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GAS-HYBRID SOURCE 14C-AMS:Application to terrestrial ecosystem, biological, and environmental research

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GAS-HYBRID SOURCE 14C-AMS:
Application to terrestrial ecosystem, biological, and
environmental research

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Discussed peripherals

HPLC/LCMS-Moving wire interface
TCEA - Analyzer
TDOC - Analyzer
GCMS
Conventional Manifold

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Executive Summary

Radiocarbon (^{14}C), a rare isotope of carbon ($1:10^{12}$) which undergoes radioactive decay, is used to answer fundamental and applied questions of interest to DOE's Office of Science Biological and Environmental Research and Basic Energy Science research portfolio. Typically analyzed by accelerator mass spectrometry (AMS), radiocarbon measurements provides the ability to determine biogeochemical process rates and due to its unique tracer applicability to identify sources and pathways of carbon at the molecular to landscape scale.

Although used to answer questions in the earth, atmospheric, and biological sciences, the application of radiocarbon analyses has yet to realize its full potential. This is in part due to the time consuming requirement of isolation of the carbon fraction to be analyzed and conversion of that fraction first to carbon dioxide and from there to solid graphite which is the preferred target matrix for analysis. Direct injection of carbon dioxide for AMS-^{14}C analysis is a promising avenue that can open up new phase spaces for higher throughput (and lower per sample cost) analyses across the biological, environmental, and ecosystem sciences.

We posit that, with appropriate investments in developing sample interfaces coupled to a direct CO_2 injection hybrid source with ^{14}C-AMS, the DOE could leverage LLNL's radiocarbon facility's technical and intellectual strengths and open up new paths for cross-disciplinary discovery and innovation while at the same time enabling radiocarbon to realize its inherent potential in the biological, earth, and atmospheric sciences. Such an endeavor could be used as a vehicle to train the next generation of inter-disciplinary DOE scientists.

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1. The Charge:

BERAC has been given a grand challenge charge to determine new and innovative tools that are needed to advance BER science in biological systems, climate, and energy sustainability and additionally to identify and/or develop new tools and their linkages to or within existing (DOE) facilities [BERAC 2010, Brinkman letter 2011]. Intellectual and technical overlap exists that allow for the simultaneous exploration of tools that address:

2.02 Establish new model organisms for relevant ecological process understanding.

2.08 Measure microbial processes and interactions in the real world and in experimental simulations.

2.12 Understand, predict, and manipulate the types and rates of ecosystem responses and feedbacks that result from and influence climate change.

2.15 Develop a complete understanding of the biogeochemical cycles important to regulating carbon flux through biological systems.

2.18 Define the fundamental microbial basis for permafrost carbon-methane transformations.

2.19 Develop designs for optimizing carbon flow for biomass production, carbon allocation, and biosequestration to reduces rates of atmospheric CO_2 accumulation and to increase terrestrial carbon storage by 50% in 20 years.

2.20 Determine carbon and nutrient dynamics in natural systems.

3.03 Develop ecosystem-observing systems to monitor biogeochemical cycles, estimate critical process parameters, and provide model tests in (ocean) and terrestrial biospheres, including subsurface soils.

3.04 Advance understanding of important biological interactions and feedbacks to identify potential tipping points and possible mitigation strategies such as carbon biosequestration.

† numbering scheme directly from the 2010 BERAC report

2. A Brief Introduction to Radiocarbon and AMS Analysis

Radiocarbon ($t_{1/2}$ 5730yrs) is produced naturally in the stratosphere when secondary neutrons generated by cosmic rays collide with ^{14}N atoms [Libby 1946; Arnold and Libby, 1949], *i.e.*, $^{14}N(n,p)^{14}C$. Upon formation, ^{14}C is rapidly oxidized to CO and then to CO_2, and is incorporated into the carbon cycle. Radiocarbon is used to radiometrically date carbonaceous containing substances to ~50,000 years BP [*e.g.,* Reimer *et al*., 2009 and references therein] and is used extensively in the earth sciences to elucidate timing and rates of late Quaternary events and processes. Due to atmospheric weapons testing activities the amount of ^{14}C in the atmosphere doubled in the mid/late 1950s and early 1960s from its preindustrial value of $^{14}C/^{12}C$ ratio of 1.176×10^{-12} [*e.g.,*

Nydal and Lovseth, 1983]. Following the atmospheric weapons test ban in 1963, the $^{14}C/^{12}C$ ratio, has decreased (Figure 1) due to the net isotopic exchange between the atmosphere, ocean and terrestrial biosphere [*e.g.*, Levin and Hessheimer, 2000; Graven *et al.*, 2011; 2012] and a dilution effect due to the burning of ^{14}C-free fossil fuel carbon, the "Suess Effect" [*e.g.*, Suess, 1955; Stuiver and Quay, 1981].
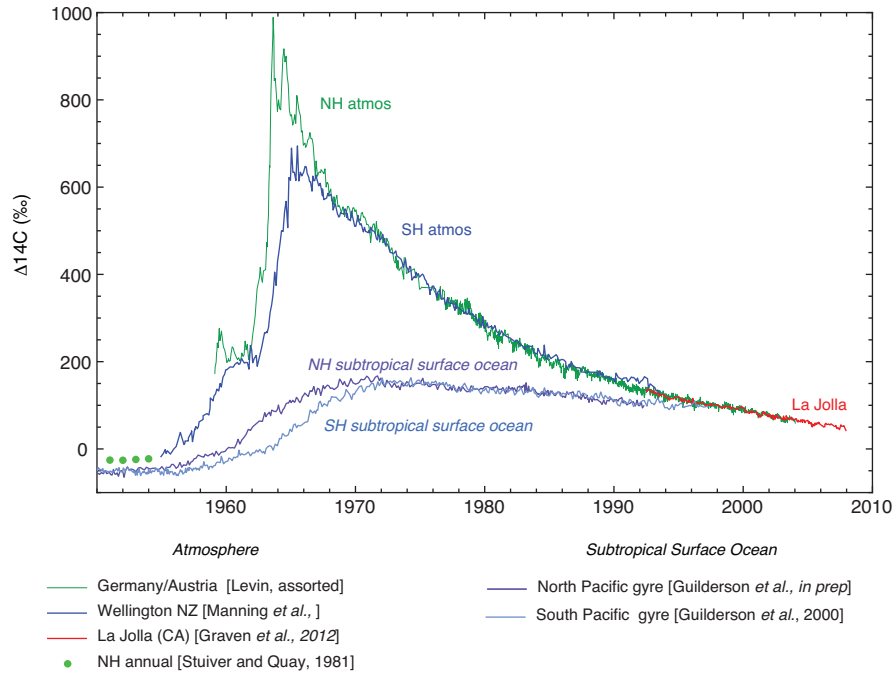

Figure 1. Atmospheric and surface ocean post-1950 history of radiocarbon as measured in atmospheric CO_2, tree-rings, and reconstructed from reef-building hermatypic corals. The decrease in atmospheric $\Delta^{14}C$ is a consequence of the net isotope and carbon exchange between the atmosphere, ocean, and terrestrial biosphere. The large seasonal amplitude in the early 1960s is a reflection of stratosphere (where much of the initial 'bomb' 14C was deposited) – troposphere exchange. The early asymmetry between the northern and southern hemispheres is due to the convolution of hemispheric atmospheric mixing and the preponderance of atmospheric weapons tests occurring north of the equator.

