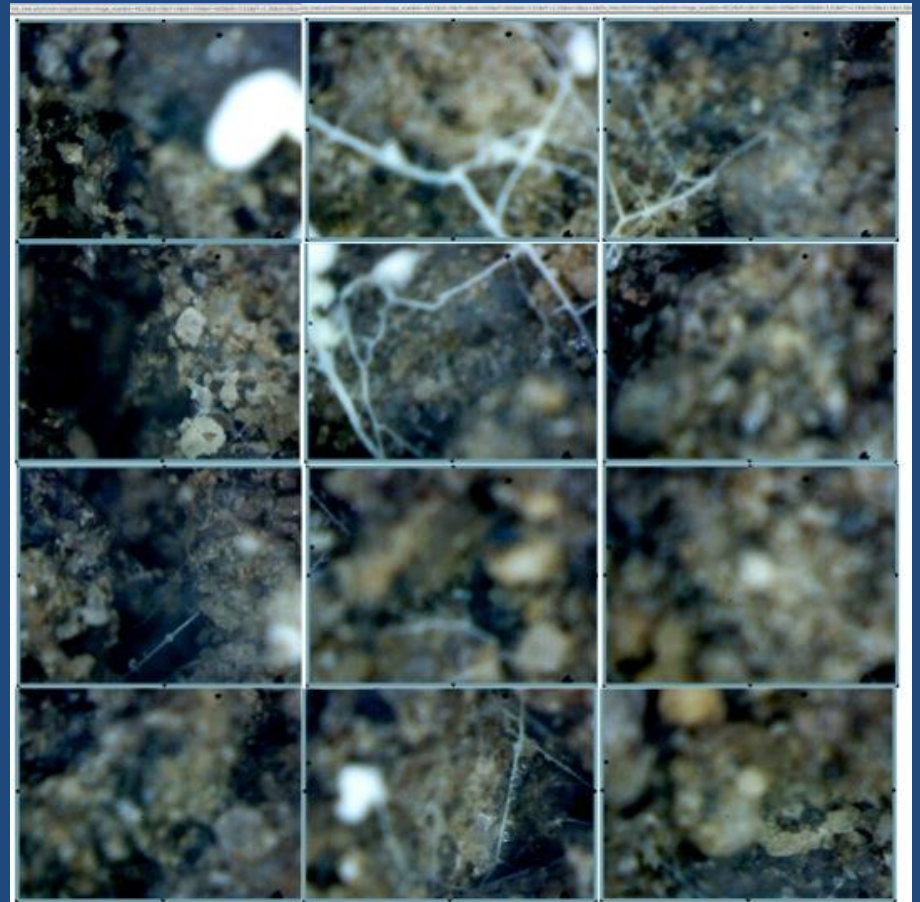


Visualizing daily *in situ* fungal hyphal dynamics: Responses in a tropical-versus Mediterranean-ecosystem forest

ESA August 2014

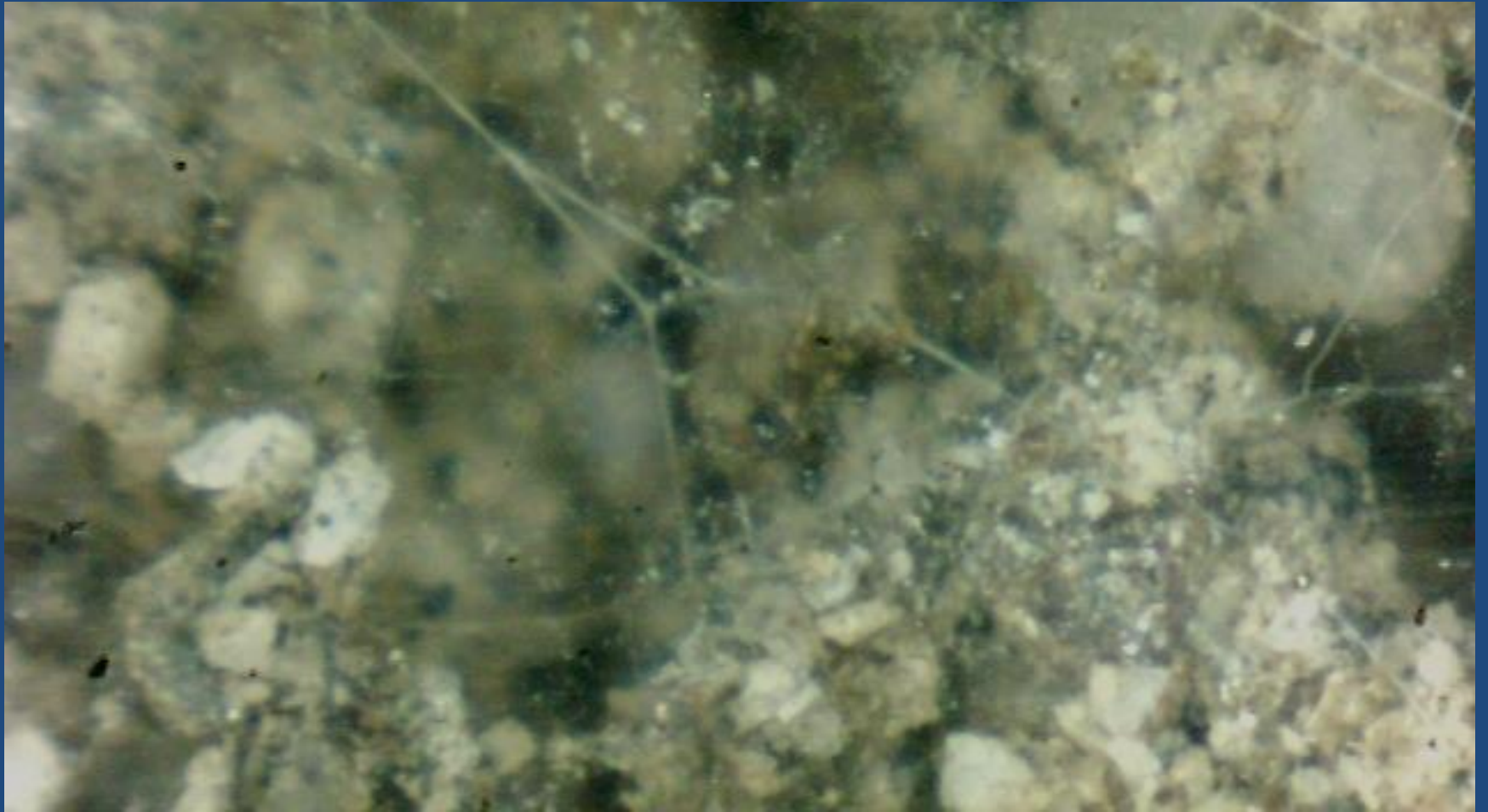
Michael F. Allen



Newly forming AM behind Region of Elongation



Expanding AMf hyphal network



RootFly Digitizing & Image Recognition

The screenshot shows the RootFly software interface. The main window displays a grayscale image of roots with red lines indicating detected root segments. A properties panel on the right shows the following details for a selected root:

- Tube: 1
- Window: 1
- Session: 213
- Root ID: 5
- Diameter: 0.34 mm
- Length: 15.80 mm
- Color: White
- State: Live
- Custom: NO_CODE

Below the properties panel, there are checkboxes for "Show Roots", "Show Dead Roots", "Show Root IDs", and "Show Diameters". A "New Root" button is also visible. At the bottom, a session list shows thumbnails for sessions 211, 212, 213 (selected), 214, and 215, all dated 5/20/9. The status bar at the bottom indicates "21.3 pixels per mm" and "28 roots in window - auto-detect has not been run".

