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**Projet de Fin d'Etudes**

# Gullies can be potential corridors of biodiversity?

## Analysis of experimental method

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**2017-2018**

**DAE5**

**Tutor : GRELLIER Séraphine**

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# Formation par la recherche, Projet de Fin d'Etudes en génie de l'aménagement et de l'environnement

La formation au génie de l'aménagement et de l'environnement, assurée par le département aménagement et environnement de l'Ecole Polytechnique de l'Université de Tours, associe dans le champ de l'urbanisme, de l'aménagement des espaces fortement à faiblement anthropisés, l'acquisition de connaissances fondamentales, l'acquisition de techniques et de savoir-faire, la formation à la pratique professionnelle et la formation par la recherche. Cette dernière ne vise pas à former les seuls futurs élèves désireux de prolonger leur formation par les études doctorales, mais tout en ouvrant à cette voie, elle vise tout d'abord à favoriser la capacité des futurs ingénieurs à :

- Accroître leurs compétences en matière de pratique professionnelle par la mobilisation de connaissances et de techniques, dont les fondements et contenus ont été explorés le plus finement possible afin d'en assurer une bonne maîtrise intellectuelle et pratique,
- Accroître la capacité des ingénieurs en génie de l'aménagement et de l'environnement à innover tant en matière de méthodes que d'outils, mobilisables pour affronter et résoudre les problèmes complexes posés par l'organisation et la gestion des espaces.

La formation par la recherche inclut un exercice individuel de recherche, le projet de fin d'études (P.F.E.), situé en dernière année de formation des élèves ingénieurs. Cet exercice correspond à un stage d'une durée minimum de trois mois, en laboratoire de recherche, principalement au sein de l'équipe Ingénierie du Projet d'Aménagement, Paysage et Environnement de l'UMR 6173 CITERES à laquelle appartiennent les enseignants-chercheurs du département aménagement.

Le travail de recherche, dont l'objectif de base est d'acquérir une compétence méthodologique en matière de recherche, doit répondre à l'un des deux grands objectifs :

- Développer toute ou partie d'une méthode ou d'un outil nouveau permettant le traitement innovant d'un problème d'aménagement
- Approfondir les connaissances de base pour mieux affronter une question complexe en matière d'aménagement.

**Afin de valoriser ce travail de recherche nous avons décidé de mettre en ligne sur la base du Système Universitaire de Documentation (SUDOC), les mémoires à partir de la mention bien.**

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# Introduction


*Figure 1 Satellite map of study site*

Soil erosion is a natural problem in every ecosystem, caused by wind, water, and human activity (Urbanization, agricultural practices, forest harvesting …). Soil erosion problem has become increasingly serious which impose threat on soil micro-environment and vegetation formation above, as well as public health of human society (Durán Zuazo and Rodríguez Pleguezuelo, 2008; Pimentel, 2006). Based on soil erosion problems in Thailand, we have proposed a hypothesis: the presence of soil seed bank and seed removal can provide a potency of gullies to support the ecosystem and to be considered as corridors of biodiversity. In order to verify the hypothesis, soil seed bank density and composition as well as seed removal characters (seed loss ratio, seed displacement ratio, etc) should be calculated through a series of experiments, executed on typical study area or in laboratory, under natural rainfall or simulated rainfall.

This report enumerates the methods implemented in other researches of soil seed bank and soil removal. Combining with conditions of our study site, some methods have been proposed with comparison of theirs advantages and disadvantages for the following experiments.

In order to accomplish this project, reference papers for the project interim-thesis have been consulted again but with attention on the method part. Besides, a series of papers concerning “soil seed bank composition” “seed removal measurement” “NIRS” were also in my reference. An Excel table has been created for noting important points in each paper and combing ideas. In the following, some schemas were also added in the thesis in other to better express opinions.

Furthermore, this research could be possibly not complete resulting from technique and time limit, that is to say, other methods for this subject that haven’t been mentioned in this report could possibly exist.

# Part 1 Soil seed bank

The presence of soil seed bank ensures the potency of revegetation after serious soil erosion events. Thus, the identification of soil seed bank density and composition is significant for revegetation research. Two broad approaches have been used to estimating seed density and species composition of soil seed banks: seedling emergence method and seed extraction method.