Except for the fact that the radiocarbon slowly decays, molecules that contain radiocarbon exactly behave chemically the same as its stable brethren: ^{14}C has the same number of protons and electrons as stable ^{13}C and ^{12}C. Utilizing isotopes are the standard for tracing exact chemical analogs of biochemicals (including nutrients and macromolecules) in biological systems and can provide quantification of molecular kinetics and dynamics in biological systems. Similar tracing exists in inorganic systems. The sensitivity for quantifying an isotopic label depends on the rarity of the isotope label, and the precision and sensitivity of the detection technique. The phase space of isotope abundance and detection allows one to tailor experimental design. Assuming equivalent efficiency and precision of detection, the more rare an isotope is, the more distinctive it is as a molecular label. Carbon 13 has a relative abundance to that of 12-carbon of 1.1% [Craig, 1957]. This is in contrast to ^{14}C which is approximately 1 part in a trillion (10^{12}) relative to ^{12}C and 1 part in 10^{10} relative to ^{13}C. By its very rarity, and assuming similar levels of detection, experiments tracing ^{14}C are more sensitive than ^{13}C by that same 10^{10}.

Very good isotope ratio mass spectrometry systems have $\delta^{13}C$ analytical precision of $\leq$ 0.1‰ versus 'routine' F14C analyses of ~3‰. Thus the practical analytical sensitivity between the techniques is 10^8 - 10^9.

The traditional method of measuring ^{14}C was to utilize sophisticated and well-shielded beta decay counters and count each individual beta particle created by the decay of individual ^{14}C atoms [*e.g.,* Anderson and Libby, 1949]. In the late 1970s novel solid state cesium sputter sources [Middleton 1974] and accelerator mass spectrometer techniques were combined that allowed for the calculation of the ^{14}C content of a sample via conventional isotope ratio mathematics [Bennett *et al*., 1977; Nelson *et al*., 1977]. Nomenclature and reporting of results using either technique have been standardized [Stuiver and Polach, 1977; Donahue *et al.,* 1990*;* Reimer *et al*., 2004]. Adoption of standard conventional isotope-geochemistry ‰ notation and conventional radiocarbon years before present [BP 1950] has, for the most part, simplified reporting in the earth and environmental sciences. In biological sciences where many of the measurements have been done with elevated tracer experiments there is a tendency for reporting to include activities as well as molar concentrations (Table 1).

14C Value	Unit
0.00	Δ (‰)
1.00	Fraction Modern, F14C
1.176×10^{-12}	atom/atom C
1.176	pmol/mol C
13.56	dpm/gC
226.0	µBq/mg C
6.108	fCi/mgC
97.89	amol/mgC

Table 1. Equivalent ^{14}C content notation

Because traditional counting waits for the decay of ^{14}C its analytical phase space is the nexus of sample size, ^{14}C abundance, and time. For example, traditional counting of a modern (F14C = 1.00) to ~ +/-3‰ precision requires 1 gram (0.08 moles) of carbon to be counted for 8 days or more. For the same modern sample a graphite sputter source combined with an AMS system could achieve the same +/-3‰ on a sample smaller than 1 mg (~83µmoles) of carbon in several minutes or less. With special handling graphite-based AMS analyses of near modern materials extends to a ~20µg (~2 µmoles) of carbon although the precision is lower. The main difference between counting and AMS is not precision and accuracy, but sample size and time of analysis. AMS has a sensitivity gain relative to counting of 10^3 to 10^9 depending on the sample size and the inherent $^{14}C/^{12}C$ ratio of the sample being measured.

3. Science Themes Which Use Radiocarbon

3.1 Carbon Cycle & Ecosystem Science

A critical scientific question is what are the present day sources and sinks of carbon dioxide (CO_2) in the natural environment and how will these sinks evolve under rising CO_2 concentrations, expected climate change, ecosystem response, and land-use change (including those used to meet energy needs via bio-fuels and feedstock). On glacial-interglacial timescales rising atmospheric pCO_2 provides a strong positive feedback to the process of deglaciation [*e.g.,* Luthi *et al.,* 2008; Shakun *et al.,* 2012 and references therein] and the amelioration of ice-age climate. Relationship between atmospheric pCO_2 and 'climate' is observed on seasonal to interannual timescales – *e.g.,* droughts in SE Asia and Amazonia during El Niño events, and the recent drought in western Europe which undid 6 years worth of regional net biosphere sequestration [*e.g.,* Bellamy *et al.,* 2005]. On multi-decadal to centennial timescales we anticipate similar relationships but because of the timescale we do not have good observations or models of the processes that influence the net exchange of CO_2 between the terrestrial biosphere and the atmosphere. Instrumental observations and primitive coupled carbon climate model simulations are consistent and support a positive terrestrial carbon cycle - climate feedback. Disagreements exist on the amplitude of the carbon cycle 'gain factor' as well as whether or not the current assessments and models correctly capture the sensitivity of the mechanisms.

Globally, more carbon is stored in belowground as soil organic matter than in terrestrial vegetation and the atmosphere combined [*e.g.,* Schlesinger 1997; Schurr *et al.,* 2007; www.gcp.org]. This vast pool of carbon is derived primarily from decomposed plant and microbial cell material [*e.g.,* Swift *et al.,* 1979, Scholes *et al.,* 1997], and the fluxes that control the size of this pool are critical to the global carbon cycle. The Intergovernmental Panel on Climate Change and DOE have both identified soil carbon sequestration as a potential means for mitigating rising levels of atmospheric CO_2, [Genomics GtL 2008] but the basic processes of SOM formation and turnover are not well understood. DOE's OBER 2008 Carbon Cycling and Biosequestration report specifically identifies the need to "more accurately predict the impacts of shifting climate conditions on carbon cycling and biosequestration in ecosystems" and incorporate this knowledge into climate models. Natural abundance or pulse-chase ^{14}C experiments can help address these mechanistic questions, by determining the age, mean residence time, and source-to-fate pathways of specific soil organic matter molecules.

Carbon initially enters the belowground soil pools as plant detritus, roots and (root) exudates. Once in the soil, this organic matter serves as a carbon source for decomposer organisms including soil animals, bacteria, and fungi. Most of this carbon is consumed and respired as CO_2, but some is converted to microbial biomass and byproducts (organic molecules), which may leave the soil as dissolved organic carbon (DOC), be used as a substrate by other microbes, or be stabilized within the soil mineral matrix. Mechanisms that result in the stabilization of soils include: climate stabilization (temperature, water content, oxygen availability); intrinsic recalcitrance due to chemical

structure *e.g.,* pyrolized carbon and lipids; physical stabilization whereby organic molecules are in association with aggregates and mineral surfaces; and microbial metabolic activity and or protection of potential substrate due to physiochemical barriers [*c.f.* Trumbore 2009 and references therein]. Because of complex interactions between soil forming factors, much of the carbon in surface and deep vadose zone soil is in reservoirs with turnover times that are multi-decadal to millennial. The controls on these longer term pools or reservoirs is a critical unknown. In the face of rising greenhouse gas concentrations, and climate change and uncertainties of the terrestrial biosphere as a future global sink or source of atmospheric CO_2 [*e.g.,* Friedlingstein *et al*., 2006; Govindasamy *et al*., 2005; Sitch *et al.,* 2008; Thompson *et al.,* 2004] understanding how organic matter is created, stabilized, and remineralized is a critical need.