The screenshot shows the Root Detector software interface. The main window displays a grayscale image of roots. The interface includes several processing steps and parameters:

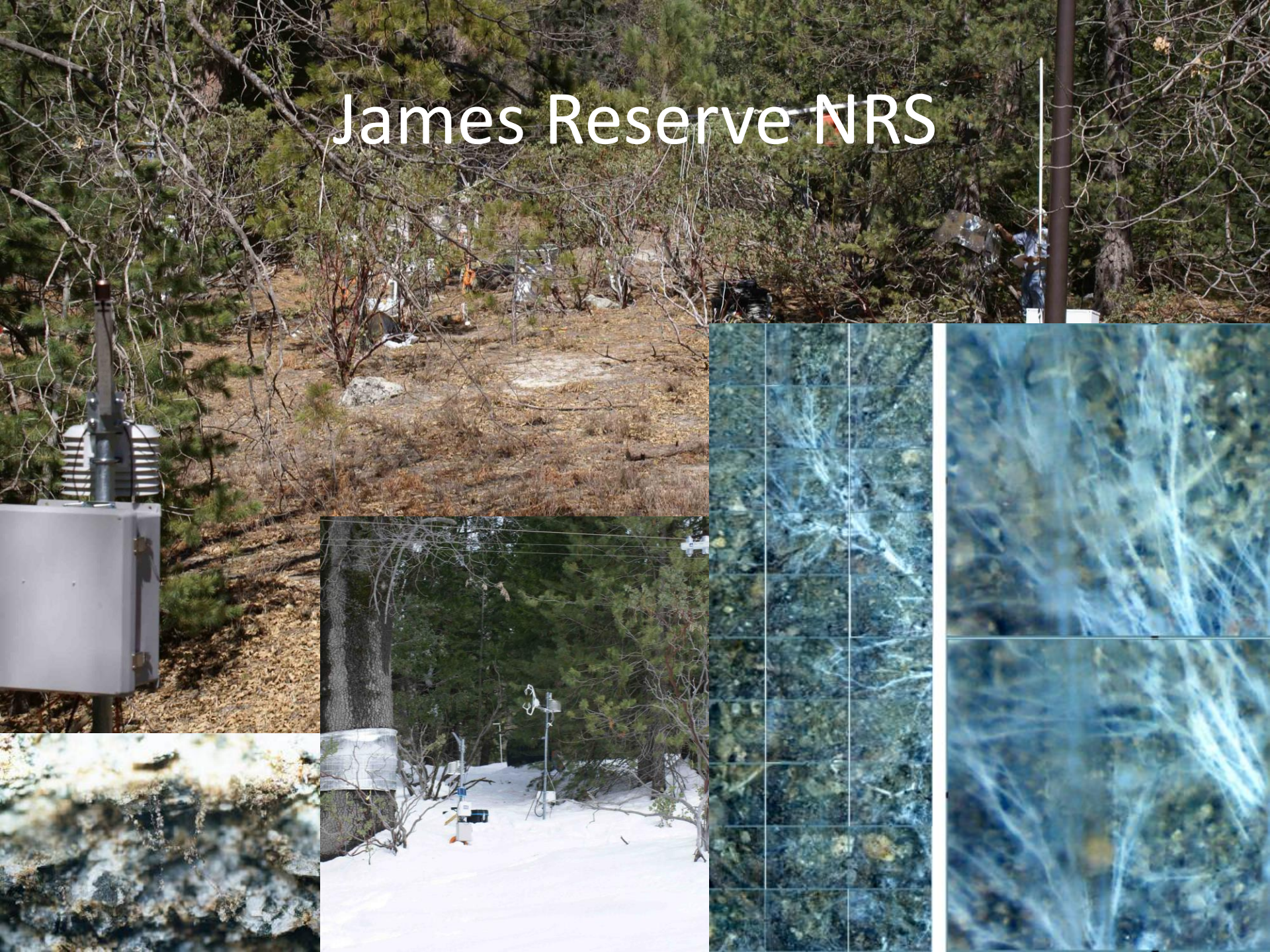
- Step 1: Select Image**
- Step 2: Click on image for each color value you want to filter on**
- Color Space in Spherical Coordinates:**
 - R from 335.7 to 425.03
 - Mean, RMS = 309.9, 25.35
 - Theta from 50.40 to 95.04 degrees
 - phi from 46.10 to 90.65 degrees
 - Colors Selected = 9
 - Background Mean, RMS = 263.3, 77.38
 - Normalise To (129, 128, 128)
 - Detect: Roots Soil
 - Manage Color Real Time
- Step 3: Filter On Colors Selected**
- Filter Method:**
 - "C" method
 - "H" method
- Guard Zone:** 15
- Ignore Borders:**
- Ignore Pixels Around Edges:** 0
- Minimum Pixels:** 10
- Maximum Ratio:** 294
- Image Width (mm):** 2.07
- Maximum Gap (pixels):** 100
- Groups Found:** 273
- Valid objects identified:** 29
- Image Size:** 640 x 480
- Analysis Time:** 00:04
- Step 4: Identify Object:**

The main image shows a grayscale image of roots, and a smaller inset shows a color-coded version of the same image where roots are highlighted in green and red.