## 1.1 The seedling emergence method

The seedling emergence method, as the method which has been used commonly, involves spreading a thin layer of soil under suitable conditions for germination, such as greenhouse or germination chamber, then identifying and counting emerging seedling to estimate soil seed bank density and composition (Price et al., 2010; Gonzalez and Ghermandi, 2012). The seedling emergence method is commonly applied because it is less laborious and useful for a large volume of soil samples. However, this approach requires space in greenhouse and a long-time seed germination monitoring (Gonzalez and Ghermandi, 2012). Besides, it can dramatically underestimate the density of the soil seed banks resulting from the seed dormancy and specific environmental requirements for germination. Therefore, it does not provide a complete assessment of soil seed bank composition (Price et al., 2010; Gonzalez and Ghermandi, 2012).

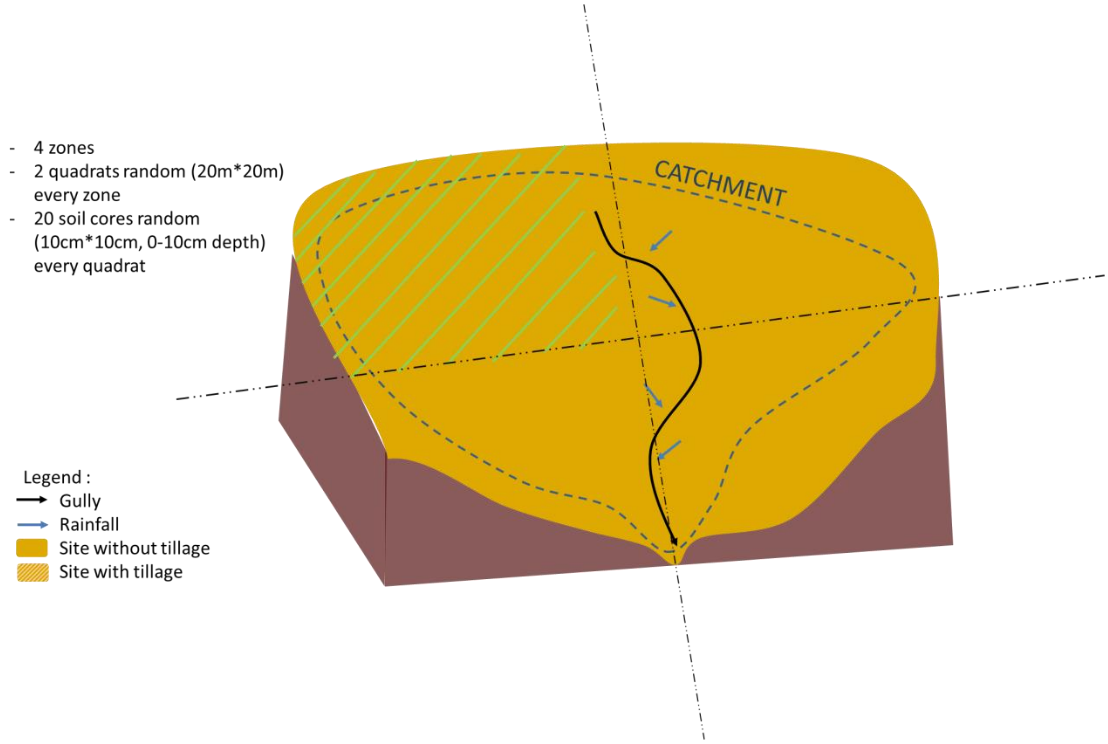


*Figure 2 Seedling emergence method*

The soil seed bank experimental method following is proposed for our study site:

At the beginning, we would divide the study site into 4 zones, regarding the gully as the vertical dividing line and also dividing horizontally the gully into upper slope and lower slope, in order to compare the soil seed bank at the site with tillage and without tillage (as Fig 2). 2 quadrats (20m*20m) would be randomly taken but be at different distance away from the gully at each zone of study site. Coordinates of each quadrat should also be recorded in order to analyze the effect of height and distance away from gully on characters of soil seed bank. 20 soil cores (10cm*10cm, depth 10cm) would be randomly collected at each quadrat when seed set was completed for the season (Weerasinghe et al. 2008).