Over the last few decades a renaissance has occurred in our understanding of the mechanisms and timescales in stabilizing organic matter in soils. Paradigm shifts have changed our view of the relative importance of above ground (litter/detritus) and below ground (fine root/root exudates) input into soil organic carbon pools on seasonal and interannual timescales [*e.g.,* Matamala *et a*l., 2003; Hanson *et al.,* 2005; Swanston *et al*., 2005; Trumbore and Gaudinski 2003]. Of particular note is the realization that it is the complex and varied interplay between physical-environmental and biological controls that regulate carbon turnover time in soil [Schmidt *et al*., 2011 and references therein, figure 2] and not "chemical structure" (recalcitrance) alone. Microorganisms can affect soil organic carbon directly via decomposition (with a portion lost via respiration) and the conversion of soil carbon to organic cell biomass that subsequently becomes an important component of soil carbon. Microbial biomass can also recycle stabilized yet labile carbon that is hundreds to thousands of years old [*e.g*., Gleixner *et al.*, 2002; Rethemeyer *et al*., 2004; 2005]. Environmental change that influences microbial metabolic activity and/or
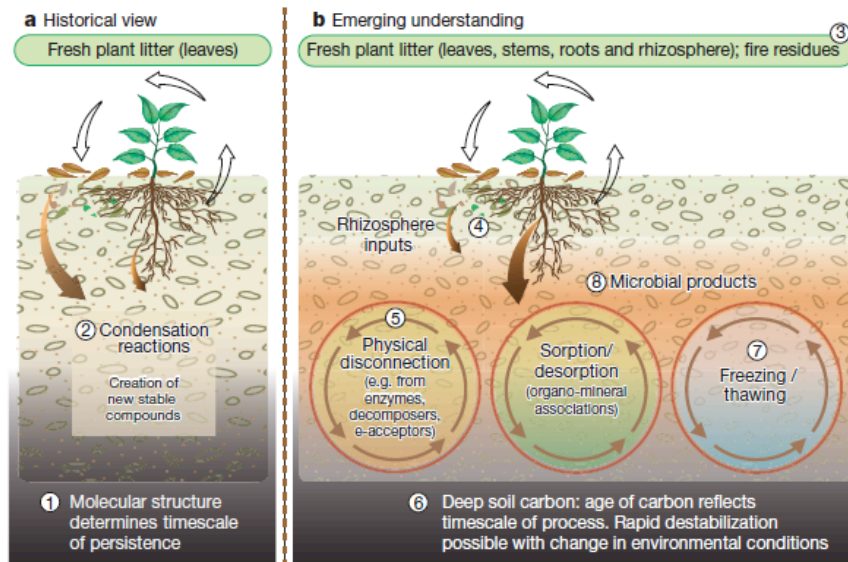

Figure 2. Evolution of processes controlling soil carbon content and turnover [from Schmidt et al., 2011].

community structure can thus strongly influence soil carbon cycling and its subsequent gain or loss impacting atmospheric CO_2 concentrations. As a corollary: changes in

metabolic activity, community structure, available substrate, and environmental conditions can also lead to changes in microbially mediated metal-ligand binding and/or the precipitation of organo-heavy metal precipitates. Additionally, such changes may produce microenvironments that make soluble other elements (Cd, As, actinides, etc).

In ecological studies "natural abundance" radiocarbon ($^{14}C/^{12}C$ ratios of 10^{-15} to 10^{-12}) is routinely used to determine:

The age of formerly living things (simply 'dating' the organism or carbon).
To determine cycling rates in vegetation and soils (*e.g.*, mean residence times)
As an indicator of source pools to carbon fluxes (*e.g.*, ancient permafrost carbon)

In low-level pulse-chase labeling experiments elevated ^{14}C-substrates (or CO_2) of $\geq 10^{-12}$ to 10^{-8} ($^{14}C/^{12}C$) are used to:

Determine cycling rates, allocation in plants, and pathways of pools of carbon that include: consumption, respiration, storage (chemical and structural), and export

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Previous research has shown that large amounts of carbon are stabilized in associations with Fe in terrestrial and marine systems [Torn, *et al* 1997, Lalonde, *et al* 2012], although due to methodological limitations, fewer studies have probed the actual structure of these associations [Keiluweit *et al in review*]. Association of organic matter with Fe surfaces may be structured as proposed by Kleber, *et al* [2005] via classic surface adsorption with a clear boundary between mineral surface and adsorbed organic matter. Alternatively, occlusion at the clay microstructure level (i.e. in the coarse clay/fine silt size range) may occur, due to either abiotic mechanisms (such as the precipitation of Fe- and Al-oxides or hydroxides [*e.g.*, Duiker *et al*., 2003] or soil biota; microbial cells, secretions, root exudates and faunal mucus act as cementing agents and are at the same time occluded within microaggregates [*e.g.*, Six *et al*., 2006; von Lutzow *et al*., 2006]. Organic matter-Fe associations may form a new mixed phase particle. The role of soil microbiota in promoting one of the other of these two structural end-members is unclear, but the end result could have a significant impact on the long-term fate of soil organic matter. To better understand the role of microorganisms in the processes of soil carbon stabilization, we need to be able to track the creation and fate of individual organic matter molecules from their source (plant or microbial tissue) through transformation processes (microbial respiration or fermentation) to their eventual fate (*e.g.,* live cells, dead cellular materials, mineral-sorbed, occluded, dissolved pools). Pulse-chase experiments are an avenue for such tracing.

In many instances and due to the increased sensitivity of ^{14}C relative to ^{13}C, low-level pulse-chase ^{14}C labeling is advantageous over ^{13}C labeling. Moreover, ^{14}C has the potential to trace carbon in plants and ecosystems up to several years (Table 2). This increased sensitivity allows for assessment of how carbon cycles through plant-soil systems under ambient CO_2 concentrations [*e.g.,* Carbone *et al.,* 2007; Czimik *et al.,*

2005] or ambient carbon (*e.g.*, litter) input rates [*e.g.,* Cisneros *et al*., 2006; Froeberg *et al*., 2007; Hanson *et al*., 2005; Trumbore *et al*., 2002].

	DAYS	MONTHS to YEARS	YEARS to DECADES	CENTURIES - MILLENIA
TECHNIQUES	Pulse-chase 13C and 14C	Pulse-chase 14C	"Bomb 14C"	Natural 14C
	Natural abundance 13C	Continuous labeling *(13C, 14C)* Natural 13C *(C3-C4 shifts)*		
CARBON POOLS	Plant metabolism	Plant metabolism		
		New growth	Growth	Growth
	Non-structural carbon	Non- & structural carbon storage	Structural carbon storage	
		Soil Organic Matter	Soil Organic	Soil Organic
	Microbes	Dynamics	Matter Dynamics	Matter Dynamics
		Decomposition	Decomposition	Decomposition

Table 2. Applications of ^{13}C and ^{14}C isotope tracing to carbon cycle science.

3.2 Revolutions in Biological Sciences

Concurrent with the renaissance in carbon cycle science, the development of new analytical tools (*e.g.,* sequencing, isotope probing and a variety of imaging techniques) has improved our understanding of cellular metabolic activity and the biochemistry of microorganisms that mediate most biogeochemical transformations. In the past decade, approaches such as nucleic acid stable isotope probing (SIP) and related culture-independent approaches (*e.g.* ^{13}C-PLFA; EL FISH, FISH MAR, isotope arrays), have become widely used means to directly connect the uptake of specific organic substrates to microbial identity. These approaches have generated many advances in the understanding of microbial bioremediation, plant-microbe interactions, food web dynamics, and microbe mediated processes (*e.g.* methanotrophy and N-fixation) yet they remain hindered by logistical drawbacks such as isotopic sensitivity, throughput, and an inability to track specific molecules. Pulse-chase labeling experiments have the potential to make significant contributions to this field by allowing a researcher to track a specific ^{14}C-labeled substrate molecule into specific microbial populations and even individual metabolites, with high sensitivity and chemical specificity.

The field of 'systems biology' uses a combination of genomic, proteomic, and metabolomic technologies to construct computational models of complex biological systems and understand how interactions between multiple cellular or community processes give rise to system functions: for example, the enzymes and metabolites in an

individual metabolic pathway [Ideker 2004]. Computational biological models, an example of which is flux-balance analysis (FBA) have been developed to predict a genotype's metabolic capabilities and system function. Historically, it has been difficult to measure intracellular and, in some cases, even extracellular fluxes of specific metabolic compounds and biosynthesis products. Thus FBA and other constraint based models are often limited by a lack of experimental data (*in vivo* and *in situ*) to optimize the models to reflect nature more accurately (*i.e.*, are biologically reasonable and reflect experimental measurements).

