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SESSION ONE
ORAL PRESENTATION C

Unlocking microbial communities in *Terra Preta*: nucleic acid extraction and purification as keys to characterizing biology in black carbon soils

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Amazonian Dark Earths, or *Terra Preta* (TP) soils, are noted for both their high fertility and black carbon (BC) content. The anthropogenic addition of charred plant material, BC, has altered soil nutrient dynamics and the unique chemistry of TP likely sustains unique microbial populations which play a role in stabilizing their fertility. Using soil from four TP sites in the Brazilian Amazon, we sought to elucidate major differences in bacterial populations in these soils as compared to soil sampled from adjacent oxisols. We used a most probable number dilution extinction method to estimate the numbers of bacteria culturable in a liquid minimal medium (R2A). For each site, numbers of culturable bacteria were equivalent to or higher in all TP soils as compared to adjacent oxisols. We subsequently used a direct cell lysis protocol to extract total soil DNA using a commercial kit (Bio101®, Qbiogene). The protocol yielded moderate to low nucleic acids concentrations from oxisol soils (6.5-65.5 ng/µl) and consistently low nucleic acid recovery (not detectable to 12.2 ng/µl) from TP soils in all cases, despite the TP soils having higher numbers of culturable bacteria. We hypothesized that the high chemical affinity of BC for charged molecules may be increasing the binding of both un-lysed cells and nucleic acids released from cells during the extraction leading to poor recovery of nucleic acids from TP soils. Robust analyses of soil microbial communities rely on obtaining representative nucleic acid from the community. Hence, developing methods to improve nucleic acid recovery from TP is essential for assessing the role of organisms in soils with high BC content. We tested five treatments aiming to maximize nucleic acid extraction from both oxisols and TP soils. Physical treatments varied bead beating time to more effectively lyse cells in the soil matrix. Chemical treatments tested the capacity of aluminum ammonium sulfate, magnesium chloride or increased phosphate concentration in the buffer to reduce nucleic acid sorption to BC. Effectiveness of each method was evaluated using a combination of direct DNA quantification, gel electrophoresis and direct counts of intact cells in soil samples using fluorescence microscopy after extraction treatments. Increased bead beating time increased cell lysis, but also increased DNA shearing. Chemical methods tested showed an improvement on commercial soil extraction kits, but leave room for further improvement. While DNA extraction has proved difficult, purification of DNA extracted from TP may pose another challenge. This study identifies some possible reasons behind reduced nucleic acid extraction efficiency in TP soils and points toward methods to improve nucleic acid yield. Reliability of studies on trophic level dynamics, community structure and plant-microbe interactions in soils containing BC will improve once DNA extracts more adequately represent the soil microbial community. An examination of the soil microbial community in TP may answer questions not only about its unique fertility, but may point toward an analogous effect of BC in other ecosystems.