

KEYNOTE: Measuring the microbial niche: Chip-SIP analysis of carbon and nitrogen assimilation

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The ecological roles of most uncultivated microorganisms must be inferred from diversity and genomic studies. To directly measure functions of uncultivated microbes, we developed Chip-SIP, a high-sensitivity, high-throughput stable isotope probing (SIP) method performed on a phylogenetic microarray (chip). The approach consists of microbial community incubations with isotopically labeled substrates, hybridization of the extracted community rRNA to a microarray, and measurement of isotope incorporation—and therefore substrate use—by secondary ion mass spectrometer imaging (NanoSIMS). Because Chip-SIP can detect isotopic enrichment of 0.5 atom % ^{13}C and 0.1 atom % ^{15}N , experiments with short incubation times and low substrate concentrations are possible.

When we applied Chip-SIP analysis to marine communities labeled with a range of substrates (amino acids, nucleic acids, fatty acids), results indicate that significant resource partitioning occurs even with these relatively simple organic substrates. Using the incorporation of labeled amino acids (AAs) as a proxy for heterotrophic activity, we found different levels of incorporation among different taxonomic groups within the same sample. Taxon-specific differences in the net incorporation of AA-derived C and N indicate that some microbial groups incorporated equimolar amounts of C and N while others took up as much as 80% more N than C. In experiments where substrates were added at concentrations spanning three orders of magnitude, microbial responses fall into three trophic types, and could also be distinguished based on activity. Two of the trophic strategies were not randomly distributed throughout a 16S rDNA phylogeny, suggesting they are under selective pressure in this ecosystem and that a link exists between evolutionary relatedness and substrate affinity. In ChipSIP studies of rhizosphere soils, we tracked microbial use of ^{13}C from labeled exudates and ^{15}N from labeled root litter. While most identified taxa consumed both ^{13}C and ^{15}N from exudates and litter, bacteria from the phylum Actinobacteria consumed more litter relative to exudates, while the fungi from the Ascomycota and bacteria from the Rhizobiales consumed relatively more exudates. Our results show that the microbial communities involved in litter decomposition differ in the rhizosphere and bulk soil, and may have implications for carbon stabilization in soil. The Chip-SIP approach expands the repertoire of stable isotope enabled methods available to microbial ecologists and provides a means to test genomics-generated hypotheses about biogeochemical function in any natural environment. Our initial results suggest that well characterized high microbial diversity on the species level as well as the genomic level result in functional differences that can be quantitatively measured in natural communities. These data considerably expand our concept of bacterial resource partitioning based on seasonal and small-scale spatial habitat use by adding relative rates of substrate utilization as a critical component of the bacterial niche.

Tracking the fate of pyrogenic organic matter decomposition in grassland soils: a stable isotope approach

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Fire is an important management tool in the tallgrass prairie ecosystem of the central United States. The prescribed fires that maintain native grass species dominance for livestock grazing removes C and N from the ecosystem during combustion, but leaves behind incompletely combusted black carbon and nitrogen residues in the soil. It is not well understood how these pyrogenic organic matter (Py-OM) residues affect C and N budgets in frequently burned ecosystems. By applying ¹³C and ¹⁵N labeled Py-OM in the tallgrass prairie, we have provided some insights into the biogeochemical dynamics of Py-OM derived C and N. We will present the results from a one year field incubation of ¹³C and ¹⁵N labeled Py-OM, including its contributions to soil CO₂ flux, soil organic matter fractions, plant N uptake and soil microbial biomarkers (phospholipid fatty acids). Initial data reveals some loss of Py-OM derived C to CO₂ fluxes in the early stages of decomposition, and a large “missing” Py-OM fraction that is not recovered in the soil or CO₂, suggesting losses through leaching or wind erosion. Evidence of microbial use of Py-OM derived C and plant Py-N uptake will also be discussed. Using stable isotopes to determine how Py-OM residues contribute to soil organic matter formation, and whether or not it is biologically utilized, provides important insight into C and N dynamics in frequently burned ecosystems.

Isotope ecology and inquiry-based learning using on-campus museum resources: examining niche partitioning between deer mice and greater sage-grouse in Wyoming.

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We used stable carbon and nitrogen isotopes to look at intraspecific differences in foraging behavior of deer mice (*Peromyscus maniculatus*) in three different habitats and interspecific differences in sympatric shrubland mice and greater sage-grouse (*Centrocercus urophasianus*) as part of a stable isotope ecology class project. We found that shrubland mice have significantly higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios relative to forest mice and sage-grouse. Further, of all mice habitats, forest mice plotted the closest to sage grouse, suggesting niche partitioning in shrublands as opposed to actual physiological constraint or preference. In general, the mice showed greater variation within habitats relative to sage-grouse. These results suggest a more generalist feeding strategy in mice than in sage-grouse in Wyoming. While preliminary, these results indicate that a robust study of these systems could enable more in-depth interpretations of the findings. This is what makes the class project model of exploration an innovative analytical strategy. By assigning a class project, graduate students with an interest in animal ecology get the opportunity to go through the process of collecting, preparing, running, and analyzing the data of samples already housed within university collections. The data is then submitted to museum's digital archive for future reference, and given enough iterations of the course, could yield a substantial resource for future work in addition to their value as an interactive and engaging pedagogical tool.