Despite their great utility, standard FBA models cannot fully predict metabolism in multi-cellular organisms. This shortcoming stems from the fact that FBA models are generally used to predict metabolic fluxes that maximize a single objective. Given that multi-cellular organisms perform a number of different functions, multi-objective flux analysis (MOFA) approaches are important to study metabolism in such systems. A handful of MOFA models have been developed [*cf.*, Handl, *et al.,* 2007; Senden *et al.*, 2009; Nagrath *et al.,* 2007; Xu *et al.*, 2009]; however, they often result in multiple optimal solutions [Mahadevan, *et al.*, 2003]. The combined use of HPLC and traditional graphite-AMS methods has been demonstrated in yeast to allow direct flux measurements of intermediate metabolites [Stewart *et al.*, 2010], thereby providing experimental constraints for flux balance analysis. This approach can provide a quantitative understanding of metabolic pathways involved in the progression of human disease and to identify metabolic bottlenecks in biofuel-producing organisms. Similar tracer-based studies could be applied to further elucidate the intersection of form and function in the stabilization of organic matter in soil pools (Figure 3).
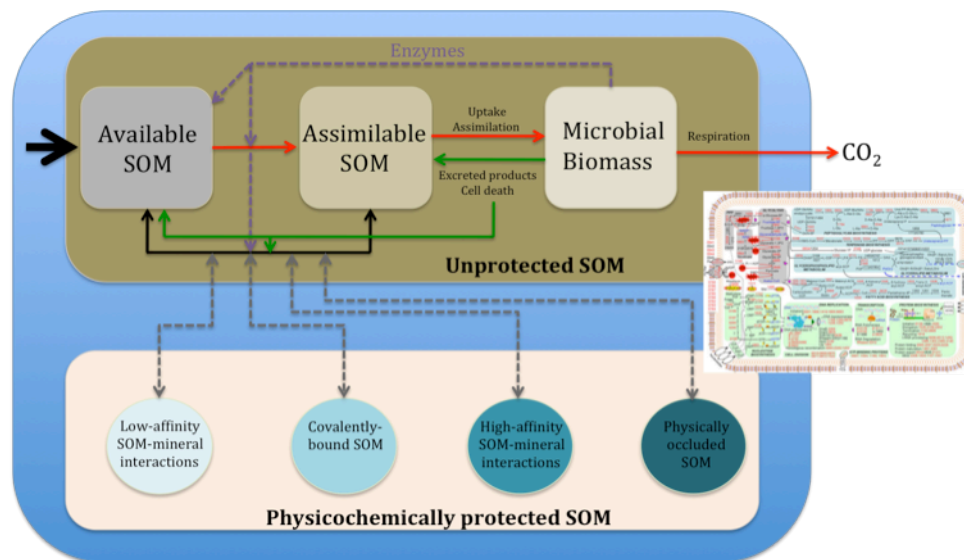

Figure 3. Schematic representation of potential microbial biomass/biochemistry - soil organic matter interactions. External factors that influence the interactions include temperature, soil moisture, pH, mineralology, structure, and vegetation. Adapted from Conant et al., [2011], courtesy E. Brodie (LBL). A similar schematic could be constructed for the interactions between microbes and various subsurface contaminants and substrates. To bridge genome and functional expression, isotopic labeling could be used to trace specific biochemical pathways.

[14]C-pulse-chase experiments are particularly useful to the biological sciences

(including biomedicine) and have been used to explore metabolism and kinetics [*e.g.*, Buchholz *et al.,* 1999; Cleland 2003; Turtletaub *et al.,* 1990; Vogel and Love, 2005], DNA damage [*e.g.*, Dingley *et al.*, 1998] and the potential of chemical therapeutics [*e.g.,* Buchholz *et al.*, 2012]. Targeted labeling using radiocarbon (^{14}C) allows for the calculation of metabolite fluxes through specific pathways in the presence of multiple carbon sources [*e.g.,* Stewart *et al.*, 2010] and provides a means to independently test and constrain FBA models. Such experimental results will provide internal and external flux constraints (on the model) and will improve the accuracy of FBA and related models' predictions.

3.3 Atmospheric Chemistry & Climate

The level of understanding (scientific basis) of the cumulative (net) radiative effects of aerosols is very low and leads to a large range of uncertainty of the net feedback in earth system models for future climate change scenarios [IPCC 2007]. Black carbonaceous aerosols are of particular importance, as they are typically less hygroscopic and therefore have potentially longer lifetimes than the well-studied fine inorganic aerosols (sulfuric acid, ammonium bisulfate, ammonium sulfate, ammonium nitrate, sodium chloride, *etc*.). Although black carbon has been referred to as "elemental carbon" it should be noted that black carbonaceous aerosols are a complex mixture of low-volatility carbon. The major sources of black carbon are from incomplete combustion of carbon fuels including fossil fuel combustion (particularly by diesel engines) and biomass burning (such as wood and vegetative burning). The carbonaceous aerosols generated by these combustion sources are also associated with primary organic carbon aerosols, as well as secondary organic carbon aerosols formed by atmospheric oxidation reactions of vapor phase and aerosol phase organics. Black carbon as well as aerosols from wood smoke (*e.g.*, biomass burning) and secondary carbon-based aerosols are all strong solar radiation absorbers and the radiation effect is only second to that of greenhouse gases [Jacobsen, 2002; 2004]. Better tools, or the wider application of existing analytical tools, are required to elucidate the sources of carbonaceous aerosols the chemical pathways of the formation of secondary aerosols and reducing carbonaceous aerosols at their respective combustion sources [*e.g.,* Bahreini *et al.,* 2012; Buchholz et al., 2002; May *et al.*, 2008; Szidat *et al.*, 2006 among many].

If it is so useful, why isn't ^{14}C (AMS) used more widely?

Large-scale studies are often resource limited. Depending on location of analysis and the effort for chemical pretreatment and preparation to graphite per sample cost range from ~$150 to in excess of $700 per sample. Although there is extant AMS-^{14}C analysis capacity in the US, access to sample preparation can be limited. Conventional AMS analyses requires isolation of the carbon fraction to be analyzed, conversion of the sample matrix to carbon dioxide and subsequent catalyzed reduction of the carbon dioxide to filamentous graphite which is the matrix analyzed via AMS [*e.g.*, Vogel *et al.,* 1987]. Establishment of satellite graphitization preparation laboratories is one means to expand access and in some cases lower sample preparation costs. Agency specific examples of "satellite" graphitization laboratories that funnel samples as graphite for AMS analysis

include the USGS (Reston, VA) and the USFS (Houghton, MI). Within the US academic community there are a small number of satellite graphite laboratories including the University of Florida (Schuur), University of Utah (Ehleringer), University of Colorado (Lehman), and the University of Hawai'i (Beilman).

Where within the DOE are AMS-^{14}C measurements made?

Within the DOE graphite sample preparation and subsequent AMS analysis occurs at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory (http://cams.llnl.gov). In addition to PI specific projects and collaborative research activities (funded by a variety of mechanisms), CAMS has an open-door policy and processes samples for the DOE (*e.g*, samples from ANL, ORNL, LANL, LBL), and the broader research community on a fee-for service basis.

