

## Advances in Biochemical Methods for Studying Organic Matter Dynamics in an Ecological Context

Organized Oral Session 44, organized by Erika Marín-Spiotta and Daniela Cusack, was held during the 2009 ESA Annual Meeting at Albuquerque, New Mexico, on 6 August 2009.

### Topics and Presenters

Application of  $^{13}\text{C}$ -TMAH thermochemolysis to the study of forest floor decay dynamics. **David Gamblin**, Purdue University; Timothy R. Filley, Purdue University  
<<http://eco.confex.com/eco/2009/techprogram/P15356.HTM>>

Single cell biogeochemistry: potential NanoSIMS contributions to ecosystem and microbial ecology. **Jennifer Pett-Ridge**, Lawrence Livermore National Laboratory  
<<http://eco.confex.com/eco/2009/techprogram/P15692.HTM>>

Using  $^{13}\text{C}$ -NMR and radiocarbon to determine mechanisms of soil organic matter stabilization in dynamic landscapes. **Asmeret Asefaw Berhe**, University of California, Merced  
<<http://eco.confex.com/eco/2009/techprogram/P15689.HTM>>

Fourier transform mass spectrometry for the study of organic matter molecular dynamics in aquatic ecosystems. **William Hockaday**, Rice University; Patrick G. Hatcher, Old Dominion University; Jeremiah M. Purcell, Shell Global Solutions; Alan G. Marshall, Florida State University; Qilin Li, Rice University; Carrie Masiello, Rice University  
<<http://eco.confex.com/eco/2009/techprogram/P16383.HTM>>

Soil organic carbon characterization by pyrolysis-gas chromatography-mass spectrometry (py-GC/MS) and tetramethylammonium-py-GC/MS: tracing plant and microbial contributions to SOM. **Catherine E. Stewart**, University of Colorado, Boulder; Jason C. Neff, University of Colorado; Theodore K. Raab, Stanford University; Marc Kramer, University of California; Kathryn L. Amatangelo, Stanford University; Peter M. Vitousek, Stanford University  
<<http://eco.confex.com/eco/2009/techprogram/P15691.HTM>>

Shifts in the composition of soil organic matter during decomposition as revealed by  $^{13}\text{C}$  nuclear magnetic resonance and fourier transform infra-red spectroscopy. **Marc Kramer**, University of California; Theodore K. Raab, Stanford University; Kathryn L. Amatangelo, University of Wisconsin  
<<http://eco.confex.com/eco/2009/techprogram/P16382.HTM>>

Quantum dots: A quantitative nanotechnological approach for studying organic nutrient dynamics in soil, fungi, and plants. **Matthew D. Whiteside**, University of California, Irvine; Kathleen K. Treseder, University of California, Irvine

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<http://eco.confex.com/eco/2009/techprogram/P15693.HTM>

Basic solid-state NMR techniques in the biogeosciences. **Caroline A. Masiello**, Rice University, Houston, Texas; William C. Hockaday, Rice University; Morgan E. Gallagher, Rice University; Jeff A. Baldock, CSIRO Land and Water; Claire McSwiney, Michigan State University; G. Philip Robertson, Michigan State University; Richard Norby, Oak Ridge National Laboratory

<http://eco.confex.com/eco/2009/techprogram/P16384.HTM>

Do enzymes link microbial communities to soil organic matter chemistry? A cross site synthesis using py-gc/ms. **A. Stuart Grandy**, Michigan State University; Michael N. Weintraub, University of Toledo; Timothy R. Filley, Purdue University; Kyle Wickings, Michigan State University

<http://eco.confex.com/eco/2009/techprogram/P18975.HTM>

Ecologists are increasingly adopting and developing new isotopic, molecular, and spectroscopic techniques in interdisciplinary ways to gain insight into ecological and biogeochemical processes. Advances in microscopy and spectroscopy, in particular, are opening the way for unprecedented opportunities for precise, spatially explicit, elemental, and isotopic analysis at the micro- and nanometer scale. These methods are greatly enhancing our capacity to visualize interactions between plants, microbes, and minerals, track the source and fate of nutrients and organic matter, and quantify rates of belowground processes. Recent technological advances have made it possible to characterize the chemical composition of organic matter in small samples, rapidly, and at more accessible costs. Particular areas of ecological research that have greatly benefited from the application of these methods are carbon (C) and nitrogen (N) cycling in terrestrial and aquatic ecosystems, trophic interactions, nutrient uptake and release, the decomposition and turnover of plant and microbially derived material, and soil organic matter formation and stabilization.

During Organized Oral Session 44 at the ESA Annual Meeting, speakers presented innovative research that applied isotopic, molecular, and spectroscopic techniques to ecological systems in new, interdisciplinary ways. The goal of the session was to promote collaborations using new methods to improve our understanding of ecological and biogeochemical processes. Speakers presented a variety of methods that addressed two general areas of scientific inquiry: (1) plant litter decomposition and organic matter dynamics in soils and aquatic ecosystems, and (2) microbial community function.

