## The enigmatic "occluded" C fraction

KATHERINE HECKMAN<sup>1</sup>, CRAIG RASMUSSEN<sup>1</sup> AND HEIKE KNICKER<sup>2</sup>

<sup>1</sup>University of Arizona, Tucson, AZ <sup>2</sup>IRNAS-CSIC, Seville, Spain

Occluded organic C (C found within soil microaggregates) comprises a significant portion of the total organic matter in soils, and is often the oldest fraction of C in soils. However, occluded C characteristics vary widely and the mechanisms controlling occlusion of C within aggregates are not well understood. This work seeks to characterize the influence of soil mineral assemblage, as a function of soil parent material, on the partitioning of C into density and aggregate fractions and the mean residence time of soil organic carbon, with special consideration given to the chemical nature of occluded C both with depth and among soils of differing mineral assemblage. We sampled a lithosequence of four parent materials (rhyolite, granite, basalt, limestone) under Pinus ponderosa and quantified the partitioning of C into density/aggregate fractions including free or non-mineral associated C, occluded C, and mineral-associated C. After separation, the chemical nature and mean residence time of C in each fraction was examined using a combination of Pyrolysis GC/MS, CPMAS <sup>13</sup>C NMR, <sup>13</sup>C and <sup>15</sup>N, and <sup>14</sup>C abundance measurements. Results indicate that occluded C is the oldest C fraction across all parent materials. The occluded fraction showed enrichment of char regardless of soil type, though protein and lipid concentrations varied among parent materials. The <sup>14</sup>C age and fractional distribution of SOC varied among parent materials, and were significantly correlated to soil mineral variables, suggesting an overarching control of parent material on C dynamics in these systems. Results have important ramifications for both land management and our understanding of soil C cycling.

## A stable isotope-based model of intracellular water dynamics

E.L. HEGG<sup>1\*</sup> AND H.W. KREUZER<sup>2\*</sup>

<sup>1</sup>Michigan State University, East Lansing, MI 48824 (\*correspondence: EricHegg@msu.edu) <sup>2</sup>Pacific Northwest National Laboratory, Richland, WA 99352 (\*correspondence: Helen.Kreuzer@pnl.gov)

Despite years of study, intracellular water dynamics are still poorly understood. NMR measurements demonstrated that *in vitro*, the diffusion of water across cell membranes can be nearly instantaneous, supporting the general assumption that intracellular and extracellular water are in isotopic equilibrium. However, the image of a cell in isotopic equilibrium with its environmental water does not address the dynamic nature of cells. O and H atoms are constantly being incorporated into intracellular water during respiration, catabolism, and anabolism, while at the same time O and H atoms from intracellular water are being incorporated into metabolites. Thus, the identity of the atoms in intracellular water is constantly changing.

Our data support a dynamic model of intracellular water composition. Experiments with cultured *Escherichia coli* cells revealed that water extracted from rapidly growing cells contained a large fraction (50-70%) of O and H atoms that were isotopically different from the growth medium water [1-2]. Furthermore, the fractions of these isotopically distinct atoms varied with the level of metabolic activity, supporting the hypothesis that they were derived from metabolic processes. Using a similar experimental approach, we determined the proportion of metabolically-derived O and H atoms in exponentially-growing and confluent rat fibroblasts. The results were remarkably similar to those obtained with *E. coli*, demonstrating the generality of our results.

Further supporting our dynamic model of intracellular water composition, we demonstrated that changes in the isotope ratio of cellular metabolites correlate with the metabolic activity of the cells. The H isotopic content of *E. coli* fatty acids is more dependent on growth medium water in stationary phase than in log phase [2]. Likewise, when we compared the isotopic composition of *Bacillus subtilis* biomass to that of its growth water, both the O and H isotopic composition of the biomass showed a smaller contribution from growth medium water in mid log-phase cells than in late log-phase cells. Thus, the rate of metabolism influences the isotope ratio of cellular metabolites.

[1] Kreuzer-Martin *et al.* (2005) *PNAS* **102**, 17337. [2] Kreuzer-Martin *et al.* (2006) *Biochemistry* **45**, 13622.