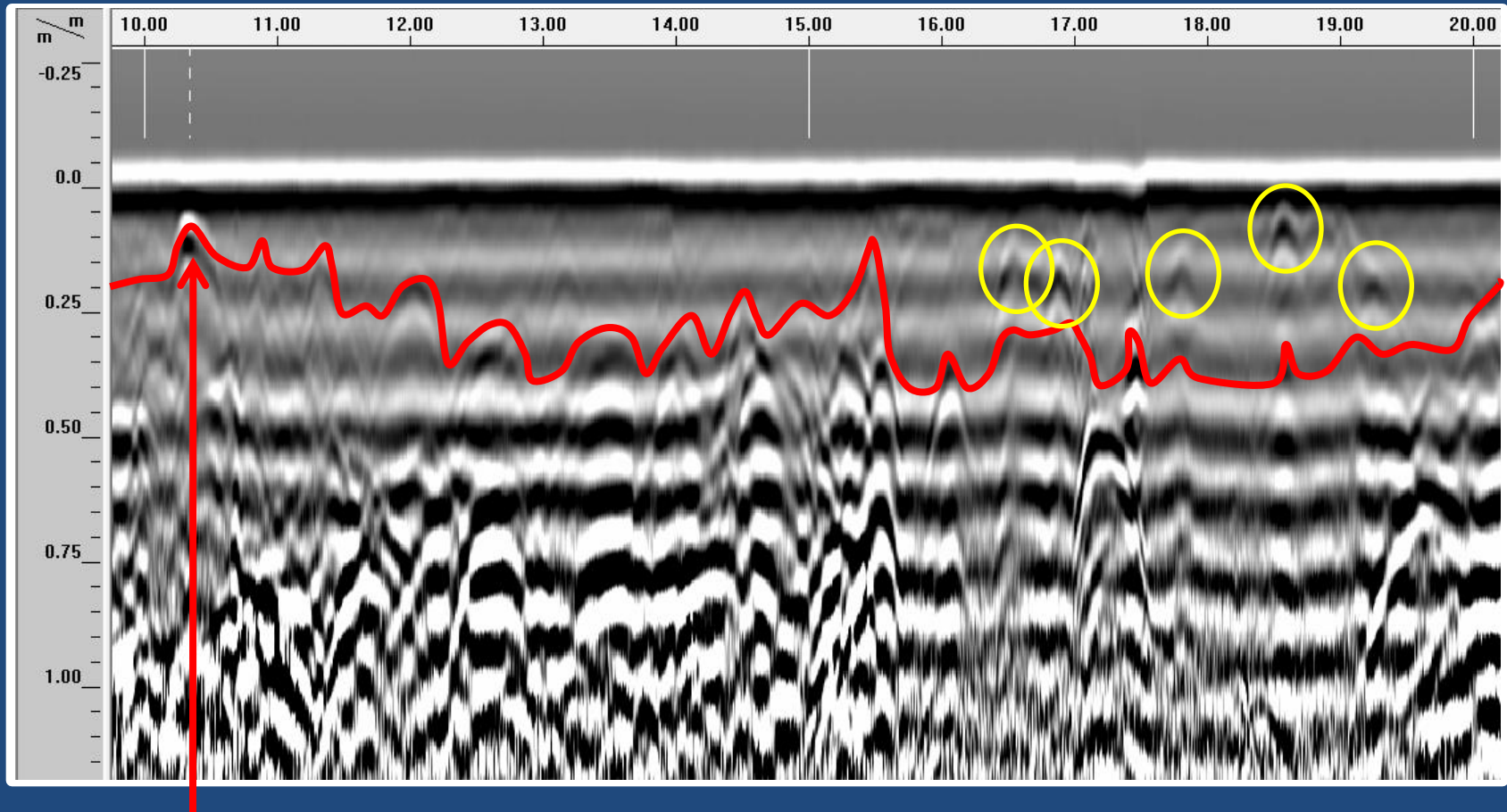
The screenshot shows the Activity and Results Log window, which displays statistical data for the root detection process:

- Using the method of semi perimeter:
 - Mean Diameter = 0.02 (mm)
 - Length = 0.12 (mm)
 - Bio Volume = 0.00 (cubic mm)
- Using the method of end points:
 - Mean Diameter = 0.03 (mm)
 - Length = 0.08 (mm)
 - Bio Volume = 0.00 (cubic mm)
- Object 265:
 - Using the method of semi perimeter:
 - Mean Diameter = 0.02 (mm)
 - Length = 0.06 (mm)
 - Bio Volume = 0.00 (cubic mm)
 - Using the method of end points:
 - Mean Diameter = 0.01 (mm)
 - Length = 0.03 (mm)
 - Bio Volume = 0.00 (cubic mm)
- Bio stats total over all valid objects in this image:
 - mm per pixel: 0.0017
 - Using semi-perimeter method:
 - Length = 16.57 (mm)
 - Bio Volume = 0.01 (cubic mm)
 - Using end point method:
 - Length = 5.70 (mm)
 - Bio Volume = 0.00 (cubic mm)
- Bio stats total over all gaps (discontinuities) in this image:
 - Length = 5.51 (mm)
 - Bio Volume = 0.01 (cubic mm)
- Combined result:
 - Using semi-perimeter method:
 - Total length = 22.08 (mm)
 - Total Bio Volume = 0.02 (cubic mm)
 - Using end point method:
 - Total length = 11.21 (mm)
 - Total Bio Volume = 0.03 (cubic mm)
- Biomass index (total biovolume/image area):
 - Using semi-perimeter method = 0.0024 (mm)
 - Using end-point method = 0.0044 (mm)
- Biomass as fraction of soil biomass index in mm divided by camera DO:
 - Using semi-perimeter method = 0.1877 %
 - Using end-point method = 0.3488 %