4. Technical Advances – CO$_2$ Source for AMS-^{14}C

Direct ionization of CO_2 for AMS-^{14}C ultimately, and to defeat the ^{14}N isobaric interference, requires negative ions (nitrogen does not form a stable negative ion) [Mueller *et al*., 1977 among many]. Direct ionization of CO_2 to CO_2^- is particularly inefficient which coupled with the low abundance of ^{14}C has precluded adoption of ionization sources similar to those used in conventional stable isotope mass spectrometry [*e.g.,* Aitken *et al.,* 1978]. Two approaches to circumvent the nitrogen interference and still use CO_2 as the analyzed matrix is to 1) first convert the CO_2 to a positive ion and then pass the CO_2^+ through a charge exchange canal converting it to CO_2^- [von Reden *et al.,* 2007 and references therein] and 2) getter CO_2 on a titanium frit simultaneously bombarding the CO_2-Ti with cesium which is thought to convert the CO_2 to graphite *in situ* followed by sputter ionization (Figure 4). The former of these approaches has been applied at the NSF National Ocean Sciences AMS facility (NOSAMS) using a microwave plasma source to initially produce the CO_2^+ [Roberts *et al*., 2007; von Reden *et al*., 2007; 2011] and the latter adapted at AMS facilities in Switzerland [*e.g*., Ruff *et al.,* 2010], England [*e.g*., Bronk Ramsey *et al*., 1997; 2000; 2004], Australia [Fallon *et al*., 2010], and also by the NIH Research Resource Bio-AMS system at CAMS [Ognibene and Salazaar, 2012; Salazaar *et al.,* 2012]. In addition to accepting CO_2, gas-hybrid sources can also analyze samples prepared as graphite targets placed in conventional cathode target holders.
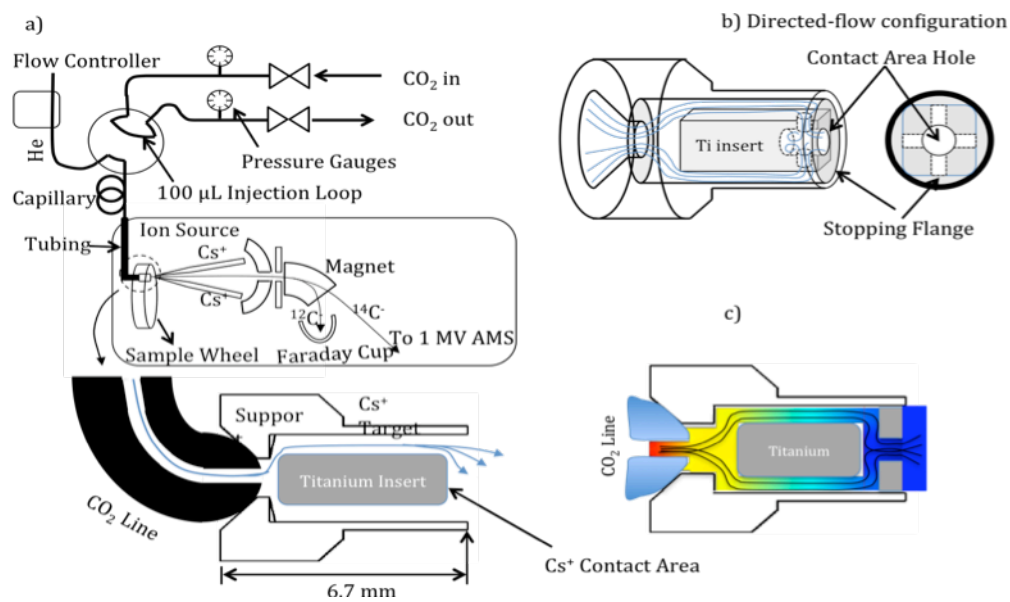
Figure 4. a) Scheme of the test system for direct CO_2 ionization: CO_2 was injected into the AMS ion source where it enters into a "Cs^+ target", in this case, a diverging-flow configuration. b) A more effective way is by directing the CO_2 through conducts into a titanium surface which is also being sputtered with a Cs^+ beam. We hypothesizes that the $Cs+$ beam energy converts the adsorbed CO_2 into C^-. The C^- was selected by a magnet and its charge was detected by a Faraday cup. c) Computer simulations of gas flow inside the "target" and how it gets in contact with the Ti surface. Salazaar and Ognibene., 2012.

The lure of direct injection of CO_2 for ^{14}C analyses is several-fold and includes: reduced sample handling and increased automation (due to not needing to manually convert samples to graphite) and the availability of off-the-shelf of "front-end interfaces" that can present CO_2 from a wide range of initial sample matrices [*e.g.*, von Reden *et al.,* 2007]. The trade off compared to analysis of solid graphite target is a loss of efficiency of ionization and subsequently, sensitivity and precision. Solid-state graphite sources have ionization efficiencies (conversion of graphite to C- ions) of several to ~40% [*e.g.,* Fallon *et al.*, 2007] which when combined with transmission and stripping (required to destroy molecular isobars $^{12}CH_2$, ^{13}CH which are injected with mono-ions of ^{14}C) yields a total system efficiency (^{14}C atoms in sample to those counted at the detector) of a few to ~15% (Figure 5). Gas-hybrid sources have ionization efficiencies of at most 3% (more frequently 1-2%) which translate to a total efficiency of ≤1%. The CFAMS system at NOSAMS has an overall efficiency of ≤0.3% [Roberts *et al.*, 2007; von Reden *et al.*, 2011]. Lower efficiency translates to lower total counting statistics and thus decreased precision as a function of sample size (total available ^{14}C atoms in the sample). Using a graphite sputter source and not even completely consuming the sample target a 100μg-C (8 μmole) near modern sample can routinely be analyzed to better than 5‰ whereas the lower efficiency of gas-hybrid sources places the theoretical counting precision limit at 1-2% (10-20‰).

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1 μg (0.08μmole) of modern carbon contains ~ 20,000 ^{14}C atoms.
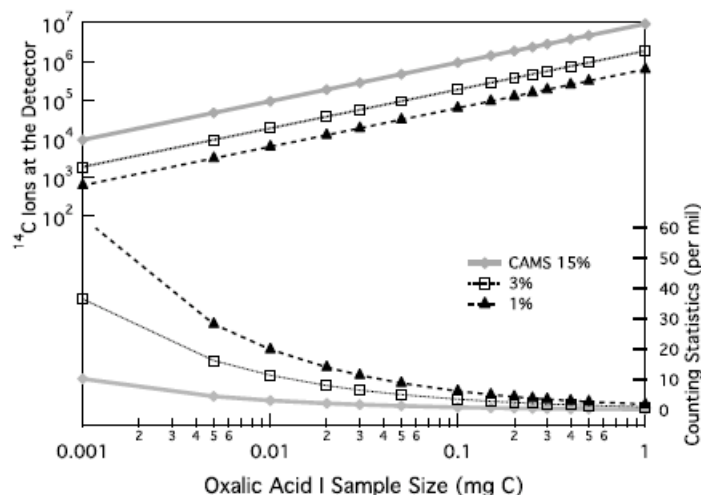**A total system efficiency of ~1% would lead to 200 counts at the detector.**

Figure 5. Total 14C counts at the detector (top panel) and the corresponding counting statistics (bottom panel) assuming complete consumption of a modern (F14C 1.04) graphite target versus sample size. The lines correspond to 15% total efficiency (CAMS Cs-sputter source and the FN AMS system), 3% and 1% overall efficiency. The 3–5% C-efficiency quoted commercial sputter based ion sources and <3% for gas-hybrid sources would, depending upon charge exchange efficiency and transmission losses, likely result in total system efficiencies in the 1–3% range. Adapted from Fallon et al., [2007].

Although less efficient than conventional Cs-sputter sources, gas-hybrid source based AMS-^{14}C can open up new phase spaces for higher throughput (and lower per sample cost) analyses across the biological, environmental, and ecosystem sciences. Addition of ^{14}C tracer (*e.g.,* pulse-chase experiments or labeled synthetic chemistry and combustion experiments) can for "small" mass samples increase the overall ^{14}C counting statistics and lead to better precision. Samples that are 1-20μg (0.08-1.6 μmoles) of carbon even without tracer addition could be analyzed to elucidate sources – *e.g.,* petrochemical based versus modern biogenic pathways or elucidate the primary source of primary and secondary carbonaceous aerosols [*e.g.,* Bench *et al.,* 2007; Buchholz *et al.,* 2002; Marley *et al.,* 2009; Reddy *et al.,* 2002; 2008; Sheesly et al., 2009; Schichtel *et al.,* 2008].

