Methods for Lab: Measurement of Hyphal Length

http://ccb.ucr.edu/lab_protocols.html

Add 0.5 to 1.0g of soil into 500ml of water. This value needs to be adjusted based on the soil type and total amount of hyphae, from preliminary trials.
Suspend the mixture for 10 to 90s in a Waring blender.
Pipette 10ml of suspension onto a membrane filter funnel. The funnel system should be compatible for a membrane that will fit on a microscope slide. The amount of suspension might also need adjusting depending on the soil.
The best membrane filter would be a 13mm diameter of 0.45μm mesh. An example is a syringe filter, <http://www.whatman.com/PuradiscSyringeFilters.aspx>.

Use a gridded filter for easiest measurement. Alternatively, a gridded ocular lens can be used. Determine the total length of grid lines observed.

For a gridded filter, this would be the total line length per filter

For ocular, it would be the total line length times the number of observation sets.

For example, count the number of intercepts, then move randomly to another location, re-count, move a 3rd time, count again. 20 to 50 observations is usually enough to generate a consistent number of hyphal intercepts. If 50 observations are used, the value is 50 X the gridded line length.

In either case, the total length of the lines must be known (or measured).
The suspension is sucked through the filter, washed with water, treated with a staining solution and then further washed with water.
The filter is placed on a slide and allowed to dry.
After drying, clear the filter using immersion oil for Millipore filters.

To calculate hyphal length, use the method of Newman 1966, as modified by Marsh 1971 for determining root length.

For Hyphal Length H:

$H = \pi N A / 2 L$

Where:
N= # intersections between the hyphae and the gridded lines (the counted value)
A is the total area of the filter
L = total line length of all the gridded lines

To calculate biomass from length, calculate the hyphal volume,
Where $V = \pi r^2 H$
Biomass can be determined as per Van Veen and Paul (1977), using a multiplier value for the hyphal moisture content.

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