Next, for the germination experiment, soil air drying is necessary in order to enhance germination, although it may lead to the loss of species with transient seed banks (Price et al., 2010). After removing gravel, litter, and roots (Li et al., 2010), air-dried soil should be sieved to 0.15mm, which is smaller than the diameter of the smallest seed in the study area, and the larger soil fraction would be used for germination to improve the germination and reduce the emergence time (Wang et al., 2017).

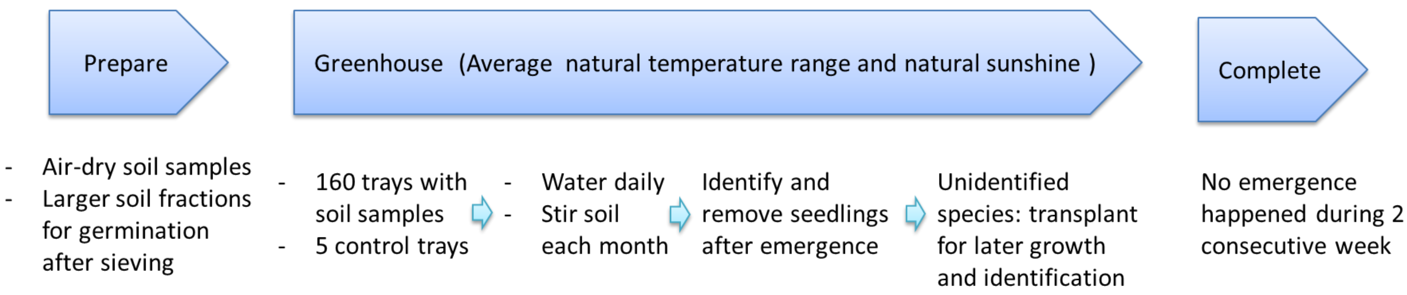


*Figure 3 Seedling emergence method flow chart*

After that, soil samples should be placed in a greenhouse and spread out on a tray which provide a soil depth of 2cm and provide adequate surface area for germination (Price et al., 2010), with a bottom pierced and with a gravel layer installed in order to prevent the soil from becoming water-saturated (Li et al., 2010). In the greenhouse, 5 control trays containing only the the gravel layer should also be placed in order to check for contamination from the greenhouse environment (Li et al., 2010; Chapple,D. 2017). All trays should be watered daily and then identified, counted and removed after seedling emergence every 3 to 4 days during the first 2 months of the seed bank assay, and once per week after emergence declined (Weerasinghe et al. 2008), all trays should also be stirred each month for un-germinated seeds' emergence (Wang et al., 2017). Unidentified species should be transplanted to bigger trays for later growth and identification (Li et al., 2010; Price et al., 2010). Experiment complete if no seedling emergence occurred during 2 consecutive weeks, the seed bank assay last totally 6 months for full germination of seeds (Wang et al., 2017; Weerasinghe et al. 2008) The temperature in a greenhouse should controlled also as the average range of study site environmental temperature and under natural light that penetrate the glass (Price et al., 2010; Weerasinghe et al. 2008)

## 1.2 Seed extraction method

On the other hand, seed extraction method provide a better estimation of total soil seed bank density and richness, Price et al. (2010) reported that seedling emergence method failed to detect nearly 90% of all germinable seeds found via the seed extraction method. Seeds are identified under microscope after being extracted from the soil considering their different size and densities, through flotation in a salt solution or other high specific gravity liquid, or a combination of both (Price et al., 2010; Gonzalez and Ghermandi, 2012). However, it is not useful for large volume of soil samples as its complicated operation and high volume of work.

For our study site, the second soil seed bank experimental method is proposed as follows:

Based on the same site division, quadrats location and soil cores selection, each soil samples from different soil core should be mixed completely and weighed out 100g in order to facilitate calculation. Then we would immerse the 100g soil sample in 1L of potassium carbonate solution (500g/L water). The mixture (soil and solution), after agitating, should be settled for 1h in order to allow the organic matter to become suspended, then soil particles and residual potassium have to been removed through a series of sieves, this would also facilitate counting and sorting of seeds because large and small seeds have been separated. Next, samples should also be dried in an oven at 60 ℃ overnight (Price et al., 2010).

