**Report Number:** DOE-MSSTATE-SC6879
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**Recipient:** Mississippi State University
**Title:** Exploratory Research - Using Volatile Organic Compounds to Separate Heterotrophic and Autotrophic Forest Soil Respiration
**PI:** Scott Roberts (formerly Jeff Hatten)
**Co-PIs:** Jeff Hatten, Karen McNeal, Janet Dewey

**Date of report:** 2/9/2015
**Period covered by the report:** 9/1/2011-8/31/2014

**Executive Summary:**
The initial focus of this project was to develop a method to partition soil respiration into its components (autotrophic, heterotrophic etc.) using the fingerprint of volatile organic compounds (VOCs) from soils. We were able to identify 63 different VOCs in our study; however, due to technical difficulties we were unable to take reliable measurements in order to test our hypotheses and develop this method. In the end, we changed the objectives of the project. Our new objectives were to characterize the effects of species and soil moisture regime on the composition of soil organic matter. We utilized the soils from the greenhouse experiment we had established for the soil VOC study and determined the lignin biomarker profiles of each of the treatments. We found that moisture had a significant effect on the carbon content of the soils with the low moisture treatments having higher carbon content than the high moisture treatments. We found that the relative yield of syringyl phenols (SP), ligin (Lig), and substituted fatty acids (SFA) were elevated in deciduous planted pots and reduced in conifer planted pots relative to plant-free treatments. Our results suggest nuttall oak preserved lignin and SFA, while loblolly pine lost lignin and SFA similarly to the plant free treatments. Since we did not find that the carbon concentrations of the soils were different between the species, nuttall oak probably replaced more native soil carbon than loblolly pine. This suggests that relative to loblolly pine, nuttall oak is a priming species. Since priming may impact soil carbon pools more than temperature or moisture, determining which species are priming species may facilitate an understanding of the interaction that land use and climate change may have on soil carbon pools.

**Summary:**
***Original Objectives, Approach, and Problems Encountered:***
The initial plan for this study was to develop and test a method to partition autotrophic and heterotrophic respiration using volatile organic compounds (VOCs). In order to accomplish this we outlined several objectives:

**1. Determine the VOCs that uniquely indicate each component of soil respiration (autotrophic, heterotrophic, decaying roots, root exudates).**

**2. Test the effectiveness of this method over a range of soil moisture conditions**.

**3. Determine if diurnal cycles affect soil respiration and soil VOC efflux.**

**4. Determine if VOCs can uniquely indicate below ground production of root biomass (I).**

**5. Test the similarity/difference in VOC and soil respiration response in a gymnosperm (loblolly pine) and an angiosperm (nuttall oak).**

In order to meet these objectives we established a greenhouse experiment in the early part of 2012 after a delay caused by the timing of funding (Figure 1). We successfully installed 4 blocks of 36 pots that had two levels of moisture treatments (high and low) and two species (loblolly pine and nuttall oak) as well as plant free controls. The experiment was allowed to grow for 14 months (until the plants outgrew their pots). During this time period we took regular measurements of soil respiration, moisture, and temperature; and semi-regular measurements of soil VOCs (see following paragraph). The results from two of our VOC analyses are shown in Figure 3. VOCs were sampled from soils of individual pots replicated over a period of 6 minutes at 8 mL/min using a pump and collected on Thermal Desorption tubes packed with tenax absorbant. VOC samples were adsorbed onto Chromosorb 106 TD tubes. The tubes were desorbed at 225° C for 5 min onto a cold trap using a Perkin-Elmer TurboMatrix 350 thermal desorber. The VOCs were then flash injected onto a 30m Perkin-Elmer Elite 225 capillary column. Initial GC conditions were 40° C with a hold time of 5 min. After the hold time the temperature ramp was 10° C/min to 90° C then 5° C/min to 140° C. After reaching 140° C, the temperature was ramped up to 15° C/min to 200° C, held for 5 min. The run was then ended.

Figure 1. Picture of 1 block of planted trees in pots (left) and cap used to measure soil respiration (right). Pots are capped around the tree and sealed with modeling clay (organic carbon free) and CO2 repiration is measured using a LiCOR LI-8100A. Soil VOCs are sampled by drawing soil atmosphere through a permanently installed probe (not shown) using a peristaltic pump.

