant-temperature environments¹².

Steinbauer and Grigsby¹³ found that among the 85 species selected from 15 families, more than 80% showed higher germination at alternating temperatures compared with constant temperatures. For most species, a single period of cold-stratification is sufficient to completely overcome dormancy¹⁴. However, in some cases, a more complex situation exists, in which different parts of the embryo differ in their depth of dormancy. In such species, consecutive periods of chilling, warming and re-chilling are required to overcome radicle and epicotyl dormancy¹⁵. Another possible reason for detecting different species with each method might be the presence of pronounced seasonal dormancy¹ and dynamic aspect of germination pattern (spring germinators versus autumn germinators).

In conclusion, based on our data in a forest ecosystem with temperate humid climate on loessic materials, artificial stratification allowed the emergence of a larger number of species from the soil seed bank, but natural stratification improved estimates of the seed bank density. We therefore recommend the use of both methods in order to optimize the estimation of both species number and density in the study of soil seed banks.

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Coseismic uplift, slow plant mortality and ecological impact in North Andaman following the December 2004 ($M_w > 9.2$) earthquake

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We present here the phenomenon of gradual decay leading to plant mortality in vast stretches (measuring several square kilometres) of vibrant mangrove forests in the coastal wetlands of North and Middle Andaman. We interpret the phenomenon as an effect of static ground uplift following the 26 December 2004 Sumatra–Andaman earthquake. The decimetre to metre scale uplift has cut-off tidal water flow into those parts of the wetlands that were elevated from intertidal to supratidal levels. The consequent thermal shock, desiccation and gradual depletion of soil salinity affected the plants in the elevated parts. However, desiccation due to lack of tidal-water inflow caused the plant mortality which took the proportions of an ecological disaster about three to four years after the earthquake. The voluminous forest debris produced by the withered plants is likely to be preserved in the coastal sedimentary sequences as peat beds and serve as palaeoseismic indicators.

Keywords: Coseismic uplift, earthquake, ecological impact, mangrove swamp, plant mortality.

WHILE discussing ground uplift in North and Middle Andaman Islands following the great Sumatra–Andaman earthquake of December 2004, we had predicted that the intertidal flora of the coastal mangrove forests that were

coseismically uplifted from intertidal to supratidal levels would perish within a few years¹. We further predicted colonization of the perished intertidal forests by sweet-water plants. To test the validity of the predictions that point to a distinct type of ecological disaster, we carried out repeat surveys of the coastal wetlands of North Andaman more than four years after the earthquake. Here we give a brief introduction to the tectonic setting of the Andaman and Nicobar Islands, and an account of what we observed in the context of our prediction. The survey revealed widespread plant mortality affecting the pristine mangrove forests of the coastal wetlands.

The 800-km-long Andaman–Nicobar chain of islands delineates the arcuate axial zone of the forearc ridge of the Indonesian subduction complex². The chain of islands is broadly parallel to the subduction zone trench, known as the Sunda–Java trench², that represents a trace of the interface along which the Indian–Australian plate is subducting beneath the Eurasian–South East Asian plate (Figure 1). A general northeasterly convergence of the plates has been inferred². Sediments deposited on the down-going oceanic plate have been scraped, and piled up by thrust faults to build up the accretionary prism and

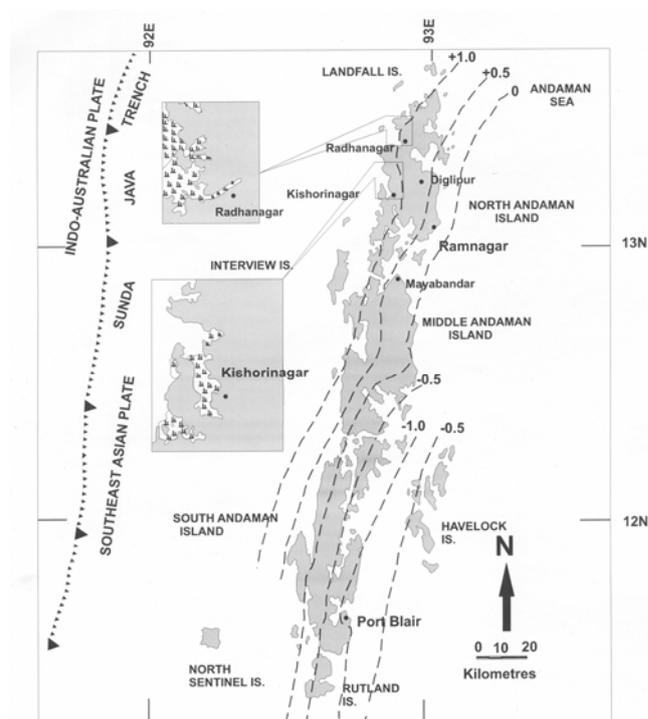


Figure 1. Map showing the location of the Sunda–Java trench² and distribution of coseismic vertical ground movement in the Andaman region, caused by the 26 December 2004 megathrust earthquake^{1,12,13}. The dashed lines represent the contours of vertical ground movement. Values assigned to the contour lines give the estimated vertical offset in metres. The ‘+’ and ‘-’ signs indicate ground uplift and subsidence respectively. The ‘0’ value contour represents the neutral line¹. (Insets) Pre-earthquake extent of the Kishorinagar and Radhanagar wetlands. The grass symbol marks the tidal swamps (wetlands) that support dense mangrove forests. The grey shade shows the land areas. IS., Island.

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