James Reserve NRS

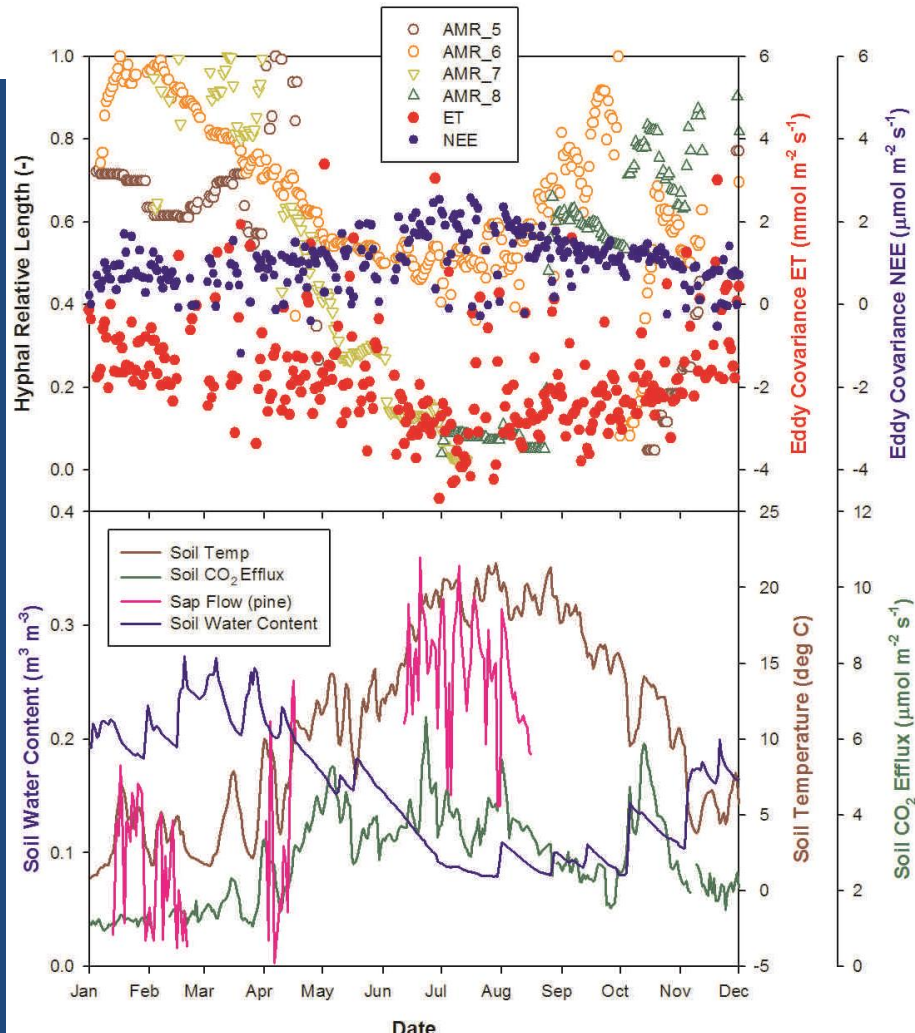
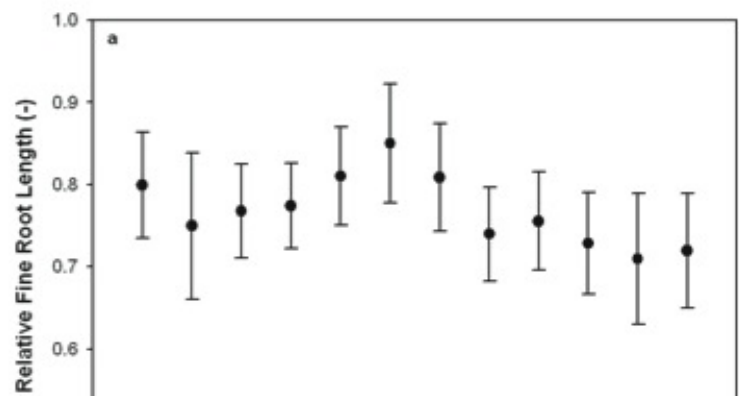


Soil Depth (per GPR)



Soil depth / bedrock interface (red line). Calibration metallic object buried at 10 cm depth (arrow). Coarse roots (yellow circles).

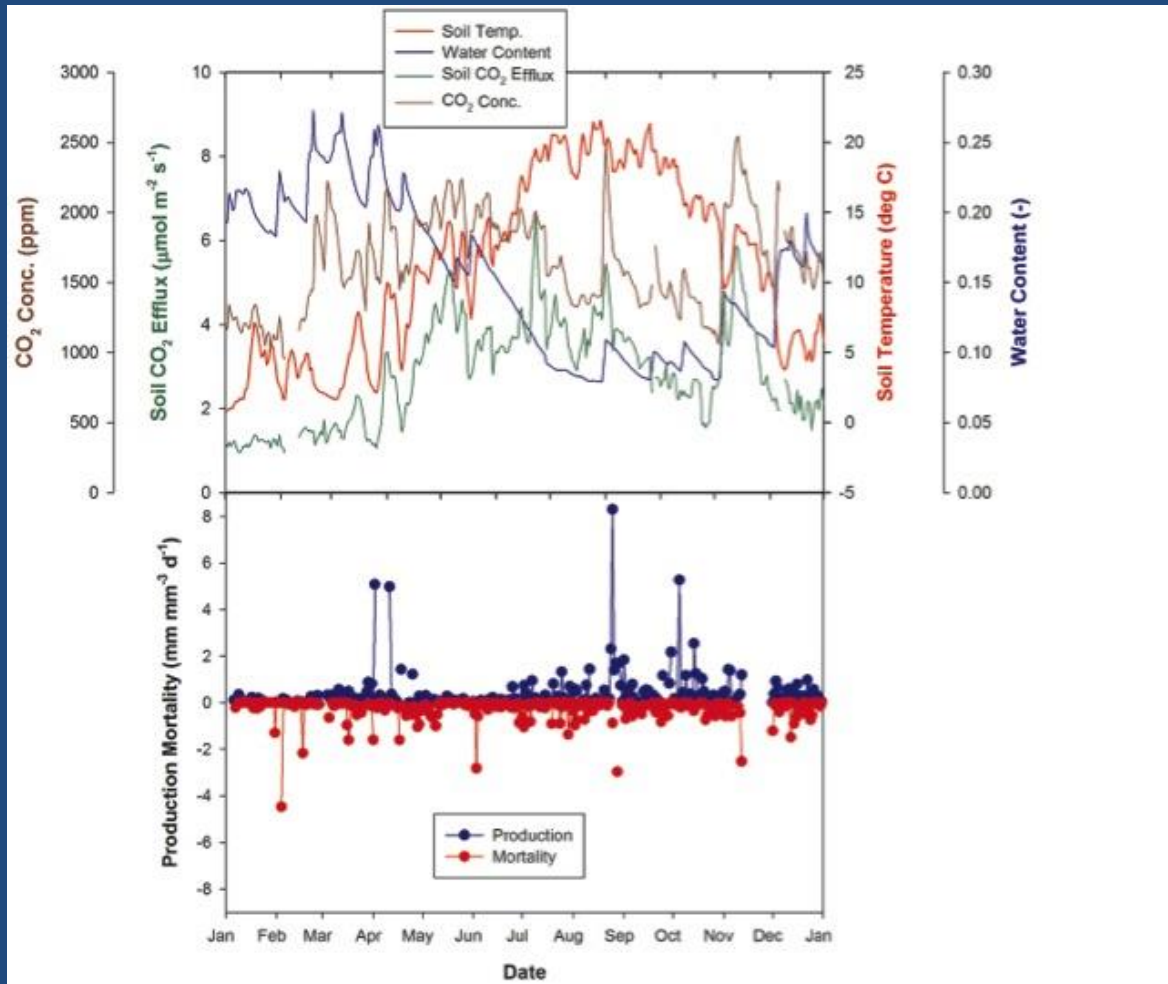
Ecosystem Responses: James Reserve: CO₂



- HL= Rs, ET, NEE
- $R^2=0.159$, $p<0.0001$
- HL= 0.697+
0.005 Rs, $p<0.0001$
0.019 ET, $p=0.461$
-0.095 NEE, $p<0.0001$