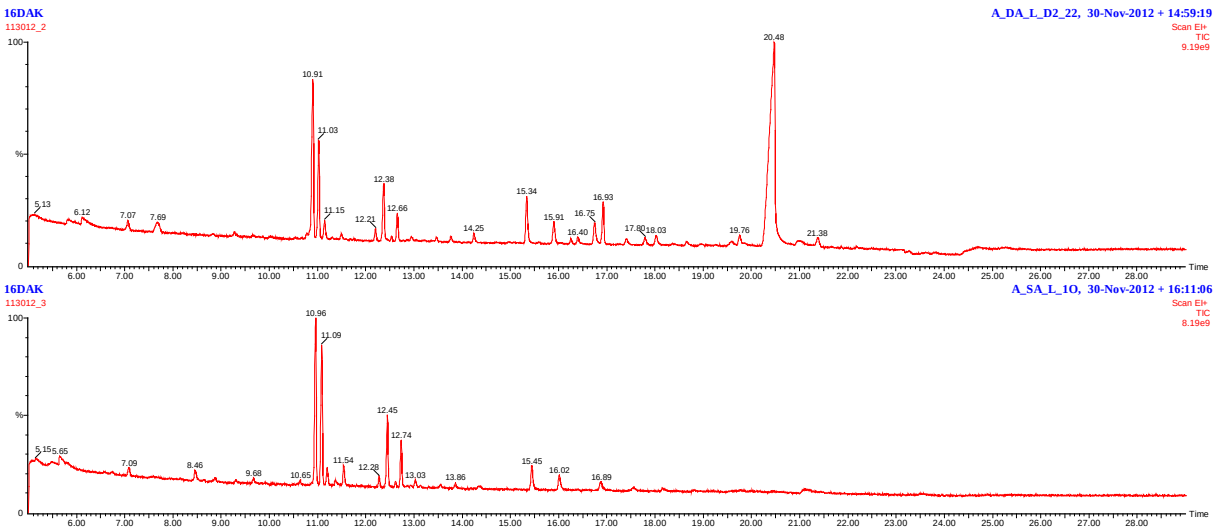


Figure 2. VOC chromatogram from a pot with a nuttall oak – deciduous tree (top) and soil-only (bottom). There are several peaks that occur in the chromatogram from the pot with the tree suggesting that the roots are producing some quantity of the VOC signal. Some of the peaks that are common between the pots also appear to be accentuated in the treatment with the tree suggesting that there may be some VOCs that are common to both soils and trees and some that may be derived from the soil only. The next step will be to quantify these constituents and determine if they correlate with some portion of the soil respiration signal.

During this time period we experienced extreme difficulty with acquiring reliable measurements of VOCs from the TD/GC/MS. We spent several thousand dollars of project funds on service visits as well as acquiring a new-used thermal desorber. In addition, Hatten purchased a refurbished used thermal desorber as part of his start-up package when starting a new position at Oregon State University. In total we went through 3 Perkin Elmer thermal desorbers on this project and none were reliable enough to provide consistent VOC measurements on this project. Essentially we ran out of time as the trees outgrew their pots, and money as we had overspent the project budget planned for these expenditures.

We were able to identify 63 different volatile organic compounds that originated in the soil or plant roots. Even with the difficulties outlined above we were able to assess VOC fingerprints from 40 samples; unfortunately these were from various periods and treatments so no clear picture could be drawn from the data collected. Is this method feasible? Hypothetically, yes there is still a good possibility that the method is feasible. There are plenty of labs measuring soil VOCs, and in the few measurements that we were successful in making we did not see any indication that this method would not work. While we were unable to assess this statistically we did find that there appeared to be unique signatures between angiosperm, gymnosperm, and soil-only treatments. However, we do not believe this method could be widely adopted unless there were an advancement in the reliability of the devices that introduce sample into the GC/MS (thermal desorbers in our case).

