



COMPASS Field Sampling Protocol Chesapeake Bay Sites

Date Created: 2 August 2022

Creator Name(s): A. Stearns

Version: v1

Date Updated: 27 Feb 2023

Editor Name(s): A. Stearns

Tree GHG Wells: Installation and Sampling

Objective:

To extract gas from trees for CO₂ and CH₄ analysis.

Contents:

- I. Experimental Design
 - II. Personal Protective Equipment
 - III. Installation
 - A. Materials
 - B. Procedure
 - IV. Sample Collection
 - A. Materials
 - B. Procedure
 - V. Corresponding Documentation
 - VI. References
-

I. Experimental Design:

At Synoptic Sites, each tree outfitted with a sap flux sensor will also have a tree gas well installed at breast height (1.3 m).

At the TEMPEST Site, the first three replicates of each tree species in each plot, outfitted with a sap flux sensor, will have a well installed at ~ 0.8 cm above the ground. Some ACRU's will have two wells installed, with the second well installed at ~ 1.3 m above the ground.

Depending on the site and location, the sample tree will have one of two sized gas wells installed, either a 8 mm I.D. or 12.5 mm I.D.

Samples taken from the 8mm wells will have a dilution factor of 2/14 since we use exetainers that are flushed at atmospheric pressure with N₂ and thus have 12 mL of N₂ inside.



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II. Personal Protective Equipment: Close-toed shoes and long pants are required at all times when working at COMPASS sites. Rubber boots are strongly recommended in the Wetland and Transition zones. Chest waders are available upon request. Eye protection is available and *is required* in the Wetland zones.

III. Installation:

A. Materials:

- Tree borer (either 12mm or 8mm) or drill bit if no core is needed
- $\frac{3}{8}$ " or $\frac{1}{8}$ " stainless steel pipe
- Hammer or weighted dead blow hammer
- If interested in saving cores for genomics add gloves, isopropyl alcohol (e.g., 70-90%), ziploc bags, and a capped pvc pipe to wash the borer with alcohol.

B. Procedure:

1. Collect a tree core or drill a hole to center of tree at breast height.
 - a. If you are interested in saving the core for genomics use gloves and clean the borer with isopropyl alcohol before each core. Place core on ice (or freeze immediately if interested in transcriptomics)
 2. Place pipe into hole
 3. Pound the pipe into the hole until half its length is in the hole
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IV. Sample Collection:

A. Materials:

- N2 flushed 12mL exetainers
- (2) 5mL syringes
- (2) 30mL syringes
- (2) 2 way stop cocks
- Needles (one box 25G x 5/8)
- Septa (8mm & 12.5mm)
- Clipboard
- Pencil



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- Sample Collection Checklist/Field Notebook

B. Procedure:

1. Check and replace all gas well septa. If you need to replace a septa (typically every 6 months or so depending on sun exposure), then let the tree equilibrate for ~10 min before sampling (or go replace all septa, and the first tree will be ready by the time you finish replacing all of them).
2. Follow sampling instructions for the appropriately sized gas well, as described below.

- **For 8mm I.D. Wells (TEMPEST & GCW):**

1. Using a 5 mL syringe with a two way stop cock/valve and needle, insert the needle into the center of the septa.
2. Draw 3-5 mL of air from the pipe/septa. This volume is approximate because some of the smaller trees will pull a vacuum and the plunger will rebound to ~2.5 mL if you try to draw 3mL.
3. Close valve and remove needle from tree well.
4. Next, open the valve while pushing on the plunger and expel sample until you have exactly 2 mL in the syringe. Close the valve.
 - i. *If you can't get 2 mL (e.g., from a small tree), then record the volume that you were able to sample*
5. Insert needle into exetainer and push sample in. We use exetainers that are flushed at atmospheric pressure with N2 and thus have 12 mL of N2 inside. So the sample has a dilution factor of 2/14.

Notes:

- i. If you pull a mixture of water and gas from a tree, what we did at tempest was expel the water and pull more sample. Repeat until you have 2 mL of gas. An alternate approach would be doing a headspace equilibration with the water. There were 1-2 trees in the seawater and fresh plots that consistently gave us water samples and the first method I described generally yielded enough gas eventually.
- ii. If you need to replace a septa (typically every 6 months or so depending on sun exposure), then let the tree equilibrate for ~10 min before sampling (or go replace all septa, and the first tree will be ready by the time you finish replacing all of them).



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- **For 12.5mm I.D. Wells (MSM/GWI):**

1. Using a 30 mL syringe with a two way stop cock/valve and needle, insert the needle into the center of the septa.
2. Draw 20 mL of air from the pipe/septa. This volume is approximate because some of the smaller trees will pull a vacuum and the plunger will rebound.
3. Close valve and remove needle from tree well.
4. Next, open the valve while pushing on the plunger and expel sample until you have exactly 12 mL in the syringe. Close the valve.
 - i. *If you can't get 12 mL (e.g., from a small tree), then record the volume that you were able to sample*
5. Insert needle into exetainer and push sample in.

V. Corresponding Documentation:





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VI. References:

TEMPEST NOTES:

June 2022 - Nick: A random assortment of trees have wells at 2 heights...we had opportunistically plugged some holes that Nate cored a long time ago, but he didn't do multiple heights on every tree. Early on when there were less sap flow trees, we sampled both heights, however for the TEMPEST event we decided to only sample the higher well on all trees.

June 2023 - We sampled both height wells during the TEMPEST Event. Nick to look at data and see if there is a significant difference in the concentration to warrant collecting from both height wells in 2024

June 2024 - We sampled both height wells during the TEMPEST Event. Unclear if Nick saw any significant difference in the concentrations, but we went ahead and sampled both