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The importance of soil seed banks in ecological studies

La importancia de los bancos de semillas del suelo en los estudios ecológicos

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A population and community perspective

The soil seed bank is the term ecologists use to designate the set of viable seeds contained in soil, on the surface, buried or associated with mulch (Leck *et al*., 1989). Seed banks play a fundamental role in the dynamics of plant populations and communities. From a functional point of view, one of the most relevant aspects is the time that seeds can remain in the soil, as this will determine the amount of reserves that species will have at any given time to attend to recruitment under regular environmental conditions or unforeseen situations of disturbance on a small or large spatial scale.

This aspect becomes particularly critical in the case of rare or threatened taxa (Ferrandis *et al.,* 2011a, 2011b). The classic work of Thompson and Grime (1979) represented a milestone in the ecology of seed banks, proposing for the first time a classification in this sense: (i) transitory banks, those of species whose soil seed reserves are depleted before a new phenological cycle is completed, i.e., before a new episode of seed dispersal occurs, and (ii) persistent banks, those in which a significant fraction remains viable in the soil for more than one phenological cycle, overlapping the dispersal of the new crop. Subsequently, Thompson *et al.,* (1997) refined the classification from the proposals of Bakker (1989) and Bakker et al. (1991), identifying three large groups from the functional point of view: (i) transitory, for species whose seed banks persist in the soil for less than one year (coinciding in general with the duration of a phenological cycle); (ii) persistent of short duration, for species whose seeds persist in the soil for more than one year, but less than five years; (iii) persistent of long duration, to define those seed banks capable of persisting in the soil for more than five years.

Obviously, the five-year border is still conventional, but it fits in well with the reality of the abundant database that the authors created on seed banks in Europe, based on the research published up to that date. Each type of seed bank has been modelled by natural selection and acts as an efficient strategy under the conditions in which the species live. In fact, one might think that under a regime of predictable disturbances in time and space (e.g., summer exhaustion of herbaceous plants in Mediterranean environments), a transient seed bank, formed shortly before they occur, results in the most efficient investment-benefit strategy (Thompson and Grime 1979, Ferrandis *et al.,* 2001). In contrast, in the face of a regime of unpredictable

disturbances, the evolutionary solution will be directed towards the accumulation of seeds in the soil (Thompson and Grime, 1979).

In ecosystems subject to recurrent fires, such as many Mediterranean shrubs, large persistent seed banks play a significant role in plant regeneration (Ferrandis *et al.,* 1999a). Similarly, this is why persistent seed banks predominate in the early stages of ecological succession, while the pseudo-stable and mature stages of vegetation are dominated by species with transient banks (Baskin and Baskin 1998, Fenner, 2000). In today's changing world, with the modification that human activity is driving on a large scale through global change, persistent seed banks can play a relevant role in the resilience of populations and communities: according to the classification of Thompson *et al.,* (1997) and its functional interpretation, short-term persistent banks would decisively drive the maintenance of populations, while long-term persistent banks would have definitive consequences on the resilience of communities. The study of soil seed banks, either in focused work on the populations of a given species or in entire plant communities, can provide crucial information for the accurate interpretation of the state, responses and dynamics of them.

Basic guide for soil seed bank analysis protocols. Sampling

The collection of soil samples (blocks) in the environment where it is wanted to evaluate the seed bank can be done with the help of a cylindrical metallic probe (3-5 cm in diameter) or a gardening shovel.

The tool to use will be the one that best adapts to the characteristics of the soil: stoniness, depth, organic matter, roots. As a general rule, for a given volume of soil, it is better to extract many small samples than a few large ones, since seed banks often have a very heterogeneous spatial distribution. If it is a matter of studying the seed bank of a given taxon, sampling can focus on those places in the field where seeds can be expected to concentrate, depending on the type of primary and secondary dispersal (e.g. under the cover of parent plants, or within a certain radius from their base, etc.). If the study covers the whole community, plots of a given size (e.g. 1 m^2) can be established from which randomly arranged extractions can be made.

There are two compatible ways to find out whether a taxon's seed bank is transient or persistent. The most obvious is to extract the soil shortly before the seed dispersal takes place: its presence in the samples will be a clear indication of the persistent nature of the bank. The other option is to analyse the vertical distribution of seeds in the soil.

Most seeds lack active burial mechanisms, so they do not usually reach strata beyond the first centimeters of the soil, unless they remain in it for a sufficiently long time. On this assumption, a correspondence can be established between the types of seed banks of the classification proposed by Thompson *et al.,* (1997) and their vertical distribution in the soil: (i) transient seed banks accumulate their seeds on the surface (first centimeters); (ii) short-lived persistent seed banks (between 1-5 years) have a representative fraction of seeds in deeper soil strata, but in a significantly smaller quantity than in the superficial stratum; (iii) long-lived persistent seed banks (>5 years) accumulate as many seeds in deep strata as on the surface. A sampling of the soil (natural, without removal) in two strata, from 0 to 4 (or 5) cm and from 4 (or 5) to 8 (or 10) cm, on the same points in each extraction, allows this correspondence to be established. If it is also carried out just before the seeds are dispersed, it makes

it possible to define precisely the persistent or transitory nature of the seed bank of a taxon. The morphological characteristics of the seeds, in addition, complement the diagnosis: the seeds of species that form persistent banks usually are of small size and tend to approximate to the spherical form, traits that the natural selection has modulated to facilitate their burial; on the contrary, seeds distant from the sphericity or with appendages that make difficult their burial form transitory banks in the surface of the soil (Thompson *et al.,* 1993).

