Identifying and quantifying biochemicals in soils and sediments are important aspects of studies of the carbon cycle. Lignin, a structural biopolymer and a component of cell walls in higher plants, is one example. As such, lignin is often used as a terrestrial biomarker in marine sediments and in degradation studies of soils. There are two common methods for identifying the presence of lignin phenols. These include the more traditional and widely used cupric oxide (CuO) method, as well as the newer method of reacting samples with tetramethylammonium hydroxide (TMAH). The TMAH reaction requires sample sizes in the 10 - 100 mg size range, which is significantly smaller than needed for the CuO method, and takes only minutes to prepare. Potential disadvantages of the TMAH method include the possible existence of a matrix effect from inorganic minerals in soils and sediments, remnant salts in marine sediments, and remnant acid from carbonate removal treatments. The objective of my experiment in Dr. Blair’s lab is to investigate the actual presence and nature of these matrix effects, and how they might affect the results of the TMAH reaction.

To test the three previously hypothesized matrix effects within the TMAH method, I have developed a sequential series of preparations and experiments. The most universal matrix effect for soils and sediments would be caused by associated inorganic minerals (typically aluminosilicate clays) reacting with the TMAH. To test this hypothesis, I set up a series of experiments to determine if commonly occurring inorganic species interfere with the TMAH reaction. The first task I accomplished in the lab this quarter was to prepare samples of varying concentration of plant material (non-woody oak) with kaolinite, a common but relatively simple clay mineral. I initially made dilutions of 2, 5, 10, and 50% plant material mixed with kaolinite, which were then ground, mixed with ~5 mL of deionized water, and freeze dried to better replicate natural conditions by binding the plant matter with the clay minerals. Samples were re-ground following freeze drying, and sub-divided for subsequent treatments described below.

In addition to matrix effects from clay minerals, marine sediments (often analyzed in Dr. Blair’s Lab) originally contain oceanic porewater (seawater), which leaves salt deposits when dried. Additionally, many samples are treated with 4N HCl to remove carbonates for elemental and isotopic organic carbon analysis. To test these effects independently and together several subsets of the original dilutions were created. To mimic marine sediment samples, portions of each original plant-clay dilutions were subjected to a salt water solution (35 ppm), followed by freeze drying prior to analysis. Marine sediment samples are also acidified to remove carbonates, which may result in a secondary matrix effect. The residual salt in these samples may amplify the acid reaction by increasing the quantity of residual acidity by producing non-volatile anions such as sulfate and borate. Thus I treated a subset of the seawater treated samples with 4N HCl for 48 hours. These sub-samples were then dried using the Thermo SpeedVac prior to analysis. While soil samples do not have appreciable salt content, these samples are often also subjected to acidification to remove carbonates. Thus, a portion of the original dilutions was also treated by the previously described acidification technique prior to analysis. A flowchart of the various treatments I have designed can be found in Appendix 1.

For approximately the first four weeks of the quarter, I spent my time in the lab weighing and mixing dilutions of plant-clay material and subjecting the sub-sets of these dilutions to the treatments previously described. Following these preparations samples were analyzed using the TMAH method to investigate any resulting matrix effects. In this method of analysis, the
samples are incubated with 150 µL of TMAH for 6 minutes, followed by sample being heated at 300° C for 30 seconds in the CDS Pyroprobe 5200; the released volatile products are passed to the Thermo Trace GC-DSQII MS (gas chromatograph/mass spectrometer). Gas chromatography is a method for separating the components of a mixture and measuring their relative quantities, while the mass spectrometer provides an identification of the components based on their masses. Before analyzing the samples I prepared, it was necessary to run standards on the GC-MS for calibration of lignin phenols (acids and aldehydes) and fatty acid methyl esters. Additionally, for each sample run, 40 µL of an internal standard (deuterated hexadecane) was added to allow for quantitative analysis. Both the retention time and the major mass fragments of expected compounds were necessary for identification in the resulting chromatograms. In addition to the prepared dilutions of plant and clay material, end members of non-woody oak and kaolinite were run independently.

The first sample I chose to analyze was the non-woody oak standard, consisting of pure plant material. Using the data provided by the standards, I was able to identify ten different compounds. Six of these compounds were lignin phenols, including: t-cinnamic acid, p-coumaric acid, vanillic acid, syringic acid, ferulic acid, and 3,5-dihydroxybenzoic acid. Three of these were fatty acids, including C_16, C_18, and C_22 fatty acid methyl esters. Finally, I was able to identify syringaldehyde, a commonly occurring aldehyde in plant matter. In order to identify these compounds within the complex mass spectrum of the plant matter, I used multiple diagnostic techniques. The relative retention time of the compounds helped me limit the number of possible peaks for any one compound. In addition, I consulted the mass spectra of each of the nine compounds. The most abundant masses were helpful in distinguishing between peaks that had similar retention times, but contained different molecules. Using the Xcalibur software, I was able to locate compounds that contained mass fragments unique to only one of two coeluted compounds. To detect fatty acid compounds, I searched retention times between 26 and 30 minutes. The mass spectra of these compounds show mass peaks 14 units apart, indicating the CH_2 groups that comprise the bulk of the molecules. The difference in retention time between these fatty acids is roughly 2 minutes, which helped me locate the other fatty acids after I found the first, C_18. While I have been able to successfully identify these first nine compounds, two of the compounds, t-cinnamic acid and 3,5-dihydroxybenzoic acid, coelute with additional compounds. I believe that these are likely carbohydrate groups, which mask the lignin phenols from appearing definitively in the mass spectrum, but further analysis will be necessary.

For the rest of the quarter, I will be working on the data resulting from the analysis of the remaining treatment sub-samples (pending). Comparison of the presence and intensity of these nine compounds from the original plant material with subsequent analysis of treated samples will help me determine any matrix effects present.
Appendix 1: Summary of Methods