**Soil seed bank germination protocol**

**Collection**

Soil samples are taken as three separate collections in one spot. Each collection is a 5cm diameter circle, taken to a depth of 3cm. PVC pipe with the correct diameters are used, hammered into the soil and then extract. All three samples are then pooled in a single container.

**Storage**

Soil samples are stored in a dark refrigerator upon collection. If cold stratification is needed for dormancy, the samples are potted as quickly as possible, covered with aluminum foil, and transferred back into a 4oC refrigerator for 5-6 weeks.

**Potting**

Pots are 10cm × 10cm square plastic pots. They are filled within 3cm of the top with potting soil (standard). Each sample (which consists of three pooled collections) is mixed thoroughly, and then a set volume of soil is measured out on top of the potting soil. The amount should be between 100ml and 200ml, depending on the available volume.

**Germination**

1. Place trays in a greenhouse (62-78 F, ambient lighting). Water trays daily to keep soil surface moist.
2. Check and photograph trays every 2-3 days. Mark newly emerged seedlings with color-coded toothpicks and record number of emerged seedlings in seedling emergence data sheet.
3. After true leaves emerge, transfer 3 seedlings per potential “species” to a separate pot to grow until flowering. Record leaf and growth characteristics and assign an ID # in true leaves ID data sheet. Place toothpick with date emerged written on a tape flag next to each transplanted individual and label pots with ID #.
4. Remove all duplicate true leaf seedlings of the same ID# after 3 individuals have been transplanted. Record number of true leafed seedlings removed and toothpick color code for each plot in true leaves data sheet.
5. Check near flowering individuals daily. Photograph and remove flowering individuals for identification and vouchering.
6. Allow trays to dry out for one week when no further seedling emergence has been observed for one week.
7. Disturb dried soil in trays by crumbling and mixing.
8. Repeat steps 4-6 with daily watering and growing out for identification.

**Supplies:**

* 5 boxes of colored toothpicks
* Counter
* Greenhouse pots and trays
* Camera
* Potting soil (for transplanting)
* Labelling tape and pen

**References**

Erin K. Espeland, Lora B. Perkins, and Elizabeth A. Leger. (2010) Comparison of Seed Bank Estimation Techniques Using Six Weed Species in Two Soil Types. Rangeland Ecology & Management 63 (2), 243-247

G. N. J. Ter Heerdt, Verweij, G., Bekker, R., & Bakker, J. (1996). An Improved Method for Seed-Bank Analysis: Seedling Emergence After Removing the Soil by Sieving. *Functional Ecology,* *10*(1), 144-151. doi:10.2307/2390273

Vandvik, V., Klanderud, K., Meineri, E., Måren, I. E. and Töpper, J. (2016), Seed banks are biodiversity reservoirs: species–area relationships above versus below ground. Oikos, 125: 218–228. doi:10.1111/oik.02022

Gross, K. (1990). A Comparison of Methods for Estimating Seed Numbers in the Soil. *Journal of Ecology,* *78*(4), 1079-1093. doi:10.2307/2260953

**Seedling emergence data sheet** (example)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Date | 7/27 | 7/31 | 8/2 | 8/4 | 8/7 |  |  |  |  |  |  |  |  |  |  |
| Toothpick color | Red | Orange | Yellow | Green | Blue |  |  |  |  |  |  |  |  |  |  |
| SB1 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| SB2 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| SB3 | 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BI1 | 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BI2 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BI3 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| R1 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| R2 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| R3 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DG1 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DG2 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DGSM | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**True leaves ID data sheet**  (example)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ID # | Shape | Margin | Venation | Other notes |
|  |  |  |  |  |

**True leaves data sheet - transplanting** (example)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Date | Plot | ID # | Quantity + color code/date | Total # removed | # transplanted /  total # transplanted |
| 8/2 | BI1 | 1 | 20 orange, 5 red | 25 | 3 / 3 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**True leaves data sheet - summary**

|  |  |  |
| --- | --- | --- |
| Plot | ID # | Emergence day |
|  |  |  |
|  |  |  |
|  |  |  |