Afterwards, viability of seed could be determined by using a dissecting microscope through pressure test, which consist applying gentle pressure with forceps to seeds to corroborate the embryo presence, and seeds would be regarded viable when they resist this pressure (Price et al., 2010; Gonzalez and Ghermandi, 2012). This testing procedure is commonly used ass it is quicker and cheaper, although it is less rigorous than other methods (Price et al.2010). Lastly, the composition and density of soil seed bank would be identified according to the result.

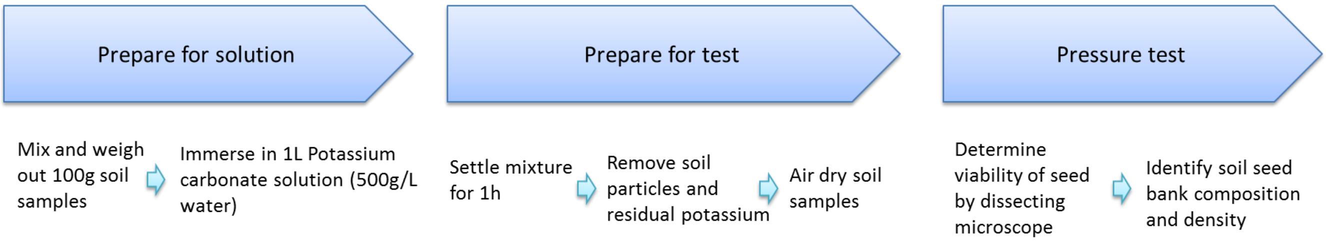


*Figure 4 Seed extraction method flow chart*

## 1.3 NIRS

Near infrared spectroscopy (NIRS), the technology that has been used in a wide selection of single seed applications as its recognized advantages such as non-destructive, high speed, no sample preparation required, affordable and flexible instrumentation, and good precision when calibrations are well developed. NIR was utilized for the first time is in the 1960s, then this technology has been developed impressively as its advantages (Lidia, 2011).

According to the study of Farhadi (2015), sorting accuracies of filled-viable, empty and petrified seeds of Larix sibirica Ledeb were 98%, 82% and 87%, respectively. Besides, sorting accuracies of viable and non-viable seeds (empty and petrified combined) reached 100%.

Despite the limitation of small seed sizes, NIRS has led to successful results, the final remaining question is what the extent of damage that a seed may suffer to be detected by NIRS. Those results demonstrate the feasibility of NIRS as seed sorting technology according to their viability and as certification of seed lots by species and origin (Farhadi, 2015). Therefore, NIRS could be a potential approach to identify seed bank density and species composition.

# Part 2 Seed removal

Resulting from overland water flow cause by rainfall events, seed removal would occur. Seed removal consist seed loss and seed displacement. Therefore, simulation rainfall has been proposed in order to estimate seed removal in laboratory, besides, the method with natural rainfall has also been applied in several studies.

## 2.1 Simulation rainfall experiment

First of all, seeds selection should be completed before the simulation rainfall experiments. Seeds from 30-40 dominant species of our study site would be collected (10 seeds for each species) during their mature periods. Besides, variety of seed shape should also be taken into consideration (Han et al., 2011, Wang et al., 2012). Then these seeds should be dyed with blue aniline in order to facilitate observation of seed removal (García-Fayos et al., 1995).

