Hi everybody! Here’s a sort of bare-bones protocol. Please feel free to edit/add/delete whatever you deem necessary. I didn’t include recipes for bleach, EtOH, or CTAB but that would be easy to do. The parts that need the most work are probably the volume displacement and penetrometer steps as I was relatively oblivious to how those were done. It might also be useful to incorporate time into this protocol - i.e. how many days it took to do each plot, etc.

1. After clearing plots of major leaf and twig debris, the logs that were selected for harvesting in year 3 on the plot maps were identified and evaluated for animal damage. If damage such as gnawing was observed or if a log did not appear to be intact enough to harvest, a different log of the same species and year was selected for harvesting.

2. Planks were removed from plots and placed on a shelf in the warehouse on the first day of harvest from each plot.

3. Logs were harvested in groups of sixteen - either sixteen from one plot or groups of eight from two plots. One thumb tack was pushed into each end of each log to designate tops. Each log was placed in a separate plastic bag. (These bags were re-used for each plot.) Large pieces of bark that lay directly beneath harvested logs were collected in the plastic sample bags along with the logs. (0.5 cm2 mesh screen was used to determine minimum size included).

4. Paper bags and small dram vials for insect collections were labeled with log and plot IDs. Any bark and soil that was loose on the logs was scraped into the sample bags. This material was passed through a piece of 0.5cm2 mesh and examined for insects and fruiting bodies. Any material that did not pass through the mesh was collected in the labeled paper bags. ID wires were cut from logs and tags were carried with the logs during processing. At the first processing stage, logs were:

a. measured for length (in cm, taken across top from tack to tack with a ruler) and top-to-bottom and side-to-side diameter (in mm, taken with digital calipers) of each end.

b. assessed for insect damage (1-4 scale\*). A piece of thin wire was used to determine the depth of insect bores.

c. assessed for presence of fungal fruiting bodies (y/n). Fruiting bodies were photographed if present (labeled with (D) in spreadsheet if Darcy’s camera, otherwise Maranda’s) and collected in coin envelopes if they were relatively fresh. Mycelium was described if present.

d. examined for insects along with the bark and debris that was scraped into the sample bags. For most logs, all insects were collected in a 1 dram vial containing 70% EtOH, but for those containing ant or termite nests, a representative sample of morphologies was collected. Insect vials were set aside to be stored at 4C.

All lab members wore gloves from this point on.

5. Logs were surface sterilized in plastic shoeboxes by rotating continuously in:

1. 95% EtOH for 5 seconds
2. 0.5% sodium hypochlorite (bleach) for 2 minutes
3. 70% EtOH for 2 minutes

Logs were placed in individual plastic shoeboxes labeled with the log ID, plot ID, and time of removal from 70% EtOH. Boxes were very loosely covered and placed in an air-conditioned room along with the tag and labeled paper bag(s) for each log.

6. Logs were left to air dry for approximately 1-2 hours (lunch time - Germinator turned on before leaving). Dryness was determined by the disappearance of EtOH odor.

7. Sieved material in bags was weighed, recorded as “wet weight excess,” and discarded.

8. In order of plot ID and dryness, logs were brought back into the lab and wet weights were recorded as “wet weight log.” Post-weighing, logs were wrapped approximately twice in Saran Wrap, enough to maintain structural integrity of the most decayed logs during processing.

9. Logs were clamped to the drill rig with the top side facing out. Eight holes were cut in the Saran Wrap with sterilized scissors. Sawdust was drilled from each hole with a Germinator- sterilized ¼” drill bit and collected in a UV-sterilized weigh boat. The 2.5 cm drilling depth was determined by a metal “bumper” attached to the drill. Top-drilled logs were then flipped over in the drill rig and the bottom of the log was drilled according to the same protocol with sterilized scissors and drill bits. Bottom sawdust was collected into a different weigh boat.

11. Logs were secured in a clamp on the floor for penetrometer assessment. The drilled holes on top and bottom were placed to the sides so that an undisturbed area could be used (penetrometer measurements were taken on the sides of the logs). The point of the penetrometer was placed as close to the center of the log as branch stubs would allow. The weight was dropped twenty times or until the point emerged from the bottom of the log. If a log splintered before twenty hits, a second penetrometer reading was taken at another point near the center of the log. The number of hits required to go through the log and the distance (mm, measured with digital calipers) that the point was driven into the log was recorded. Saran Wrap was removed before being passed on to measure volume.

10. Logs were weighed immediately after penetrometer measurements were taken to determine mass loss due to drilling and penetrometer. Logs were secured for volume displacement measurement by either pinning with probes, or attaching wire to thumbtacks, depending on level of decay. Logs were then immersed in approximately 1.5L of water placed on a large-scale balance. Logs were held under water until all bubbles were released. If necessary, logs were rocked side to side to release trapped air. The mass on the scale was recorded as log volume. Logs were removed from the water and allowed to drip for approximately 10 seconds. The negative value on the balance was recorded as the amount of water absorbed. Thumbtacks were removed.

11. Logs were wrapped in labeled paper bags and placed in a drying oven set at 100-105 C. We found that it helped to write the date placed in oven on the bags.

12. Drilled sawdust was weighed to ensure that enough material (~1.5g) was available for DNA and enzyme cryovial allocation. Weigh boats with covers indicating top (t) or bottom (b) were placed at the allocation station along with log ID tags. One set of cryovials were labeled with the following scheme for DNA and enzyme allocation: SPECIES (A-X) PLOT (1-8) TOP/BOTTOM (t/b) REPLICATE (y/z) [see 2012 species decoder]. 0.5 ml of sawdust was allocated into each tube for DNA extraction using a Germinator-sterilized scoop. This scoop was re-used for packing enzyme cryovials with sawdust to 0.5 g. The working surface was wiped down with 70% EtOH and/or 0.5% bleach after DNA top sawdust allocation to reduce the amount of airborne sawdust. 1.0 ml of 2% CTAB was pipetted into each cryovial. Tips were changed between top and bottom tubes. A Germinator-sterilized scoop was used for bottom sawdust. Tubes for DNA extraction were stored in an air-conditioned room while those for enzyme analysis were stored in a -20 C freezer. Enzyme tubes were transferred to a -80 C freezer at UMSL on dry ice.

13. After at least three days in the drying oven, logs were removed and weighed for “Dry weight.” Date of removal was recorded. Tacks should be removed before weighing.

14. After all logs cycled through the drying oven, planks were removed from the warehouse, cleaned, and placed in the oven. Logs were stored at room temperature (no ac) in the lab until drilling for chemical analyses.

\*Insect damage assessment scale

**0**: No observable damage/1-2 shallow (0-2 mm) holes, indicating no insect damage

**1**: Little observable damage/3-5 shallow (3-5 mm) holes distributed over an area of several cm²

**2**: Definitive damage/5+ shallow (3-5 mm) or deeper (5+ mm) holes distributed over a broad area; observable damage across log

**3**: Lots of damage/many deep (5+ mm) holes distributed over a broad area; observable damage across log

**4**: Too much damage to remove from site - we should not have anything classified as a 4 on our spreadsheet.