***Departure from Planned Methodology, Other Accomplishments, and Other Major Findings:***

The greenhouse study that we established allowed us to examine other processes with the appropriate measurements. We developed a new set of objectives to examine the role of species

and soil moisture in stabilizing and destabilizing soil organic matter. We redesigned our study to examine how the soil carbon of these treatments changed over the 14 month experiment. We were also able to leverage non-project funds to hire an undergraduate researcher (Andrew Shaman) who conducted the analyses and presented the findings at two conferences. CuO oxidation biomarkers were used to examine compositional changes to the soil organic matter.

We found that moisture had a significant effect on %C of soils with the low moisture treatments having higher %C than the high moisture treatments. There was, however, no difference in %N. We found that the relative yield of syringyl phenols (SP), ligin (Lig), and substituted fatty acids (SFA) were elevated in deciduous planted pots and reduced in conifer planted pots relative to plant-free treatments (Figure 3). There were no significant differences between the moisture treatments; however the higher moisture treatments of the nuttall oak soils

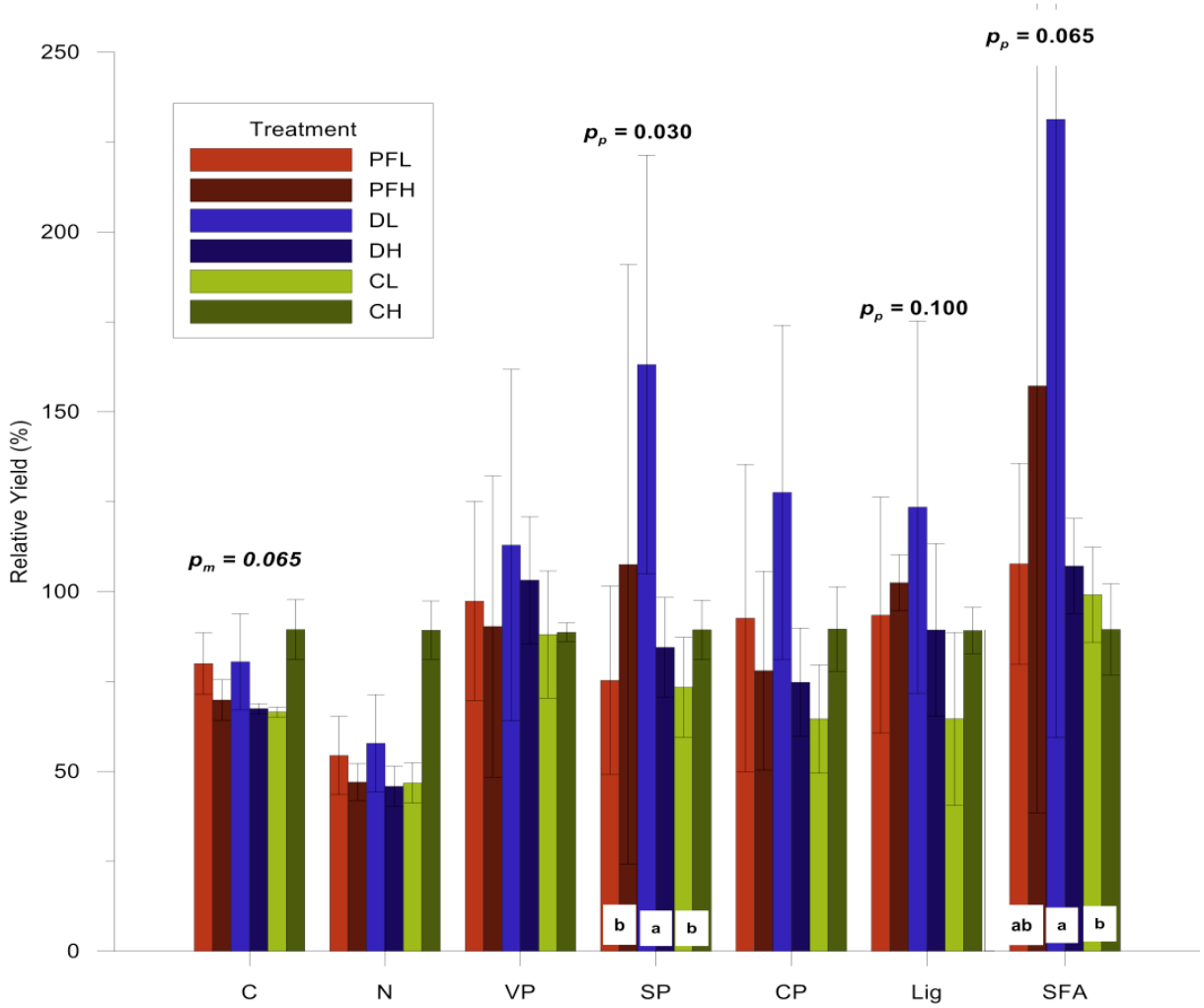


Figure 3. Mean carbon loss (C) and relative yield for nitrogen and other CuO products. We calculated relative yields to determine if compounds were selectively lost, preserved, or produced relative to bulk carbon from values less than, equal to, or greater than 100%, respectively. %RY = [(Yt/Yc)*(1-%OCL)]*100, where Yt and Yc are the constituent yield of treated and pre-treatment soils, respectively, and %OCL represents the percent carbon loss incurred in the soil sample.

typically had higher relative yields of CuO oxidation products. Results suggest nuttall oak preserved lignin and SFA, while loblolly pine lost lignin and SFA similarly to the plant free treatments. Since we did not find that the carbon concentrations of the soils differed between species, the nuttall oak probably replaced more native soil carbon than loblolly pine. This could imply that relative to loblolly pine, nuttall oak is a priming species. This research will be detailed in a manuscript in preparation for the journal Biogeosciences.

**Publications:**

*Indicates student author