5. Potential Interfaces for a Gas-Hybrid Ionization Source

5.1 High-Performance Liquid Chromatography – Moving Wire Interface (MWI)

CAMS' Biomedical Research Group has developed a system to directly analyze elutant from an HPLC using a gas-graphite hybrid CO_2 source and a moving wire interface (MWI: Figure 6) [Thomas *et al.,* 2011; Ognibene *et al.,* 2012]. The development of the MWI was driven by the need for basic analytical tools for biomedical studies with a focus on metabolites, enzyme dynamics, elucidation and quantification of biochemistry pathways, and developing data-sets that are appropriate to test flux balance models [*e.g.,* Bucholz *et al.,* 1999; Miyashita *et al.,* 2001; Stewart *et al.,* 2010; Vogel *et al.,* 2001]. The MWI is based on the design of Sessions *et al.,* [2005] which has been successfully used for stable $\delta^{13}C$ analyses of specific analytes with masses as small as 90

nanograms. Coupled to an HPLC allows for the discrete analysis of eluted non-volatile compounds or a spectrogram of compounds. Compounds that are amenable to HPLC separations include but are not limited to: sugars, amino acids, nucleic acids, proteins, sterols, ether-linked isoprenoids, ester-linked phospholipids, long and short chain polyamines.
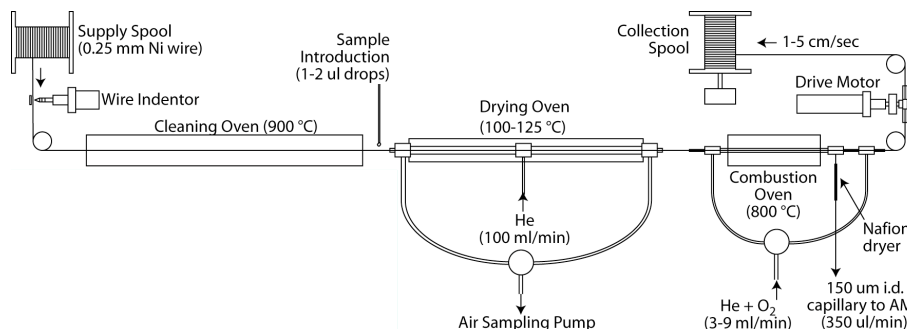

Figure 6. Schematic of the MWI. Samples can be introduced from an eluted stream from an HPLC, a "column-less" HPLC (i.e., a pre-chosen single compound), or manually dropped onto the moving wire (e.g., aqueous and colloidal solutions).

The design and initial optimization of the MWI focused on tracer-based applications in the biological sciences where 1-2μl drops of HPLC separated analytes are, via a micro-capillary needle, placed on the wire, dried, and converted to CO_2. An indenter generates dimples on the nickel wire which significantly improves sample adhesion. The MWI produces a temporal resolution similar to that of a conventional HPLC ultra-violet adsorption detector [Figure 7] and has been adapted from the design of Sessions *et al*., to have 100% transmission of CO_2 with no splitting of the CO_2 stream [Thomas *et al*., 2011]. The introduction of the sample stream could be adapted to handle larger volumes or to increase the total amount of carbon and increase precision via repetitive sampling (Figure 8).   In theory, particulates or colloids in solution could be introduced via the MWI.

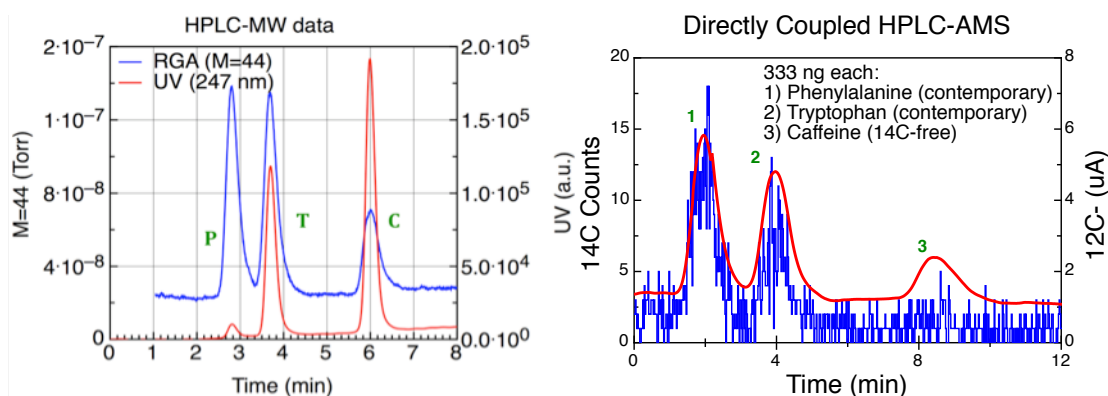*Figure 7. (LEFT) 1 ul injection containing 0.333 ug each in methanol-water of phenylalanine (P), tryptophan (T) and caffeine (C) through the MWI and output to either a conventional UV detector (red line) or a residual gas analyzer (RGA – blue line). (RIGHT) Real-time trace of a synthetic mixture of P, T, C ^{14}C counts detected (blue line) and ^{12}C current (red line).*
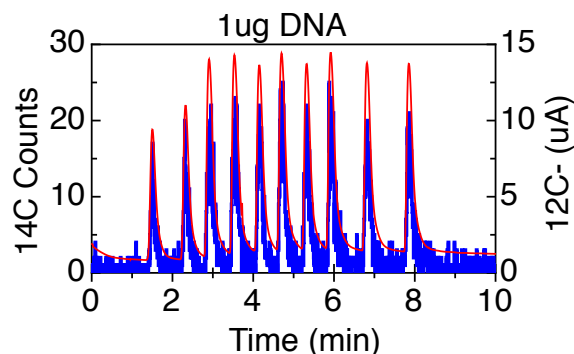
16

Figure 8. Initial measurements of (human) DNA: <1μg of DNA in manually placed repetitive 1 μl drops. F^{14}C is 1.04 ± 0.05. The reproducibility of the individual drops is consistent with counting statistic limitation of so few ^{14}C atoms analyzed.

5.2 Total Carbon / Elemental Analyzer (TCEA)

Elemental analyzers are ubiquitous instruments and used to quantify the inherent carbon content of analyzed material. In the last decade they have been frequently utilized as a front-end peripheral to conventional isotope ratio mass spectrometers (δ^{13}C, δ^{15}N). Samples, placed in tin capsules, are converted to CO_2 in a helium-oxygen stream. The oxidation of the tin capsules is an exothermic reaction which raises the effective combustion temperature. The combustion gases are purified by passing the gases through a copper oxide bed, further purified over a bed of lead chromate (to trap sulfur) and the resulting pure gases (CO_2, H_2O, N_2) are chromatographically separated in a column. The temperature of the column is modulated to trap CO_2 and H_2O (<50°C) whilst N_2 and the carrier gas (usually He) pass through. The column is then heated to release CO_2 (> 80°C). TCEAs have been successfully interfaced with gas-hybrid ion sources [*e.g., Ruff et al., 2011*].

Samples that are amenable to TCEA elemental (%C) or δ^{13}C analysis are amenable to TCEA-Gas-Hybrid AMS analysis. These would include macro and bulk samples, physically or chemically separated soil fractions (*e.g.,* free light, occluded light), other sources of particulate organic and inorganic carbon, bulk chemistry isolates (*e.g.,* lignin, cellulose). In elemental analyzers very low percent carbon matrices (*e.g.,* acid insoluble heavy fraction) have a history of incomplete combustion, memory between samples, and physically clogging the column. For these reasons such matrices may not be amenable to direct injection TCEA-CO_2-^{14}C but would require conventional sealed tube combustion and subsequent analysis (see section 5.5).

In addition to regular precision (3-4‰) analyses on full sized (~80μmole) samples an EA feeding a gas-hybrid source gives the opportunity for (inexpensive) lower precision analyses (1-2%). Replicate samples (3-4‰) at the plot scale have been used to assess plot-size variability [*e.g.,* Hanson *et al.,* 2005; McFarlane *et al.,* 2012; Swanston *et al.,* 2005] and have determined that variability from plot to plot is a factor of five (5) larger. There is an underlying assumption that this variability translates across scales but the expense for preparation of graphite targets has precluded landscape scale quantitation.