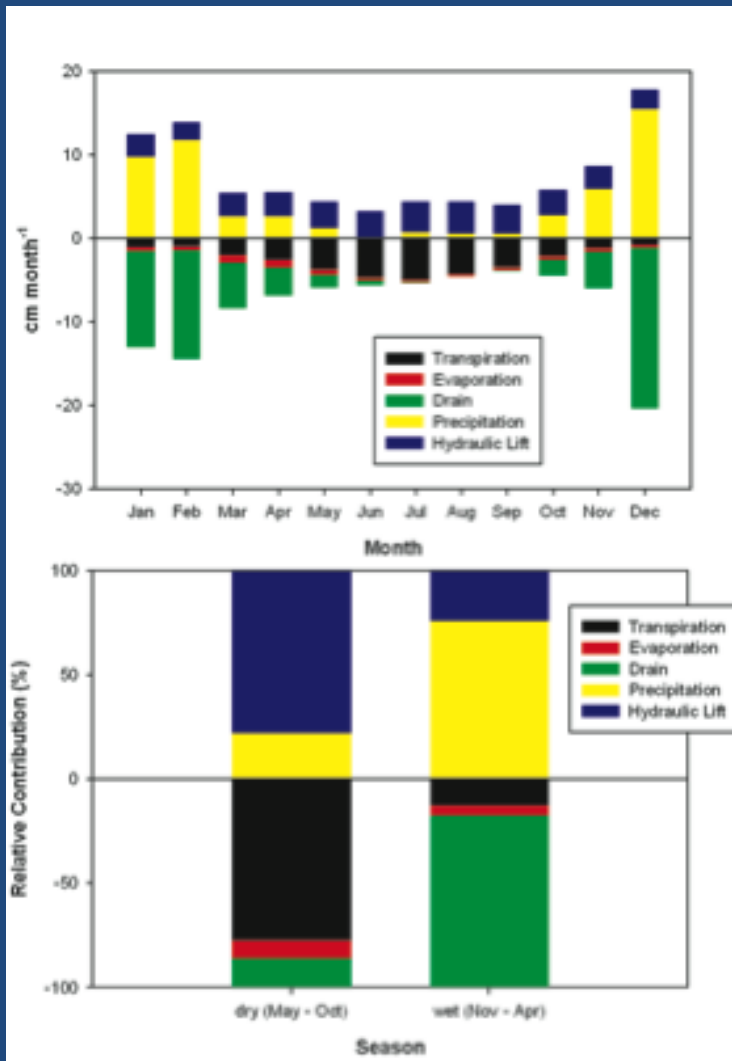
James Reserve: MF responses

- $HL = -1.22 (0.165\theta - 0.14T)$ $R^2 = 0.40$, $p < 0.0001$



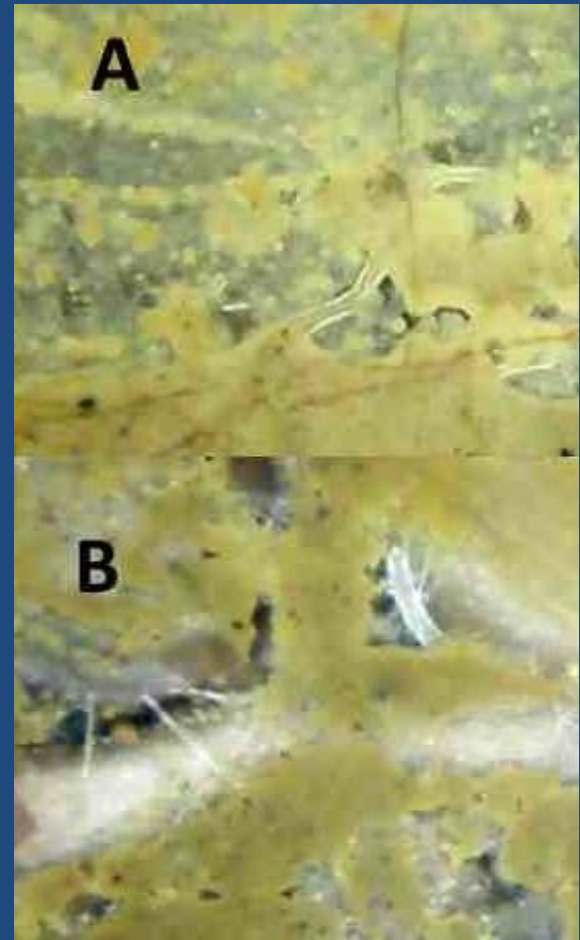
EM allocation in Forest (g C/m²/y)

