Title
Linking minirhizotron images to soil physical properties and microbial diversity (TER 2)

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Linking minirhizotron images to soil physical properties and microbial diversity
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Introduction: Minirhizotron images and mycorrhizal fungal diversity

Automated Minirhizotron (AMR)
• The AMR has demonstrated better image quality than conventional manual minirhizotrons.
• The AMR is capable to record images at user-defined intervals that will be able to capture short-term ecological changes.
• We have analyzed >60,000 images from manual minirhizotrons taken at the James Reserve since June 2005.

Mycorrhizal fungal diversity at the James Reserve
• Mycorrhizae (the association of a mycorrhizal fungus and a plant root) are important for plants because they participate in nutrient and water uptake.
• The spatial and temporal variation of mycorrhizal fungal diversity remains unknown and the James Reserve provides an excellent test-bed to explore spatial and temporal patterns.

Problem Description: Roots and fungi: who are they; where they are; how long they persist?

Soil sensors, AMR and molecular identification
• AMR images and environmental variables
  • Which are the factors associated with root and fungal growth? How long does a root or rhizomorph live?
  • Continuous measurements of soil temperature, moisture and CO2 coupled with the analysis of minirhizotron images is helping to answer these questions. Differences in microbial diversity in space and time could influence environmental variables such as soil respiration.
  • One main challenge is to design image software recognition to sort from AMR images.

Proposed Solution: Environmental observation and mycorrhizae identification

Molecular identification process
• Field collection of soil cores
  • Soil hyphae
  • Roots
  • DNA extraction
• DNA sequences are analyzed using BioEdit software
• PCR products are cloned
• Soil hyphae DNA
• PCR products ready for sequencing
• PCR amplification of fungal DNA
• Clones are screened, purified and prepared for sequencing
• DNA sequences match with known sequences using BLAST (NCBI database)

Minirhizotron images and soil respiration
• PCR products
  • Root
  • Rhizomorph
  • Hyphae
• Molecular identification
  • We need to understand temporal and spatial variation of mycorrhizae diversity and changes in root colonization/infection, soil colonization, and spores production.
  • Morphological identification is limited for arbuscular mycorrhizae spores and ecto-mycorrhizae roots.
  • Combining AMR images, morphological and molecular identification is an alternative solution to track spatial and temporal changes in mycorrhizae diversity.

Figure 1. Prototype of AMR with USB microscope will generate >10 GB of image data along with soil sensor array (e.g. soil temperature, moisture and CO2 sensors).

Figure 2. Image of root, soil particles and AM (arbuscular mycorrhizae) hyphae taken with the AMR using the USB microscope.

Figure 3: EM root tip morphotypes.

Figure 4: All images.

Figure 5. Molecular identification process done at the James Reserve using PCR amplification of fungal DNA ITS 1 and 2 of Ribosomal DNA.

Figure 6. EM morphotypes present at the James Reserve in the organic and mineral soil. Preliminary results suggest that morphotypes richness did not vary with soil depth but we observed a differential vertical distribution of percent EM morphotypes root colonization.

Figure 7. Lifespan probability from March-October 2005 and December 2005-June 2006. Probability to find a dark root (DR), light root (LR), dark rhizomorph (DRh), light rhizomorph (LRh) and hyphae (H) in the sequence of minirhizotron images was higher for the second period of the survey.

Figure 8. Counts of root tips, rhizomorphs and hyphae per frame of a minirhizotron tube at the James Reserve from March 2005 to June 2006. Preliminary results suggest that 40% of the variation in soil respiration was explained by the combination of root and hyphae counts for the period studied. Rhizomorph counts were higher than root and hyphae counts but have higher variation. Further analysis should be done to partition sources of soil respiration (e.g. carbon respired from roots or from soil sources) using 14C methods.

Figure 9. Relationship between monthly average of soil respiration and monthly root count. We found a significant positive relationship (r²=0.71, p=0.000) between soil respiration and root count. During warmer months (June, July and August) there were more roots and more soil respiration. During colder months (December, February, and March) we found less roots and lower soil respiration. Preliminarly results suggest that soil respiration might not be dependant only on soil temperature, but soil water content and root production might be important factors of CO2 efflux at the James Reserve.