Analysis of seed content in soil samples. There are two general techniques for counting soil seeds: (i) those based on the emergence of seedlings and (ii) those based on the physical separation of seeds. The first group of techniques requires the cultivation of soil samples. Each emergent seedling corresponds to a viable seed, which will have to be taxonomically identified and then removed, so as not to interfere with the germination and/or emergence of other seeds and/or seedlings. Soil samples should be grown in an enclosure limited to contamination by the arrival of exogenous seeds or disturbance by animals. An umbraculum (enclosure covered with mesh) usually gives better results than a greenhouse, since it effectively reproduces the natural conditions of temperature and light that govern the processes and phenology of germination. Soil samples should first be passed through a coarse-light sieve to remove stones and plant debris, and then spread individually over some type of container (trays), forming a thin layer (no more than 1-2 cm) to facilitate the emergence of small seedlings, on a seedless substrate base (sterile peat, for example) that allows the seedlings to take root until they are identified and removed. The transplant of seedlings to individual flowerpots when they cannot be identified early gives good results, when freeing the samples of developed plants that difficult the manifestation of other seeds. The samples should be cultivated long enough to ensure that the seed bank has been able to express itself fully, which may require up to a complete phenological cycle. Thus, the difficulty in identifying the seedlings, the lethargy of the seeds and the space and time necessary for germination and emergence of the bank in the samples are the main limiting factors of this method.

Ter Heerdt *et al.,* (1996) proposed to do a pre-screening of soil samples by washing on a small light sieve (0.25 mm), so that, without losing seeds, the volume of soil to be handled is reduced and germination is stimulated. With this methodology, the cultivation time required for the expression of the complete seed bank of nemoral ecosystem communities in Central Europe was estimated to be around 6 weeks. However, the effectiveness of this protocol will depend very much on the type of seed dormancy of the dominant species in the community. For example, Ferrandis et al. (1999a, 1999b), in the study of cistáceas-rich Mediterranean shrubs that form huge persistent seed banks with physical lethargy, had to cultivate samples between 1 and 2 years.

The protocols for physical separation of seeds are based on the detection and extraction of seeds from soil samples, usually with the help of sieves and magnifying glass. The factors limiting their effectiveness are the size of the seeds and their viability. Ferrandis *et al.,* (1999a) determined that in the fraction of soil passing through the 0.5 mm mesh sieve, imprecision in seed detection makes this methodology unfeasible. Above this size, it is possible to locate and extract the seeds faithfully, although in general it is very time-consuming. Moreover, not all seeds with a good external appearance are in fact viable: the embryo must be diagnosed by excision of a representative sample of the extracted seeds, either based on their colour and turgidity, or by staining with tetrazolium salts (Ferrandis 1999b). Therefore, physical separation of seeds will be a good option if we work with relatively

large seeds. In general, this method is reserved for studies focused on specific taxa, of which we have information on the appearance of the seeds (possibility of identification), size (greater than 0.5 mm) and especially if they have seminal lethargy difficult to overcome. In these cases, the samples are filtered with sieves of light of inferior mesh and superior to their dimensions, to reduce the volume of soil to process. In other cases, especially when the focus of our study is the seed bank of an entire community, we will turn to emergency seedling techniques.

Table 1. - Methodological protocols for the detection of contained seeds in soil samples (adapted from Ferrandis et al. 2011a)

Physical separation	Seedling emergency
Sifted. Washing of the sample by a sieve with a light mesh above the seeds under study, to remove coarse elements and organic remains (roots, bulbs, leaf litter), and on another with a light mesh below, which retains the seeds.	Seedling recognition. Field observation, or sowing of seeds in pots, to recognize the seedlings of the taxon in the early stages of development.
Drying. Drying of the sample at room temperature, on the washing sieve itself	Sifting of the sample on a sieve with a larger mesh size than the seeds being studied, to eliminate the coarse elements and organic debris (roots, bulbs, leaf litter), which could hinder the emergence of seedlings; it can also be effective to wash the samples on a fine sieve, which retains the seeds and allows the volume of the sample to be reduced, in order to shorten the growing period.
Scrutiny of the sample. Spreading of the soil sample, pro fractions or complete, on a tray, Petri dish, or similar; search and extraction of seeds with the help of tweezers and magnifying glass if necessary.	Greenhouse cultivation. Spread the sample in a tray, as thinly as possible (never more than 1-2 cm thick) on a layer of seed-free substrate; water regularly (micro-spraying is recommended) to keep the soil constantly moist.

Feasibility analysis. The external appearance of the seed is not an accurate indicator of its viability. It is recommended to inspect the appearance of the embryo (colour and state of hydration); tetrazolium stains can also be used; perform the test with a representative sample of the seeds extracted from the soil.	Seedling count. Periodic review of samples; counting and removal of seedlings as soon as they are identified; when the seedling emergence ceases, it is recommended to remove the soil (taking care not to mix it with the sterile base substrate layer, to promote the germination of seeds that may be buried too deep.

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