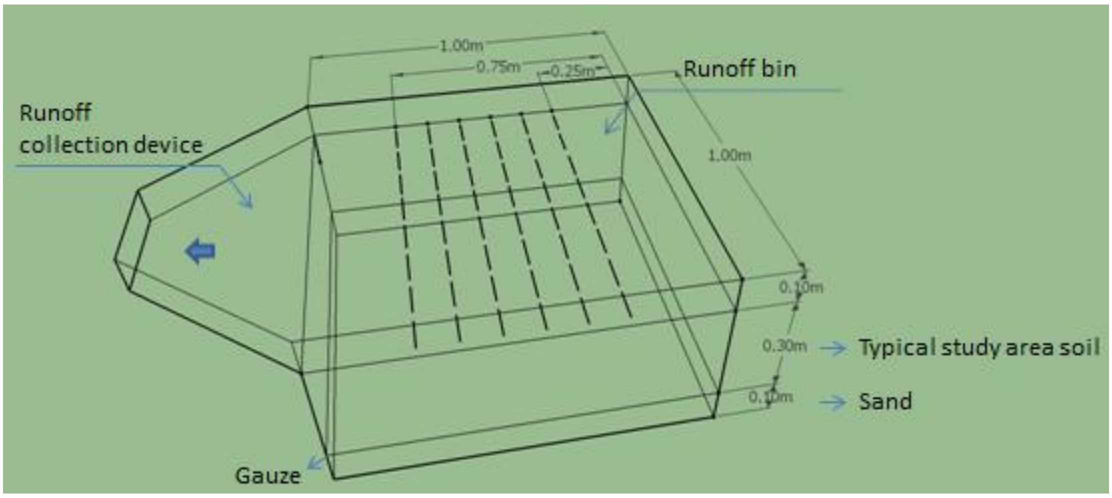


*Figure 5 Runoff bin for seed removal experiments under simulation rainfall experiments*

Afterwards, these seeds would be brought in laboratory for future simulation rainfall experiments. With lateral sprinkling automatic rainfall simulation system, raindrop composition and distribution should be realized evenly and closely to nature rainfall, rainfall intensities should similar to those of the heavier rainstorms registered in our study area(García-Fayos et al., 1995). Runoff plot(1m*1m*0.5m) would be installed with 10cm sand as bottom layer (sand should be covered with gauze) and 30cm of soil from typical study area(Han et al., 2011). As shown in Figure 5, the plot consist 2 parts: the runoff collection device to collect runoff and seed loss during simulated rainfall; the principle part that provide space for placing seeds. In order to reduce heterogeneity of soil surface, one day before the rainfall simulation experiment, a pre-rainfall with an intensity of 30 mm/h would be executed until runoff occurred (Jiao et al., 2011).

Next, seeds would be placed in the center of each plot, that is, 25cm-75cm from the top down plot of soil surface in lines with 10 intervals. According to seed size, 2-3 species should be placed in a line, 10 seeds of each species should also be placed separately and evenly in a line, besides, larger seeds should be placed in down line to not to affect the runoff path (García-Fayos et al., 1995; Wang et al., 2012; Han et al., 2011). Then simulation rainfall experiments would be performed on slope with gradient of 10°, 15° and 20°, under rainfall intensity of 50mm/h, 100mm/h and 150mm/h. Each experiment would be replicated 3 times, that is, 27 experiments totally. Each simulation rainfall would last 60 minute. After each rainfall event, soil should be replaced by unused soil (Han et al., 2011).