James Reserve Forest Dependent on Deep Water

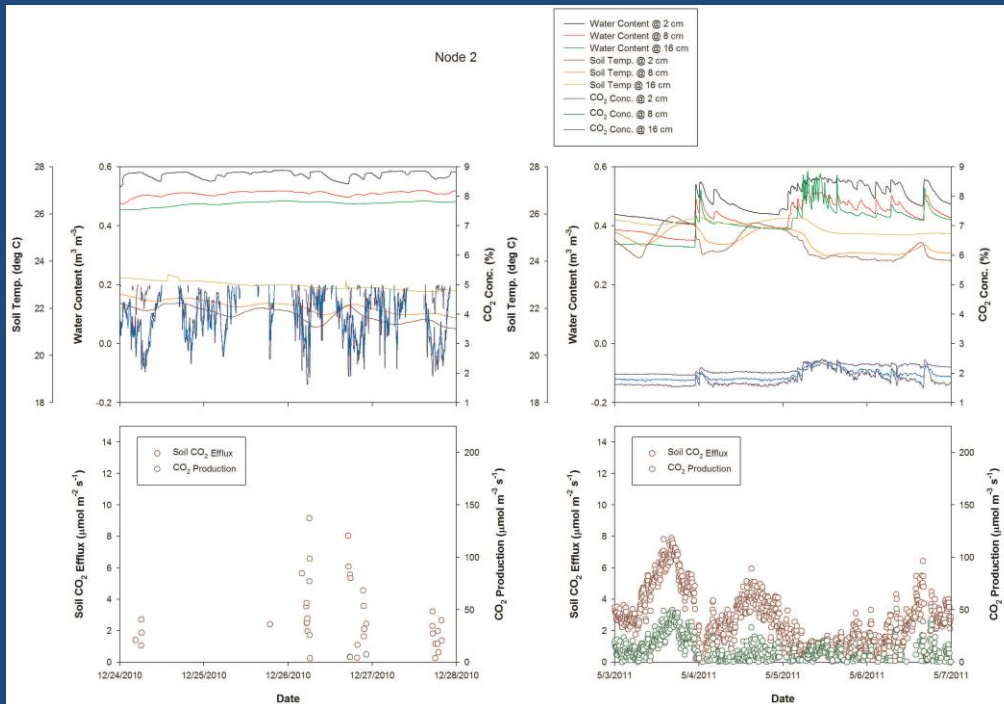
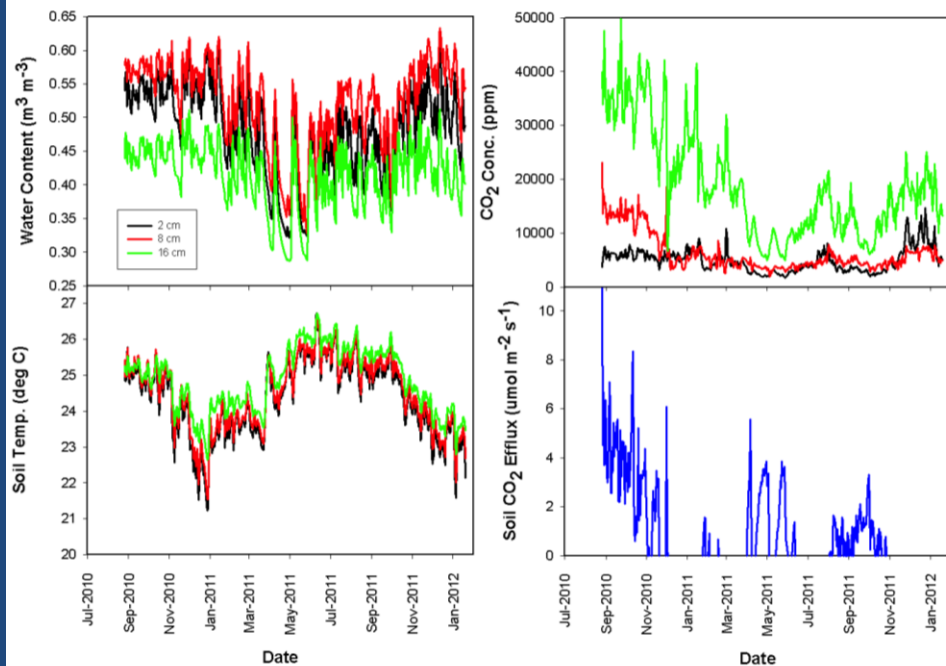
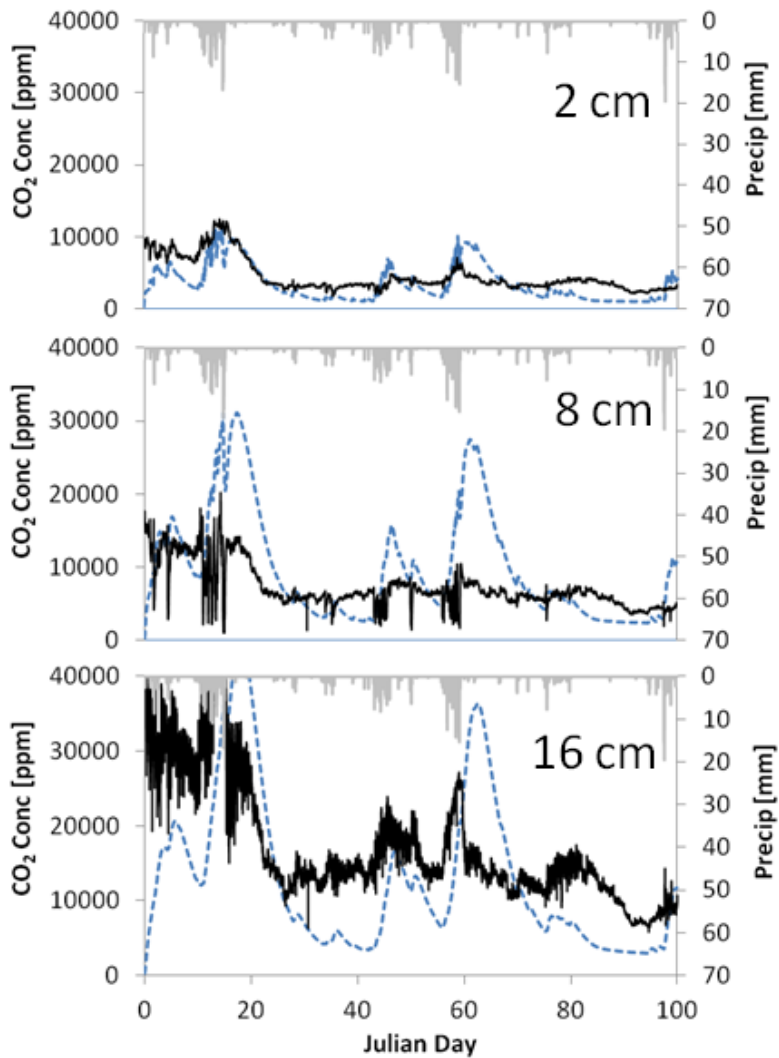


- Water in the rocks below the soil sustains ET and NEE late into summer, and, through hydraulic lift, fine roots and some hyphae.