1) Methods for organic matter characterization and dynamics

*<sup>13</sup>C-nuclear magnetic resonance (NMR) spectroscopy*

<sup>13</sup>C-NMR spectroscopy is a nondestructive method that identifies compound functional groups (e.g., alkyl, aromatic, carboxylic, carbonyl ...), which have been shown to behave differently in the environment (Kögel-Knabner 1997, 2000, Preston 2001). Further information on specific compound classes can be gained from <sup>13</sup>C-NMR spectra by applying a molecular mixing model (Baldock et al. 2004). Advantages of solid-state NMR are that it does not typically require chemical pretreatments or large quantities of sample. Masiello explained technical language associated with NMR methods (called “experiments” in NMR jargon), and the main differences between the most commonly used for solid

analysis, cross-polarization, and direct-polarization. Masiello emphasized the need to use appropriate standards with known C concentrations for a more effective and quantitative use of  $^{13}\text{C}$ -NMR. When applied to ecological questions, NMR has proven to be important in advancing our understanding of chemical changes during litter decomposition, especially concerning the lignin molecule vs. the operationally defined Klason “lignin” fraction (Preston et al. 2009), and of the widespread presence of black carbon, or charcoal, in soils and sediments (Hammes et al. 2007). In addition, NMR (and FTIR, see below) has contributed significantly to changing our paradigm on “humification”, by showing that SOM is composed of mixtures of relatively simple molecules.

Coupling NMR spectroscopy with isotopes can greatly enhance our understanding of ecosystem dynamics. Berhe presented results from a study combining  $^{13}\text{C}$ -NMR spectroscopy, to characterize plant litter and SOM chemistry, with radiocarbon measurements using accelerator mass spectrometry, to measure turnover rates and residence times. Together, these approaches revealed the effect of erosion on soil C stability in dynamic hillslope environments. Berhe’s research also showed that the application of physical density fractionation, which separates the bulk soil C pool into different fractions, based on their location in the soil matrix and on the degree of mineral association, can greatly enhance our understanding of stabilization mechanisms controlling C and nutrient retention or loss in soils.

Kramer discussed analytical biases between different methods and emphasized the need to use multiple techniques to gain the most complete information. He compared results using solid-state  $^{13}\text{C}$ -NMR and **Fourier transform infrared (FTIR) spectroscopy**. Biochemical changes during early stages of decomposition of an angiosperm and a fern in a tropical rain forest (samples also analyzed by Stewart using TMAH-pyGC/MS; see below), were consistently detected by both NMR and FTIR. However, agreement between both methods decreased as decay progressed and in the smallest soil size fraction samples. Thus, understanding analytical biases is important for accurate data interpretation.

*Tetramethylammonium hydroxide (TMAH) thermochemolysis combined with pyrolysis gas chromatography–mass spectrometry (pyGC/MS)*

PyGC/MS provides a chemical fingerprint of organic matter by identifying specific compounds produced after thermal and chemical degradation of the original sample. Certain sample types break down into predictable compounds that can indicate source material, as well as relative degradation state along a plant litter decomposition continuum (Grandy and Neff 2008). Stewart presented the use of pyGC/MS and TMAH thermochemolysis to partition SOM into different sources: microbial vs. plants, and angiosperm vs. gymnosperm. Her research tracked the persistence of plant species-specific indicator compounds, in particular lignin-derived isomers and fatty acids from plant waxes, in tropical rain forest soils. The addition of TMAH thermochemolysis greatly increased the detection ability of pyGC/MS, providing a more complete picture of SOM chemistry and its potential sources.

Gamblin and co-authors (presented by Filley) used TMAH thermochemolysis and pyGC/MS to measure chemical changes during plant litter decay. These methods provide advantages for analyzing specific plant components, such as lignin, cutin, and suberin, and so can also be used to identify organic matter derived from leaves and roots. However, methodological constraints and analytical biases need to be addressed when interpreting results. Filley discussed in detail method improvements using

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<sup>13</sup>C-labeled-TMAH, which greatly increases the method's sensitivity.

Combining pyGC/MS with enzyme activity assays, Grandy presented data linking changes in SOM chemistry with microbial function in a variety of soil, ecosystem, and land use types. In particular, Grandy's research found significant statistical relationships between the presence of specific lignin derivatives and nitrogen-containing compounds, as determined by pyGC/MS, with decomposer community composition (fungi to bacteria ratios) and/or the activity of specific hydrolytic and nitrogen-cycle enzymes. Grandy's data revealed consistent trends in SOM chemistry related to soil properties, soil physical fractions, and enzyme activities. The combination of these methods was also useful for detecting changes in chemistry and microbial function due to differences in land use.