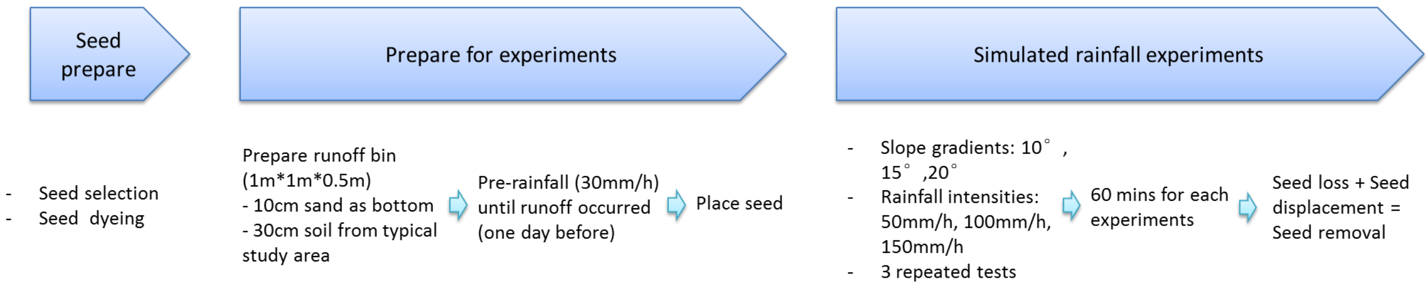


*Figure 6 Seed removal experiment under simulation rainfall flow chart*

Seed removal consist both seed loss and seed displacement. Seed loss could be counted from the number of lost seed including the seeds in the runoff samples, in the sediment samples and also on the outlet of V-shaped collecting device after each rainfall simulation experiment. Then the total seed loss ratio (SLR=the lost seeds/the total experimental seeds×100%) could be figured out. Furthermore, the number of displaced seed and the distance that each seed travel from its original position should also be measured in order to calculate the seed displacement ratio (SDR=the displaced seeds/the total experimental seeds×100%) and seed displacement distance (SDD) (Wang et al., 2012; Han et al., 2011).

The seed removal experiment under simulation rainfall provides an approach to estimate both seed loss and seed displacement. This approach is conducted in the laboratory, which is easier to realize comparing with seed removal experiment under natural rainfall. Anyway, the error of this approach is more obvious especially at the "seed-counting" period. Furthermore, some details in this approach are still not completely adequate, for example, seed species and quantity, soil bulk density, simulated rainfall intensities, slope gradients of plot bin, etc., resulting from the absence of information of our study site.

## 2.2 Natural rainfall experiment

### 2.2.1 Seed removal experiment with runoff plot

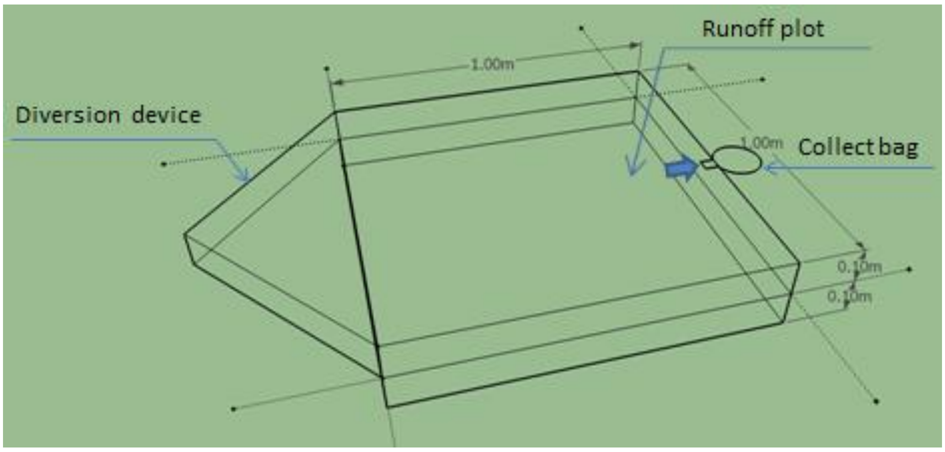


*Figure 7 Runoff plot for seed removal experiment under natural rainfall*

According to the seed removal experiment under natural experiment that realized by Wang et al. (2017), 3 typical slope with-tillage and without-tillage should be selected respectively at our study site, in order to estimate seed removal caused by overland runoff and also to compare the seed removal with tillage and without tillage. After reporting coordinates and slope gradient of these slopes, 3 runoff plots (1m*1m) should be placed on each slope, that is, 18 runoffs plot totally. These runoff slopes should be installed from half to one year before the execution of seed removal experiment, to reduce errors caused by soil disturbance during experimental device installation.

Runoff plot would be inserted 10 cm into the soil and extended 10 cm above the soil surface. The runoff plot composition consist 3 parts: the diversion device above the plot, the principal part, and the bag at the lower side of the plot, with a mesh hole which is smaller than the diameter of smallest seed in our study site, in order to collect the seed removed by runoff (as Fig 7). The bag should be replaced in 3 days after each erosive rainfall event. After air-drying and sieving bag contents, the number of seeds per species would be counted in order to obtain the seed loss number and seed loss ratio resulted from overland runoff under natural rainfall.