J.A. Hatten, Shaman, A.Z.*, S.D. Roberts, K.J. Krapfl, J.C. Dewey, and K.S. McNeal. 2014. The role of tree species and soil moisture in soil organic matter stabilization and destabilization. American Geophysical Union Fall Meeting. December 16-19. San Francisco, CA. (Poster)

J.A. Hatten, Shaman, A.Z.*, S.D. Roberts, K.J. Krapfl, J.C. Dewey, and K.S. McNeal. 2014. The effect of tree species and soil moisture on soil lignin biomarkers. Soil Science Society of America Annual Meeting. Nov. 2-5, 2014. Long Beach, CA. (Poster)

Shaman, A.Z.*, and J.A. Hatten, S.D. Roberts, K.J. Krapfl, J.C. Dewey, and K.S. McNeal. 2014. Characterization of lignin biomarkers in planted and plant-free pots. Society of American Foresters and the International Union of Forest Research Organizations. October 5-11. Salt Lake City, Utah. (Poster)

Shaman, A.Z., J.A. Hatten, S.D. Roberts. 2014. Characterization of soil organic matter in planted and plant-free pots. Mississippi State University Graduate School Summer Undergraduate Research Symposium, Starkville, Mississippi. June 24. (Poster)

Hatten, J., K. McNeal, C. McLaurin, C. Templeton. S. Roberts. and J. Dewey. 2013. Exploratory Research - Volatile organic compounds as indicators of belowground processes: Partitioning heterotrophic and autotrophic forest soil respiration. Terrestrial Ecosystem Sciences Principle Investigator Meeting. Bethesda, Maryland., May 14-16. (Poster)

Dewey, J., J. Hatten, S. Roberts, and K. McNeal. 2012. Using volatile organic compounds to separate heterotrophic and autotrophic forest soil respiration. Terrestrial Ecosystem Sciences Principle Investigator Meeting. Washington, D.C., April 23-24. (Poster)

J.A. Hatten, Shaman, A.Z.*, S.D. Roberts, K.J. Krapfl, J.C. Dewey, and K.S. McNeal. In-preparation. The role of tree species and soil moisture in soil organic matter stabilization and destabilization. Manuscript in preparation for Biogeosciences.

**Web site:** None to date

**Networks or collaborations fostered:** Many scientists were interested in our method to partition auto- and hetero-trophic soil respiration. Several offered their research sites to us to

conduct a field trial; however as a result of the unreliability of the VOC measurement we were never able to take them up on their offer.

**Technologies/Techniques:** We demonstrated a clear hurdle in this technique would be to develop a reliable method to measure soil VOCs.

**Inventions/Patent Applications:** None to date