Chasing methane: a novel approach to coupling methane isotopic measurements in air and water, and implications for tracking oil and gas emissions.

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Methane is an important greenhouse gas with more than 20 times the radiative forcing as carbon dioxide. The recent boom in oil and gas drilling and production has given rise to concern over fugitive emissions both in air and water during drilling, hydraulic fracturing, well completion, transport and distribution of product. Since methane can occur in ground water (up to 22.7 mg L⁻¹ at STP) from either biogenic or thermogenic sources, carbon isotopic signatures can be used to help determine potential connections between ground water potential methane sources, including oil and gas operations. We have developed a method to quantitatively extract and measure methane concentration and $\delta^{13}\text{C}$ of methane in ground water using cavity ring down spectroscopy (CRDS). An important part is that the measurement can be made both in the lab *and* from a mobile platform that can be driven to water sources. Because the measurement can be made very quickly it will serve as a very useful reconnaissance tool to determine where to make more detailed water quality measurements that can allow more definitive investigation. More importantly, the same Picarro instrument is used in air-mode to make atmospheric measurements while driving down-wind of potential methane sources while simultaneously collecting GPS position and wind speed. In near real-time, concentration data are plotted to Google Earth and using isotopic data Keeling plots are calculated to determine isotopic source signatures. Sources can include methane from natural gas, landfills, feedlots, or wetlands, but each will have slightly different isotopic values. In this manner, water measurements are coupled with the Picarro mobile methane air analysis to allow determination of $\delta^{13}\text{C}$ methane end members that can inform mixing models to determine any linkage between air emissions from gas wells and presence of methane in ground water.

The simulation of stable water isotope ratios in an atmospheric global climate model.

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Stable water isotope ratios are geochemical markers that have been analyzed extensively from numerous sources to help reconstruct past climates. However, most paleoclimate reconstructions using isotopes involve inverting the isotopic data into the geophysical variable of interest (e.g. temperature), and these inverse methods have to make assumptions which may not always be valid. An alternative to this approach is to implement water isotopes into a Global Climate Model (GCM), and then compare the isotopic output from the model directly to the isotopic data. This can avoid the issues with using inverse methods, and can add confidence to paleoclimate simulations. Stable water isotopes can also provide information on the current climate, as new measurement techniques can allow one to observe the atmospheric water vapor isotope ratios from satellite. These new global data sets can provide an additional constraint on hydrologic processes, and in-turn can help improve model parameterizations, particularly sub-grid scale cloud and land-surface processes. In light of these potential scientific benefits, stable water isotope ratios are implemented in the National Center for Atmospheric Research (NCAR)'s Community Atmosphere Model, Version 5 (CAM5), which will eventually become the atmospheric component of the isotopically-enabled Community Earth System Model (iCESM). The added physics needed to simulate isotope ratios in an atmosphere model are discussed, and a CAM5-produced climatology of water isotope abundance in the atmosphere is presented. These simulated values are then compared to numerous observations, including satellite data, and the sensitivity of the model climatology to different initial and boundary conditions is shown. Finally, results from different isotope-enabled models, such as NCAR's ocean model, are shown to indicate the research possibilities of a fully-coupled climate model.

Carbon routing in the polar bear: insights from the lipid and protein pathways

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For the polar bear (*Ursus maritimus*), life in the Arctic poses many challenges – water balance, seasonal allocation of dietary resources to growth and storage, and acquisition of sufficient prey to satisfy nutritional requirements. The latter is of great interest as this species hunts primarily ice seals and the Arctic is experiencing rapid declines in sea ice. As a result, the reductions in habitat over the last few decades have been associated with declining body condition, vital rates, and population size. A better understanding of the nutritional ecology of free-ranging bears would be beneficial to conservation efforts. We are complementing ongoing field studies of the Chukchi population with controlled feeding studies of grizzly bears maintained on high fat resources to simulate polar bear diets. High lipid diets pose unique challenges to diet reconstruction inferred from bulk stable isotope analysis, mainly because assumptions related to routing are difficult to satisfy. To overcome some of these challenges, we are parsing out the protein and lipid pathways and complementing with compound specific isotope analysis of individual amino acids. Inferring polar bear diets from stable isotopes has great promise because prey diversity is low, lending well to mass balance modeling approaches. However, a priori knowledge of tissue-diet discrimination factors is critical and our unique approach should provide better accuracy leading to improved diet estimates.

Update on the NEON stable isotope measurements

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The National Ecological Observatory Network (NEON) is a continental-scale observatory designed to gather and provide 30 years of ecological data on the impacts of climate change, land use change, and invasive species on biogeochemical cycles, biodiversity and natural resources. NEON is a project funded by the National Science Foundation, with many other U.S. agencies and NGOs cooperating. Our presentation will focus on (1) the large number of atmospheric deposition, soil and plant biogeochemical, and hydrological measurements that will be made as part of NEON, (2) the stable isotope analyses that are planned for terrestrial and aquatic sites, and (3) the opportunities for collaboration between the stable isotope community and NEON. All data generated by NEON will be publicly available via a web portal to conduct analyses of pattern and process at local, regional, and continental scales.