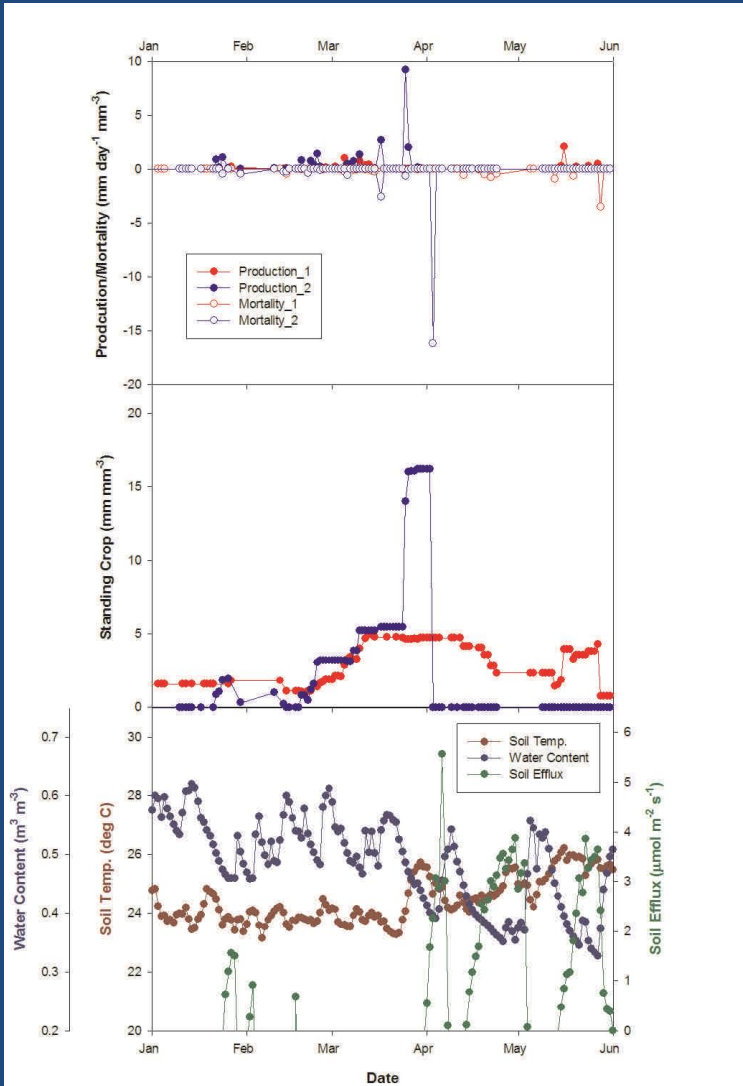
La Selva Biological Station



La Selva CO2

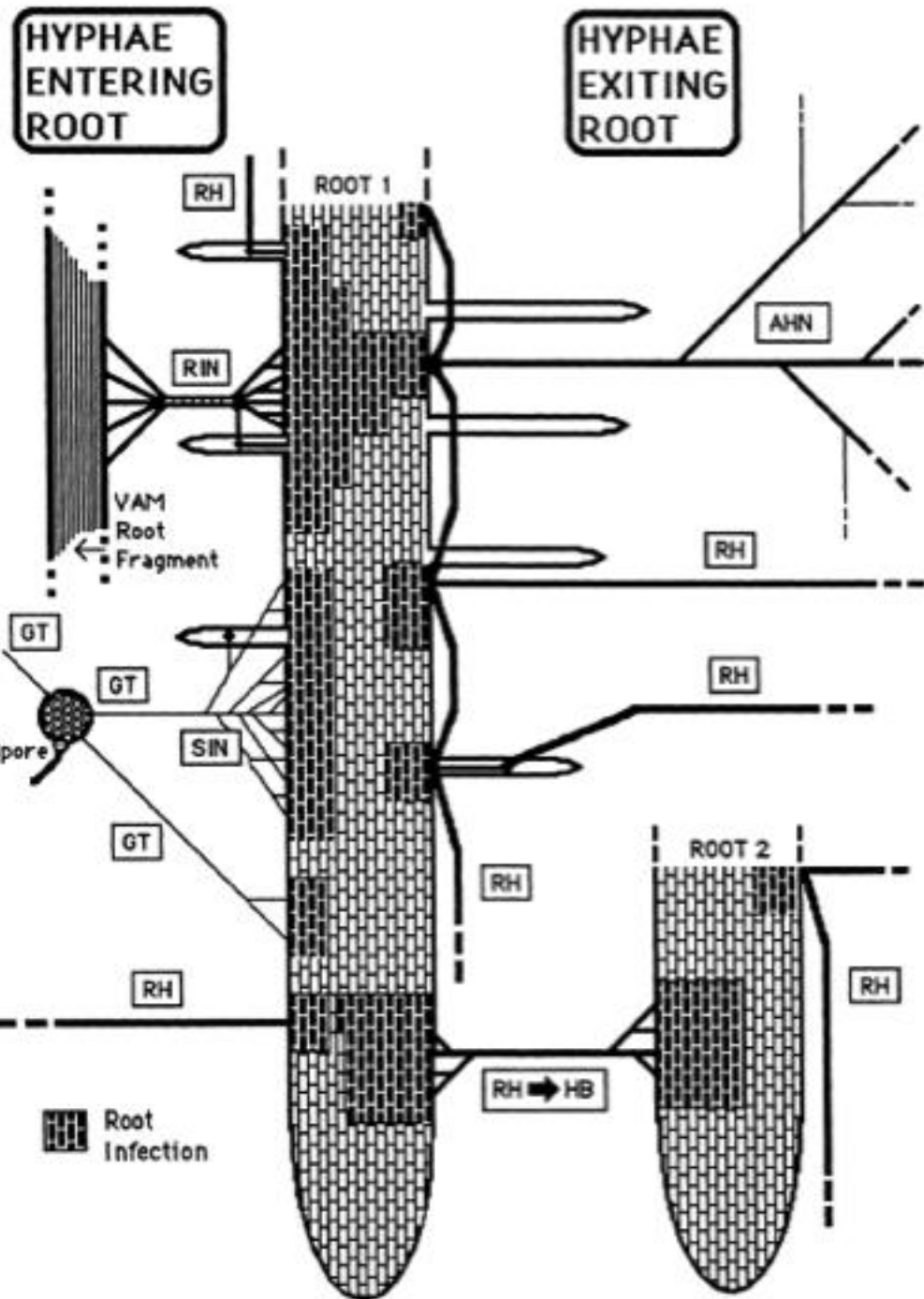


La Selva

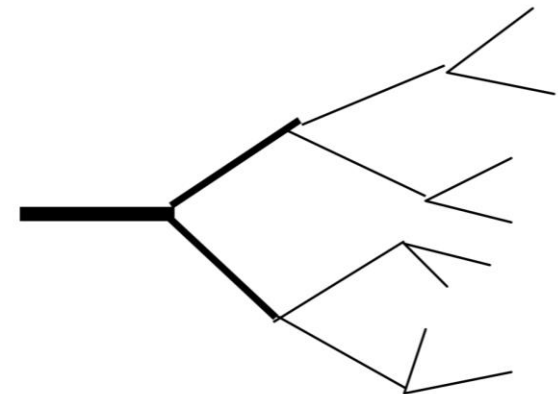


- $\delta RL = 72 (-18\theta - 1.4T)$
- $R^2 = 0.47, p = 0.0026$
- $HL = 191 (-100\theta - 5.22T)$
- $R^2 = 0.40, p < 0.0001$
- At 8cm depth
- $CO_2 =$
 $17988 \theta, p < 0.0001 +$
 $1427 T, p < 0.0001 +$
 $-220 \text{ Root}, p < 0.0001 +$
 $-65 HL, p < 0.0001$

Friese & Allen 1991, Allen et al. 2003 Developmental Model



AM hyphal dynamics



Hyphal length (h)
 a = segment length = 0.5 cm
 $h = a(1) + a(2) + a(4)$

geometric series
 a = segment length = 0.5 cm
 r = branch ratio = 2
 n = # branches, 1-8

