A Journey from Roots to Bulk Soil: Organic Matter Characterization in the Biosphere 2 Tropical Rainforest

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Introduction

- Tropical soils are major reservoirs of organic carbon (OC) and are estimated to contain 1/3 of the global carbon (C) stock.[1] Soils in the rainforest area of Biosphere 2 were started with mixed organic and inorganic materials from Wilson Pond soil (silt loam from cattle pond), gravel sand, coarse organic material, and compost found in Arizona and the US.[2]
- The rhizosphere is the area of the soil around plant roots that possesses a distinctive community of microorganisms and is influenced physically, biologically, and chemically by root growth and activity. Plant roots release up to 25% of the photosynthetically fixed C they produce as organic compounds, such as lignins, tannins, proteins, lipids, and other secondary metabolites, into the soil.[3] These exudates along with other plant and animal residues and living microbial biomass constitute soil organic matter (SOM).
- The microorganisms present in soils break down and transform organic material into usable food and energy sources necessary to undergo their biological processes. By doing so, carbon dioxide is released into the atmosphere.
- Since CO_2 released from soils contributes to a positive feedback loop of greenhouse gases, this study seeks to characterize organic metabolites from root, rhizosphere, and bulk soil samples in the tropical rainforest (TRF) of Biosphere 2 (B2). This will provide further insight into soil composition and microbial mineralization of OM, which will serve as a baseline study for the B2 Water, Atmosphere, and Life Dynamics (WALD) campaign taking place in the fall of 2019.
- The B2 WALD project will monitor drought and rewetting experiments in order to understand ecosystem responses to extreme weathers predicted with climate change.

Methodology

- **Sampling:** In late April 2019, soil samples from the tropical rainforest in the Biosphere 2 were collected. One-meter surface cores were taken in 4 replicates from 8 sites. These were split with a hot knife in 4 depth groups: 0-10cm, 10-20cm, 20-50cm, 50-75cm and allocated into sterile bags. Tweezers were used to separate roots from rhizosphere and the bulk soil. Soils (bulk and rhizosphere) and roots were placed into 2mL and 50mL centrifuge tubes, respectively, for further analysis.
- Extraction of Organic Matter:

1) Weighing
   - Weighed ~1g of bulk soil, rhizosphere, and grinded roots
   - Added 5mL double deionized H_2O per tube for 1:5 ratio

2) Water Extraction on the Samples
   - Sonicated for 2hrs
   - Centrifuged for 20min
   - Supernatant was transferred to a new tube
   - pH was adjusted to 2-3 by adding 0.01M HCl

3) Solid Phase Extraction (SPE)
   - Cartridges were conditioned with MeOH
   - Samples were loaded into cartridges
   - Cartridges were washed with 0.01M HCl to remove impurities
   - Filtered air was used to dry the cartridges
   - Samples were eluted into 1.5mL vials using MeOH

Figure 1. Location of sampling site in TRF.

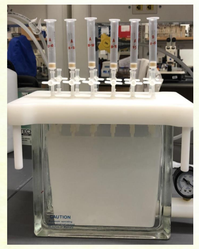

Figure 2. SPE set-up.

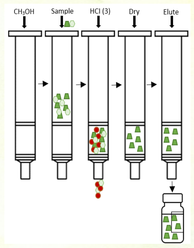

Figure 3. Solid Phase Extraction process to obtain desired compounds from soil water extracts.

Sample Analysis

- A mass spectrometer converts molecules to ions so that they can be moved about and manipulated by external electric and magnetic fields in order to measure the characteristics of individual molecules.
- Fourier Transform Ion Resonance Cyclotron Mass Spectrometry (FTIRC-MS) was used to analyze the extracted samples.

Figure 4. FTICR-MS is based on the circular movement of charged particles in a strong magnetic field (cyclotron movement). The cyclotron frequency depends directly on the mass-to-charge ratio of the ions. The periodic movement of ion packets is recorded and converted to a frequency spectrum with a Fourier transform, which is converted to mass spectrum after calibration.

Data Analysis

The data collected from the mass spectrometer was cross checked through a database for organic compounds and a list containing the molecular formula of the detected compounds was generated.

