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How deep can ectomycorrhizae go? A case study down to 4 meter depth in a Brazilian eucalyptus plantation under rainfall reduction

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Most often, it is recognized that mycorrhizae are only occurring in the first layers of soil and decrease drastically with depth. Unfortunately, the vertical distribution of mycorrhizal associations remains poorly understood and very few studies have investigated these symbiosis in very deep layers, the majority of studies focusing on the topsoil. In this work, we characterized, in a 5-year-old *Eucalyptus grandis* plantation in Brazil, changes in fungal community within soil profiles and whether these changes are influenced or not by a water stress using a through-fall reduction experiment. This research was conducted at the experimental station of Itatinga (ESALQ-USP). Root samples were collected between May and June 2015. Fine roots (< 2 mm) were sampled carefully layer per layer during the digging of a pit (1.5 x 4 meters) in order to prevent any contaminations between two consecutive soil depths. Ten soil layers have been sampled from 0 to 4 meter depth. An Illumina sequencing of the Internal Transcribed Spacer (ITS2) was carried out on DNA extracted from root samples to study fungal community composition and diversity. We showed that the fungal communities were dominated by ectomycorrhizal fungi (ECM). The fungus *Pisolithus* represented more than 80% of the fungal community. For

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the first time, both ECM sequences and ECM root tips were observed along the profiles down to 4 meters. By subsampling the root tips of *Pisolithus*, we showed that *Pisolithus* populations responded strongly both to the depth and to the rainfall reduction. This work represents a step forward in the microbial ecology with the evidence, for the first time, of the occurrence of ECMs down to a depth of 4 meters. These results highlight the importance to expand these researches to other tropical perennial plants of major interest in order to evaluate the genericity of our observations made on eucalyptus.

Keywords: deep soil, ECM fungi, *Pisolithus*, tropical forest plantations, eucalyptus, drought stress