Inexpensive and rapid lower precision analyses could easily be applied at the landscape scale to capture the full range of variability and provide additional insights into the heterogeneity of carbon sequestration and transport through ecosystems. This would have the obvious benefit of increasing the library of carbon data that can be utilized by regional and global models under future International Land Model Benchmarking (I-LAMB) and Carbon-Land Model Intercomparison Projects (C-LAMP) and the IPCC Assessment Report process.

5.3 Total Dissolved Organic Carbon (TDOC) Analyzer

Until the last decade dissolved organic carbon has been an underappreciated mechanism for moving carbon across landscapes and between reservoirs: *e.g.,* land to stream/river, or from land to the ocean. This realization is in part due to the availability of off-the-shelf instrumentation and established protocols to quantify DOC concentrations and particularly the increasing use of radiocarbon analyses to determine the source of DOC. Radiocarbon analyses of DOC has been applied to elucidate and document the rapid transformation of carbon across the Amazon landscape and to show that a significant portion of the carbon fixed by vegetation is shunted from the forest to the Amazon River and from there to the ocean and atmosphere inside of 5 years [Mayorga *et al.,* 2005]. With large stores of "^{14}C-old" carbon as peat and permafrost [*e.g.,* Schuur *et al.*, 2007; MacDonald *et al.,* 2006; Beilman *et al.,* 2008] ^{14}C is an incredibly useful tracer to document DOC exchange across the Arctic landscape [*e.g.,* Benner *et al.,* 2004; Dutta *et al.*, 2006; Guo *et al.*, 2007; Neff *et al.*, 2006; Raymond *et al.*, 2007; Zimov *et al.*, 2006 among many].

One common "off-the-shelf" TDOC analyzer is high-temperature catalytic oxidation TDOC analyzers. This technology combines high temperature combustion (>600°C) with an oxidizing catalyst such as platinum and usually an infra-red gas analyzer (NDIR) for peak quantification. TDOC analyzers have been coupled to stable isotope ratio mass spectrometers to determine the δ^{13}C value of DOC [*e.g.*, De Troyer *et al.*, 2007; Kaldy *et al.,* 2005; Panetta *et al.,* 2008]. This requires a sequential system that takes the TDOC effluent and directs it through a reduction and gas chromatography column prior to release (injection) into the IRMS. Delay and trapping of the TDOC effluent prior to passing to the chromatography column allows for sharpening of the CO_2 peak and, in the case of low concentration samples, repeated injections of aqueous DOC. Addition of a cobalt-silver oxide trap is used to capture halogens from thermally decomposed salts [Panetta *et al.,* 2008]. This arrangement allows a significant number of sample injections before catalyst regeneration or fouling due to the deposition of salts. A similarly modified TDOC analyzer would be a viable front-end interface for the gas-hybrid ^{14}C-CO_2 source. Such a system would facilitate research planned under NGEE-Arctic and is anticipated for NGEE-Tropics.

5.4 Gas Chromatography Mass Spectrometry (GCMS)

Gas chromatographs are ubiquitous instruments used to identify different volatile substances within a sample. Applications span the full breadth of the biological, chemical, and environmental sciences and analyzed isolates include but are not exclusive

to: n-alkanes, n-alcohols, fatty acids, isoprenoids, sterols, hopanoids, lignin, phenols, PAHs, and PCBs. For more than twenty years GCMS instruments have been directly coupled to stable isotope mass spectrometers and the $\delta^{13}C$ values of individual compounds (termed CSIA for compound specific isotope analysis) have been used to elucidate environmental and biogeochemistry processes responsible for the composition and concentration of compounds across terrestrial and marine environments [*e.g.,* Freeman *et al.,* 1989; 1994; Hayes *et al.,* 1990]. Radiocarbon analyses of specific compounds (CSRA) has built upon CSIA and has been used as a tool to determine the source of carbon used by an organism (apparent age of the reservoir) and to add another constraint to the molecular and or metabolic pathways [*e.g.,* Eglinton *et al.,* 1996; 1997; Ingalls and Pearson, 2005; Pearson *et al.,* 2000; 2001].

Within the atmospheric chemistry community the ability to apportion carbon sources between fossil fuel combustion based sources and precursors and those that are emitted naturally from the biosphere has transcended from 'bulk' carbonaceous material [*e.g.,* Reddy *et al.,* 2002; Marley et al., 2009] to analysis of PAHs and black carbon [*e.g.,* Gustafsson *et al.,* 2009; Sheesley *et al.,* 2007]. Radiocarbon identification of the initial (carbon) source of condensation nuclei of secondary aerosols is a key need to determine aerosol life cycles and their role in radiative forcing. In addition to field based observations and experiments (*e.g.,* MILAGRO, CALNEX-CARES) using isotopic labeling of fuel sources and/or additives can allow one to follow fuel carbon in specific molecular structures through the combustion process. Isotope tracing of combustion processes was used to prove that the ester structure of the additive dibutyl maleate was less effective in suppressing soot than oxygen content would have predicted [*e.g.,* Buchholz *et al.,* 2002; 2003; 2004].

5.5 Conventional "Cracker" Manifold

Sample introduction into the ion source of "old fashioned" stable isotope mass spectrometers was done via a manifold inlet system. A number (12-24) of individual sealed glass ampoules (6mm) containing purified CO_2 were evacuated and isolated via pneumatic valves. After cracking of the tubes via a plunger (manual or automated) the operator (or computer software) would open the valve and allow the sample CO_2 to be collected in the sample bellows and from there to IRMS analysis. A similar approach for gas-hybrid CO_2-AMS[14]C analysis would allow for analysis of a wide array of sample matrices from a variety of isolation techniques. Conversion of the sample matrix to CO_2 would be performed via individual sealed tube combustion. Examples include but are not limited to specific chemical isolates and various low %C fractions which are not amenable to TCEA combustion (such as acid insoluble mineral associated carbon).

6. Analytical/Technical Challenges for Gas-Hybrid AMS-[14]C

The main analytical and technical challenge to fully take advantage of a gas-hybrid CO_2 source for ^{14}C analyses utilizing off-the-shelf or custom designed peripherals that are routinely utilized in the stable isotope community is to increase the source

ionization efficiency. The initial ionziation ($CO_2 > C^-$) is the limiting factor in the relationship between sample size and precision and thus modest gains in the efficiency directly translates to increased precision of analysis or decreased sample size to meet the same precision (refer to figure 5). To increase the ionization efficiency, experiments and modeling of alternative frit designs and gas flow are needed to explore, identify, and implement more optimum designs.

Wet lab experimental and chemistry space for natural abundance and tracer-^{14}C are not suitable for co-location. Nearly every natural abundance ^{14}C laboratory has taken pains to locate their space in a building separate from those where ^{14}C-tracer is used. It is not appropriate to site tracer incubations and microbial experiments in the same space where *e.g.,* physical-chemical fractionation of soils occurs. Cross-contamination issues that are prevalent in 'shared use' wet-lab space directly translate to peripheral devices.

The MWI was designed to trap all volatiles and excess carrier is shunted through a series of absorbent traps to collect CO_2 (elevated with ^{14}C). The nickel wire is one-time use and all inlets are heated to minimize adsorption of CO_2. Experiments carried out by the BioAMS group have shown that the protocol development is suitable for natural abundance and low-level tracer samples to co-use the MWI and the gas-hybrid source. Additional work is needed to confirm suitability of a single shared-use HPLC. Similar methodological protocols and/or physical modifications will be required for other 'shared' use peripherals to negate cross-contamination and spurious results. If methods and protocols cannot be developed (*i.e.*, experimental tests show cross contamination) then an 'easy' route is to have dedicated tracer and natural abundance instrumentation (HPLC, TCEA, TDOC).