For each compound, average Nominal Oxidation State of Carbon (NOSC) was calculated from the number of electrons transferred in OC oxidation half reactions based on the equation below:[4]

$$NOSC = -((4*C + H - 3*N - 2*O + 5*P - 2*S)/(C)) + 4$$

Gibs free energy was estimated from NOSC following equation:[4]

$$\Delta G°Cox = 60.3 – 28.5*(NOSC)$$

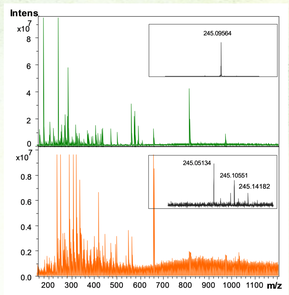

Figure 5. FTICR-MS spectrum of root, and bulk soil samples, represented by green and orange colors, respectively.

Results

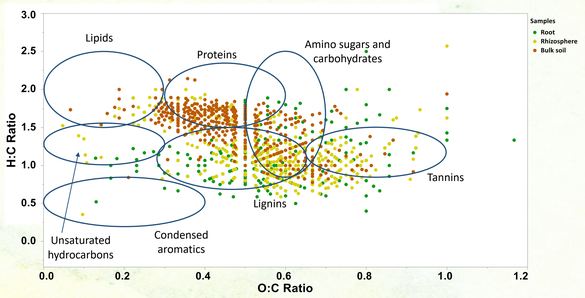

Figure 6. Van Krevelen diagram[5] representing major chemical classes detected in root, rhizosphere, and bulk soil samples at 0-10cm depth from the average of two replicates.

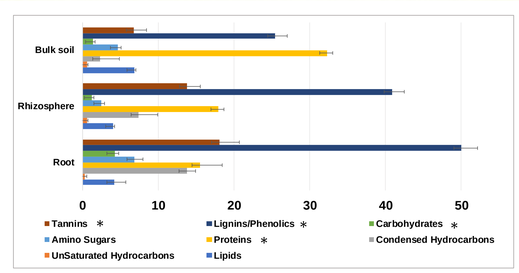

Figure 7. Relative abundances of FT-ICR MS compound classes detected in root, rhizosphere, and bulk soil samples at 0-10cm depth. (*) indicates statistical differences between root and rhizosphere samples when compared to bulk soil by one-way ANOVA ($p < 0.05$).

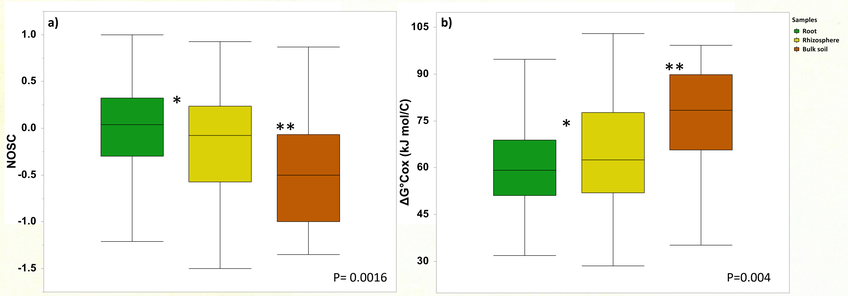

Figure 8. a. Nominal Oxidation State of Carbon for root, rhizosphere, and bulk soil samples at 0-10cm representative of redox activity. b. Gibbs Free Energy of organic carbon (OC) oxidation under standard conditions (ΔG°Cox) indicates the thermodynamic favorability of the compound, with lower values of ΔG°Cox for a compound meaning it is more prone to undergo degradation.

Highlights

- We observed major differences in OM composition throughout the sampled roots, rhizosphere and bulk soil, with root and rhizosphere displaying similar chemical composition when compared to bulk soil.
- High amounts of lipids and proteins in the bulk soil are indicative of microbial biomass residues, suggesting that organic matter is utilized and transformed by microbes as amino sugars made available through root exudates are rapidly taken up by soil microorganisms and used to form new biomass communities. Organic compounds such as lignins, tannins, and condensed hydrocarbons reveal plant inputs in surface soil.
- During microbial mineralization, large molecules are broken down and transformed, but not all can be utilized by the microbes. Therefore, a large amount of recalcitrant and humified compounds are being accumulated in the soil.
- Gibbs free energy is representative of thermodynamic favorability of the compound to undergo degradation. Comparing NOSC and ΔG°Cox values calculated for root, rhizosphere and bulk soil samples, we can conclude that OM in roots (plant material and root exudates) have the lowest bioenergetic potentials and they are picked up by microorganism first to undergo transformations. Moving from rhizosphere to bulk soil, the bioenergetic potential of the OM increases, indicating the refractory nature of the OM in the bulk soil.
- Future directions: The data generated by this study will serve as a foundation for the B2 WALD campaign and future research on soil organic matter composition and transformations to understand OM cycling in face of projected extreme climatic conditions.

Acknowledgements

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