Quantifying Ectomycorrhizal (ECM) Associations

ECM roots are usually quantified by washing roots from soil cores. Assuming that roots are young and healthy, each mycorrhizal root tip will contain an active Hartig net zone. These tips can be counted to quantify the intensity of the association, and their numbers are usually expressed in % ECM per root length. (Brundrett, *Mycorrhizas for Forestry and Agriculture*)

We will be using the gridline intersect method, simultaneously measuring length of mycorrhizal roots and counting the total number of mycorrhizal tips.

Materials

- Large Tray
- Tweezers or Fine Forceps
- Weigh Boats
- DI water
- Petri Dish with grid (1cm by 1cm)
- Microscope
- Counter

1. **Collecting Roots.** Lay your tray out on top of a table. Dump your soil sample onto the tray, and evenly spread soil (this will help expose roots). Using your hands, comb through the soil to find roots. Select 0.05 mm diameter or less roots from the soil.

2. **Washing the Roots.** Set up a 3-Bath system. Fill three weigh boats with DI water. Using fine forceps, dip your root carefully into the first weigh boat. Most of the soil will be removed during this step. Gently rub your finger against the root to remove any excess soil, being careful not to disrupt any mycorrhizal material that may be attached to the root. Dip your root into the second weigh boat, and rinse off any remaining soil. Finally, dip your root once again into the last weigh boat to remove the final remnants of soil.

3. **Setting up roots for examination.** Once you wash your root, lay it out horizontally along the grid. Keep adding roots end to end on the grid until you have filled up all the horizontal axes as shown below:
4. **Identifying Ectomycorrhizal Fungi.** ECM fungi are usually identified by branching patterns and appearance. ECM are attached to the root and branch out. ECM have a “Y” shape to them when they branch out. Page 188 of *Mycorrhizas for Forestry and Agriculture* has a good diagram to help you identify ECM.

5. **Counting ECM and Root Length.** Setup your counter, so that one counter counts the ECM at each intersection, and one counter counts just Root, and then one counter totals the two. Count each intersection on your Petri Dish. You may do this under a microscope.

6. **Calculations.** Colonization is calculated percentage per root length, by dividing the number of intersections with ECM by the total number of intersections assessed.

**References**

Brundrett M., Bougher N., Dell B., Grove T. and Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32. 374 + x p