#### *Fourier transform ion cyclotron resonance (FT-ICR)*

FT-ICR mass spectrometry (MS) measures fine-scale differences in the chemical composition of dissolved organic matter (DOM) in soils and sediments, and in aquatic ecosystems (Hockaday et al. 2009). Hockaday used this method to study changes in DOM molecular composition caused by photochemical and biological degradation during riverine transport. Hockaday discussed some practical considerations for the use of FT-ICR MS in ecological research, including several ionization pretreatment techniques, which increase detection of the complex mixture of molecules that compose organic matter in water samples. Hockaday described FT-ICR facilities available at Woods Hole National laboratory and highlighted the willingness of many methods specialists to expand their research through collaborations with ecologists.

#### 2) Methods for study of microbial community function

##### *Quantum dot fluorescence*

Whiteside introduced nanoscale semiconductors known as quantum dots, which were originally developed to track cancer cells. These fluorescent dots can be attached to molecules and followed through metabolic uptake. In Whiteside's study, quantum dots were bound to glycine and chitosan to observe uptake of organic molecules by plants and fungi. Epi-fluorescent spectroscopy, and confocal microscopy techniques were used to quantify the uptake of labeled substrates, and their movement over time was observed using raster image correlation spectroscopy. Whiteside encouraged other researchers to incorporate this method, which can be applied both in the laboratory and in the field and is relatively inexpensive, for greater understanding of microbial activity in situ in field studies.

Nano-scale secondary ion mass spectrometry (NanoSIMS) and enhanced element labeling-catalyzed reporter deposition fluorescence in situ hybridization (EL-FISH)

NanoSIMS, which combines high-resolution microscopy with secondary ion mass spectrometry, provides unprecedented opportunities to visualize the spatial organization of individual biological and physical components in environmental matrices (for example, soils), and collect information on their elemental and isotopic composition at the nanometer scale. NanoSIMS can provide spatially explicit maps of the incorporation of isotope-labeled substrates into subcellular structures. Pett-Ridge introduced a novel approach, EL-FISH, which combines stable isotope imaging based on NanoSIMS

with rRNA-based in situ hybridization (Behrens et al. 2008) to link metabolic activity of individual cells with microbial phylogenetic identity. Pett-Ridge presented pioneering nano-scale research on nitrogen fixation, links between gene expression and incorporation of labeled  $^{15}\text{N}$  into microbial subcellular structures, and soil submicro-aggregate composition. These methods can provide great insight into the role of microbial communities in driving ecosystem-scale processes.

### Conclusions and recommendations

These methods highlight our ability to study ecological processes at micro and nanometer scales using new isotopic, spectroscopic, and microscopic techniques. A challenge the speakers recognized is linking new detailed data collected at the micro- and nanometer scales, which provide important information on mechanisms, to emergent patterns in ecosystem processes. Geostatistical models will likely prove to be very useful tools in integrating data from different methods across different spatial and temporal scales. A common theme of the talks in this session was the recognition that different methods have inherent analytical biases, so that greater understanding can be reached by the applied combination of multiple methods. A further challenge is the development of standard calibration materials for methods like NMR and NanoSIMS. The two general themes presented here, microbial metabolism and organic matter characterization, could also be linked to a greater extent. For example, study of mycorrhizal uptake of organic compounds via quantum dots could be linked to SOM chemistry via pyGC/MS. It is an exciting period in the development of the ecological sciences, in which we are demonstrating our ability to develop high-tech methods, and synthesize and apply large data sets across multiple scales to understand the natural world.

For ecologists, a likely major barrier to using these methods effectively is unfamiliar chemical jargon and theory. Most university courses on NMR, for example, discuss applications and samples that are very dissimilar to those of interest to ecologists. It can be a daunting task for students to self-educate on these methods using published research papers, which usually assume at least intermediate understanding of their theoretical foundations. There is a need for more courses to teach about these specialized organic matter characterization methods geared toward ecological questions, such as those offered by the University of California, Irvine, the University of Florida, and by the University of Utah on radiocarbon and stable isotopes.

These methods are becoming more mainstream in ecological research due to decreases in cost and greater access to instruments in more campuses and user facilities. Research awards for access to specialized instruments are also available for researchers at all levels, from graduate students to more established senior principal investigators. For example, the Department Of Energy's Pacific Northwest National Laboratory Environmental Molecular Science Laboratory has annual calls for research proposals that could benefit from the application of high-end instrumentation, including multiple NMR magnets. There are a limited number of NanoSIMS facilities in the world, and fewer of them specialize in ecological samples, so direct collaboration with associated scientists is currently the best way to access this technology. Specialized e-mail list serves, like ISOGEOCHEM, run by Cornell, the University of Vermont, and Texas A&M, are valuable sources of information on appropriate methods and location of instruments. We encourage students wishing to use these methods in their research, who may not have access to specialized instruments in their department or campus, to seek out collaborations with experts at other institutions and across fields.

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