7. Proposed Plans for the 1MV AMS System at CAMS

In 2011, following workshops in 2009 and 2011 with NIH and the community, the LLNL Biomedical AMS (BioAMS) research group was requested by NIH to submit a supplement to the existing P41 National Research Resource for Development of Biomedical AMS. The purpose of the supplement was to procure a new ^{14}C spectrometer focused on online real-time AMS analysis of biological samples. The current NIHRR BioAMS system is housed in CAMS' ~10000 square foot AMS facility (Figure 9) where direct biological samples (serum, urine, etc) are not routinely analyzed (*i.e.*, samples have to be converted to graphite elsewhere prior to AMS analysis). The new system to be installed in FY-2013 will be housed in a BSL-2 facility at LLNL. With the installation of the new system the 1 MV BioAMS system, gas-hybrid source, and HPLC-MWI has the potential to become dedicated to earth and atmospheric science research.
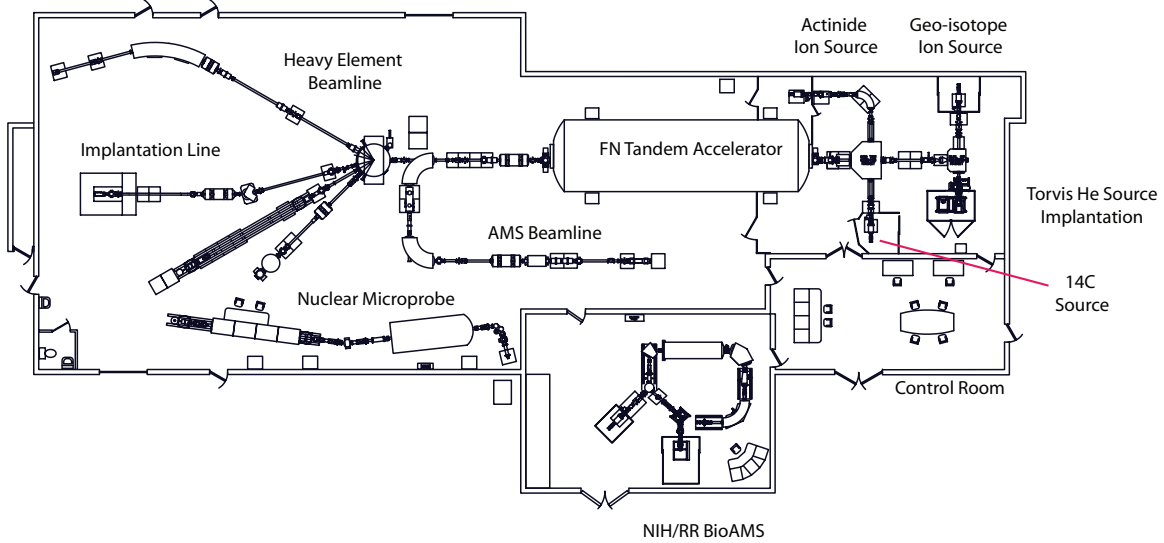
Figure 9. CAMS' AMS facility includes a multi-use FN tandem accelerator for AMS and implantation studies (materials science, isotope production), a nuclear microprobe, and the NIH/RR BioAMS system.

Due to space limitations and to facilitate the use of the BioAMS system with multiple peripherals that could be utilized for BER and BES research we propose the following:

1) Optimize the existing low energy injection system (Figure 10)
      a. Remove existing high-intensity Cs sputter source
      b. Simplify low energy optics by removing 45°ESA
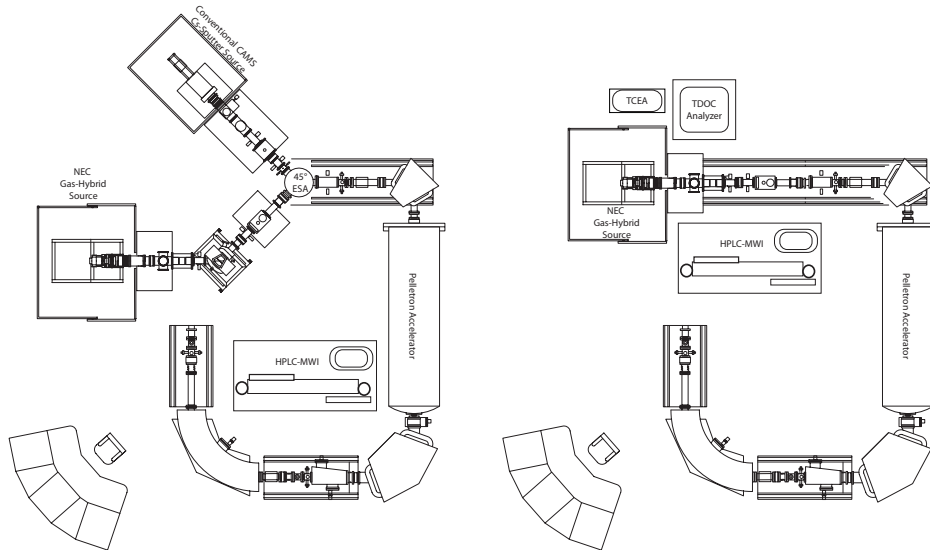
Figure 10. (LEFT) Existing geometry of the BioAMS spectrometer with two ion sources: a conventional high intensity Cs-sputter source, and a gas-hybrid source. (RIGHT) Proposed modification of the low-energy end of the spectrometer so that multiple peripherals could provide CO_2 to the gas-hybrid source. The gas-hybrid source can also accommodate conventional graphite targets, so the capability to measure graphite targets will not be lost.

2) Procure, modify, install, test (experiments will need to be performed to determine and ameliorate sample memory effects, fractionation, etc), interface to the existing software/data-acquisition system and to develop protocols for:

    a) TCEA analyzer
    b) TDOC analyzer
    c) GCMS analyzer
    d) Cracker manifold system

3) Alternative frit design and gas-flows experiments

      The ionization efficiency of the gas-hybrid source is the limiting factor for the (counting statistic based) precision of sub-optimal sized samples (*ie.*, less than a few hundred micrograms of carbon). Thus any gain in the total efficiency of $CO_2 > C^-$ directly translates to better precision on smaller samples.

Resource requirements:

1) **Redesign and modification of the low energy system**: 0.5 FTE. Effort would include ion optics modeling (2 months) and 4-person months for re-alignment of the low energy beam line, repositioning of major steering elements, testing, and if needed, final readjustments. No new major hardware would be required as flanges, gate valves, beam profile monitors, einzel lenses, and vacuum pumps would be re-used from the existing low energy beamline. Total time of project ~6 months.

2) **Sample interfaces for the gas-hybrid AMS-^{14}C:** 2 FTE. The prioritized installation sequence of interfaces are TCEA, TDOC, and then the GCMS. The cracker manifold is comparatively low technology and has a comparatively low total effort and cost to bring on-line. Major hardware purchases would include the TCEA, TDOC and GCMS analyzers, and the manifold. Additional purchases would include fittings, controllers, and gas-line plumbing (carrier gas, effluent, and cryogenic trapping) to interface the effluent from the analyzers to the gas-hybrid ion source. A CO_2 isolation finger would be installed between the sample interface (TCEA, TDOC, GCMS) and the gas-hybrid ion source. CO_2 evolved from the interfaces would either bypass the isolation finger (^{14}C-chromatogram mode) or be collected here (repeat injections for single compounds, DOC). Collected CO_2 would, via a capillary, be leaked into the gas-hybrid source. Experiments will need to be performed for each of the individual interfaces to determine and ameliorate sample memory effects, fractionation, and interface-specific backgrounds. Total time of project 36 months.

3) **Alternative frit design, modeling, and gas-flows experiments**: 1 FTE. Design and fabrication costs of frits and targets will be incurred. Duration 24 months.

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Tom Guilderson	Lawrence Livermore National Laboratory
Katherine Heckman	United States Forest Service
Marco Keiluweit	Lawrence Livermore National Laboratory
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