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1. Theories on the formation of humus have changed over time due to technological advances and better characterization of physicochemical patterns in the soil environment. For reference, look at the Lehmann and Kleber 2015 paper on Canvas.

**Part A.** Describe the historical view of humus, also known as the Polyphenol theory. **(4pts)**

In the polyphenol theory, lignin decomposition products and other microbial products bind together in order to form large macromolecules containing several phenyl rings.

**Part B.** More current views on humus formation have identified two competing interpretations, namely Selective Preservation and Progressive Decomposition. For each of these theoriesdescribe the process of humus formation **(6pts)**

Selective Preservation: In selective preservation, plant and animal residues consist of labile and stable molecules. The labile molecules are broken down by bacteria while the more stable molecules are broken down by fungi. Each type of molecule is broken down into smaller and smaller particles until they are eventually released as CO­2.

Progressive Decomposition: In progressive decomposition, plant and animal residues are not broken into labile and stable categories but are thought to be uniformly broken down form residues, to large molecules, to small molecules, to monomers.

**Part C.** Describe the Soil Continuum Model, indicating how it varies from the above theories and how it builds on the above ideas to generate a more holistic model.

(HINT: Think of this as a compare/contrast questions – what makes this model similar and different from the theories you have already discussed?) **(5pts)**

In the soil continuum model, molecules are progressively broken down into smaller and smaller molecules much like the progressive decomposition theory. The difference between this theory and the two proceeding models is in the SCM model, the availability of molecules is in flux due to factors such as adsorption and formation of aggregates.

2. In class we discussed the diversity of archaeal groups. Think about extremophiles and answer the following questions.

**Part A.** Define what it means to be an extremophile. **(2pts)**

An extremophile is a microbe from the achaea group that has evolved the ability to survive in extreme environments that most microbes cannot survive in.

**Part B.** Pick one type of extremophile (pH, temperature, salinity, etc.) and describe a habitat that may be favorable or selective for this group. **(4pts)**

Sulfolobus is an organism that is well adapted to survive in high temperatures and low pH. These organisms can survive in places like volcanic hot springs.

3. Find one published paper from a scientific journal that includes **data on two of the following** microbial parameters: **(1)** soil microbial biomass, **(2)** soil microbial diversity, and/or **(3)** soil microbial activity using methods that we have covered in class. Attach a PDF of the paper to the email when you send your completed assignment. For this paper report the following:

**Part A.** copy and paste the abstract of the paper, and include a citation below. **(2pts)**

This study reports the effects of long-term elevated atmospheric CO2 on root production and microbial activity, biomass, and diversity in a chaparral ecosystem in southern California. The free air CO2 enrichment (FACE) ring was located in a stand dominated by the woody shrub *Adenostoma fasciculatum*. Between 1995 and 2003, the FACE ring maintained an average daytime atmospheric CO2 concentration of 550 ppm. During the last two years of operation, observations were made on soil cores collected from the FACE ring and adjacent areas of chaparral with ambient CO2 levels. Root biomass roughly doubled in the FACE plot. Microbial biomass and activity were related to soil organic matter (OM) content, and so analysis of covariance was used to detect CO2 effects while controlling for variation across the landscape. Extracellular enzymatic activity (cellulase and amylase) and microbial biomass C (chloroform fumigation-extraction) increased more rapidly with OM in the FACE plot than in controls, but glucose substrate-induced respiration (SIR) rates did not. The metabolic quotient (field respiration over potential respiration) was significantly higher in FACE samples, possibly indicating that microbial respiration was less C limited under high CO2. The treatments also differed in the ratio of SIR to microbial biomass C, indicating a metabolic difference between the microbial communities. Bacterial diversity, described by 16S rRNA clone libraries, was unaffected by the CO2 treatment, but fungal biomass was stimulated. Furthermore, fungal biomass was correlated with cellulase and amylase activities, indicating that fungi were responsible for the stimulation of enzymatic activity in the FACE treatment.

**Part B.** Briefly describe the experimental study, including objectives and/or hypotheses. **(3pts)**

This study aimed to test the effects of 8 years of elevated CO2 treatment on root growth and microbial biomass, activity, and community structure. They hypothesized that elevated CO2 increases root biomass, which will increase microbial biomass and activity, thus altering the microbial community.

**Part C.** Outline the methods used in the study to measure microbial biomass, diversity, and/or activity (1,2,3 above). In this description, be sure to discuss the importance or relevance of these methods for addressing the objectives of the study. **(5pts)**

This study used substrate-induced respiration using glucose to assess microbial activity and biomass. Microbial biomass C was measured using the chloroform fumigation-extraction method with some modifications. Microbial activity was measured by looking at extracellular enzymes that degrade carboxymethylcellulose and amylase activity also by CFE.

**Part D.** Using your understanding of the pros (advantages) and cons (disadvantages) of the methods used, critically discuss if the techniques used were appropriate for addressing the study objectives. If you think the approach is appropriate, be sure to explain why. If you do not find it appropriate, indicate an alternative method that may have been better. **(5pts)**

The major pitfall I can see of using SIR and CFE would be overestimation of carbon inputs from CFE. This is due to the solubilization of non-cellular carbons within the soil. Since they used two separate methods for carbon biomass estimation, I think these two methods would be fine. The methods described for measurements of enzymes were not described in the paper.

4. For each of the following species answer **(a)** what phylum it belongs to, and **(b)** what functional roles this bacterium is known for in the soil. **(15pts)**

**A. Cytophaga *hutchinsonii*** A. Bacteriodetes B. Phylum broadly known to degrade cellulose.

**B. Frankia *alni*** A. Actinobacteria B. Nitrogen fixing bacteria on roots of alder

**C. Pseudomonas *fluorescens.*** A. Proteobacteria B. Grows near plant roots and produces antibiotics to guard against plant pathogens.

**D. Nitrosomonas *europaea.*** A. Proteobacteria. B. oxidates ammonia to nitrite.

**E. Desulfovibrio *desulfuricans*** A. Proteobacteria. B. In soil, this bacterium reduces sulfur.

5. Discuss the relationship between phylum-level classification and functional diversity. **(4pts)**

Phylum-level classification groups organisms based on sets of broadly similar traits. As taxonomic ranks become more specific, the traits that the organisms within these ranks share increase. In bacteria, it is often difficult to discern past the phylum level of classification due to difficulties in culturing.

Lipson, David A., Richard F. Wilson, and Walter C. Oechel. "Effects of elevated atmospheric CO2 on soil microbial biomass, activity, and diversity in a chaparral ecosystem." *Applied and Environmental Microbiology* 71.12 (2005): 8573-8580.