$h = \sum_{i=1}^n [a(r^i - 1)] / (r - 1)$
 $h = 122$ cm/entry point

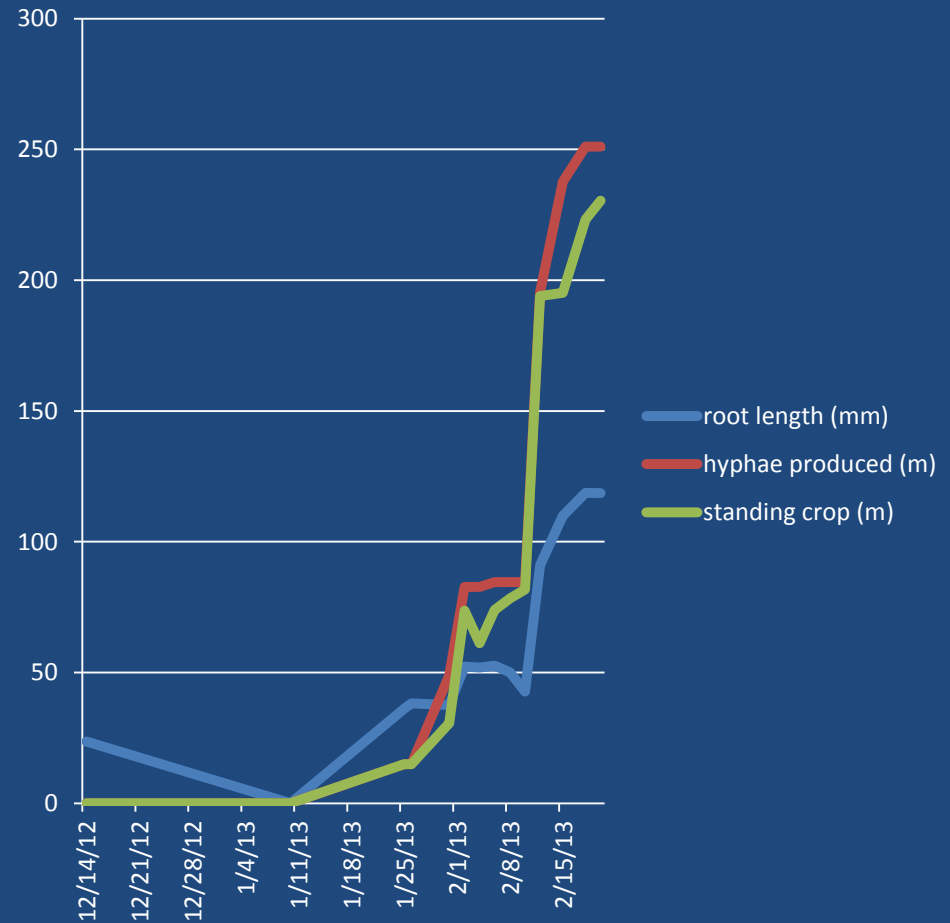
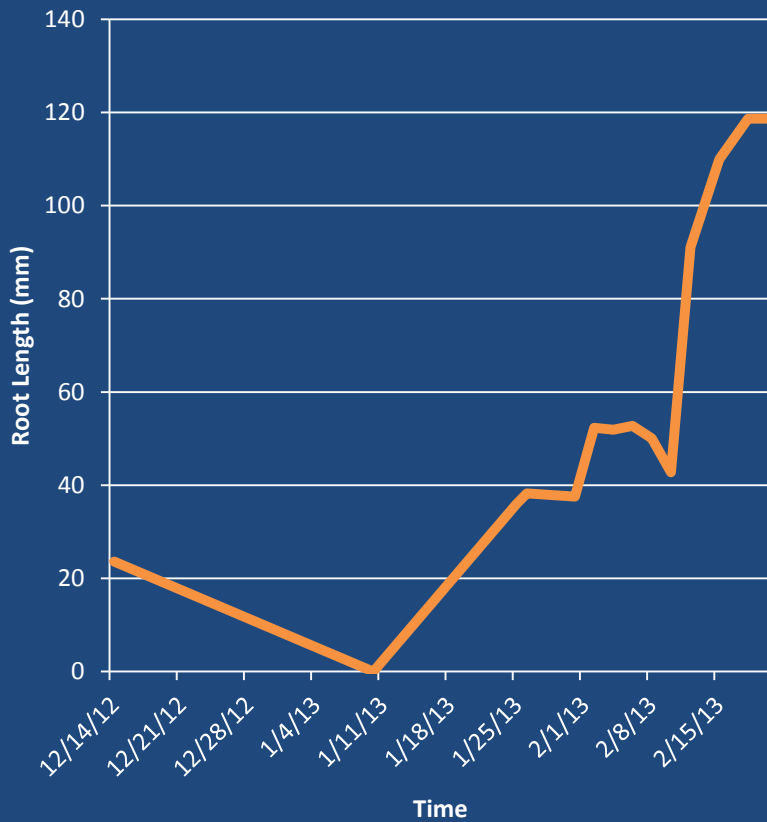
Dynamics of one location: tube surface 67.5mm^2

Motte Reserve, NRS

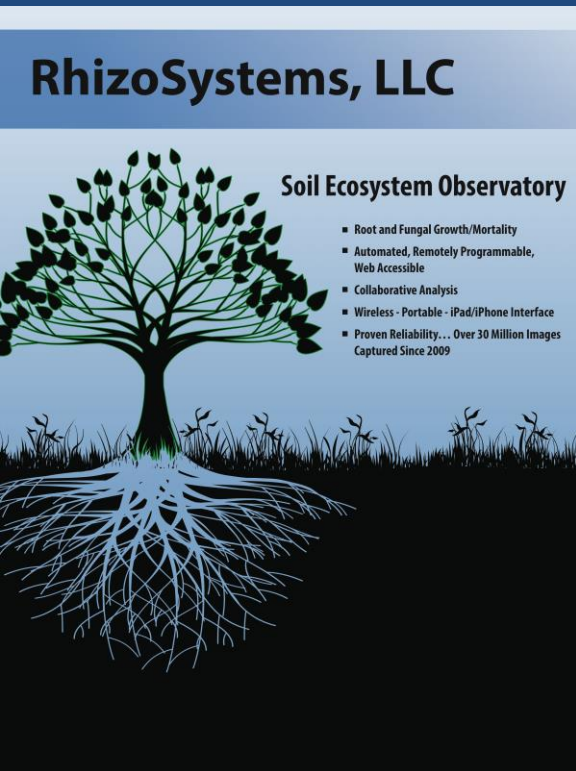


Modeling AMf Hyphal Production

Root Length Sums over Time



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