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Fungal mycelia in soils - a new method for quantification of their biomass

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All plant-bearing soils are interwoven with fungal hyphae. Their structure and function are affected by environmental factors like drought, which might be a stress factor of increasing importance in many world regions due to climate change. The fungal mycelium in soil is important both for mycorrhizal symbiosis with plant roots and for litter decomposition, and thereby also for carbon turnover in soils. However, the mycelium biomass has been difficult to assess. Here we describe a simple and feasible method to quantify the biomass of fungal mycelium. We report on a manipulation study in the field where drought stress has been induced. The experiment was performed in a Norway spruce (*Picea abies*) 20 years old stand planted on former agricultural land, with a control plot and a roofed plot where precipitation was excluded. To investigate the fungal mycelium, nylon nets (mesh size 1 mm, width 7 cm and length 25 cm), were inserted vertically into the soil down to 20 cm depth. The nets were left in the soil from October to June, removed and replaced by new nets that were left in the soil from June to October. After removal, by cutting a block of soil around each net, the nets were cleaned from residual soil and scanned using the image scanner CanoScan 9000F. The resulting images were analyzed using the image processing software ImageJ. The image analysis was based on the distribution of grey values in the individual pixels which characterize the different components in the image (voids, hyphae, the nylon net, and soil). Based on the repeated visual evaluation of hyphal coverage in the net segments, we obtained an exponential equation allowing us to determine automatically the coverage of net windows by hyphae in percentage for each net scanned. In this way we can compare the hyphal coverage in the control and the drought-exposed plots. Based on the hyphal coverage scans together with hyphal dry weight on clean nets, we account for the soil particles adhering to the nets. Using this analysis method, the hyphal mat coverage in mm^2 on any net is quantified and the hyphal biomass on the net can be calculated and compared between treatments. Also, the hyphal biomass per cm^3 soil at the spot where the net has been inserted can be assessed. In addition, DNA from net-bound hyphae may be extracted to determine the identity of fungal species at different soil depths for the individual treatments.