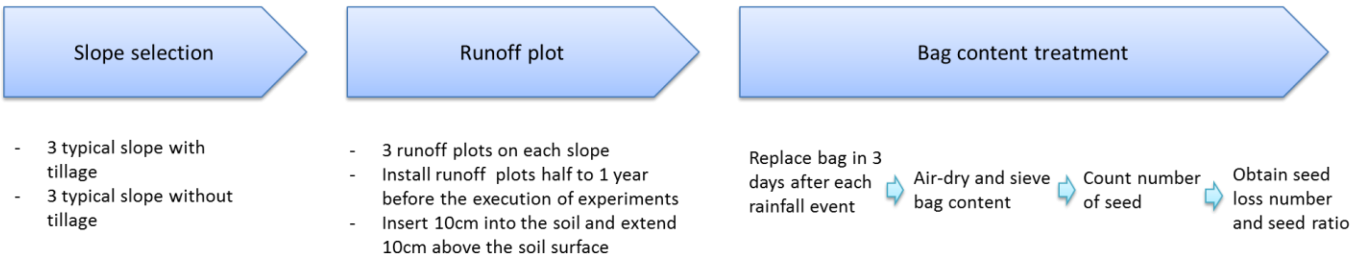


*Figure 8 Seed removal experiments under natural rainfall flow chart*

Seed removal experiment under natural rainfall is more rigorous since its conditions are more similar to those of actual seed removal situation. However, it is time-consuming, laborious and difficult to prepare resulting from occasionality and uncontrollability of natural rainfall. Besides, this approach provides only seed loss estimation instead of seed removal (seed loss and seed displacement). Furthermore, for the description of method above, the plot site selection should be more detailed with further consideration combining with information about topography and actual soil situation of our study site.

### 2.2.2 Fluorescence marking method

According to a report of Isselin-Nondedeu et al, (2006), the fluorescence marking methods were also used for measuring seed removal, that is, mark seed by fluorescence color and then realized them from starting lines, in order to evaluate the proportion of seeds that had moved down from the starting line. Observation of seed fates lasted for a 90-day period in order to track seeds across the slope from a starting line to the bottom. Finally, seeds were counted with noting their locations.

This method is therefore valuable reference on our study, however, the lack of other related and similar report as reference has restricted the analysis and proposition of experiment methods.

# Conclusion

According to those description of experiment methods above, the table following has been created in order to more obvious comparison.

*Table 1 Comparison of 4 experiment methods*

| | | Advantages | Disadvantages |
|---|---|---|---|
| **Soil seed bank** | Seedling emergence method | - Commonly applied<br>- Less laborious<br>- Useful for large volume of soil samples | - Require greenhouse and monitoring<br>- Underestimate soil seed bank density<br>- Incompletely assess soil seed bank composition |
| | Seed extraction method. | - More accurate and complete | - Not useful for large volume of soil samples<br>- Large workload<br>- operate complicatedly |
| **Seed removal** | Under simulation rainfall | - estimate both seed loss and seed displacement<br>- Easier to realize in laboratory | - Big error<br>- More details needed according to study site |
| | Under natural rainfall | - more rigorous and accurate | - More laborious<br>- Difficult to predict natural rainfall<br>- Estimate only seed loss<br>- More details needed according to study site |

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# Gullies can be potential corridors of biodiversity?

### Analysis of experimental method

## Abstract

Soil erosion, caused by wind and water is a natural problem that attires more and more attention from all over the world, revegetalization and recovery of gullies show positive aspect for controlling soil erosion and biodiversity restauration. We have proposed a hypothesis: the presence of soil seed bank and seed removal can provides a potency of gullies to support the ecosystem and to be considered as corridors of biodiversity. The objective of this report is to analyze and propose method for measuring soil seed bank density and composition as well as seed overland removal by overland runoff after rainfall. For soil seed bank, we described two approaches: seedling emergence method and seed extraction method. NIRS technique has also been mentioned. For seed overland removal, experiments were proposed to be completed under simulation rainfall and natural rainfall. However, the methods proposed in this report should be more detailed with information of study site.

## Key word

"soil seed bank" "seed bank" "seedling emergence method" "seed extraction method" "seed removal" "natural rainfall" "simulation rainfall" "NIRS" "fluorescence marking method"