

## Final Technical Report

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**Title: Ecosystem Controls on C & N Sequestration Following Afforestation of Agricultural Lands**

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The controls affecting soil organic carbon (SOC) and soil organic nitrogen (SON) dynamics and their sequestration are receiving increasing attention from both scientists and the public as impacts of global change start to affect our environment and natural resources. Soil organic matter (SOM) studies are conducted not only for sequestration and ecosystem sustainability, but also for food security and global change effects. The need to understand the role of soils in ecosystem functioning for 1) soil nutrient storage in a changing climate, 2) increased, organic agriculture production, and 3) increased food needs, even as biofuel production utilizes an expanded acreage of our soil resources, all point to the need for a better knowledge of the controls on SOM dynamics. In our project, we proposed to continue analysis of our available soil samples and data, and to develop new studies to answer the following objectives:

*Objective 1) Broaden field based studies of ecosystem C and N compartments to enhance current understanding of C and N sequestration and dynamics.*

*Objective 2) Improve our understanding of mechanism controlling C and N stabilization and dynamics.*

*Objective 3) Investigate the interrelated role of soil temperature and organism type and activity as controlling mechanism in SOC dynamics and sequestration.*

The objectives were interrelated. Data analysis and publication of presently available data, on our transect of sites, constituted an important part of our research. Today's questions concerning the role of soil organic matter (SOM) in soil fertility, ecosystem functioning and global change can only be addressed through knowledge of the controls on SOM stabilization and their interactions while understanding that soils are governed by unifying principles (Fierer et al. 2009) that make it possible to interrelate studies and concepts on different soils and landscapes.

We concentrated analytical studies on Sand Ridge and Emiquon, IL for Objective 1; Akron, CO, Hoytville, OH, Halsey, NE, and KBS, MI for Objective 2; and Halsey, NE, Sand Ridge, IL and a mean annual temperature gradient ranging from 2°C in Saskatchewan, CA to 25.6°C in tropical Brazil for Objective 3. In addition to our traditional approaches of field measurements of SOC and plant biomass, <sup>13</sup>C and <sup>14</sup>C tracers, long-term incubation and fractionation of SOM, we used four additional methodologies.

- 1) Pyrolysis-molecular beam mass spectrometry (py-MBMS) to chemically characterize fractions based on their sensitivity to breakdown in heat in an anaerobic atmosphere.
- 2) Fatty acids to measure the interacting controls of Ca, N and vegetation on microbial diversity.
- 3) The use of mid-infrared fourier transform, mid-infrared spectroscopy (MiDIR) to characterize functional groups of different soils and fractions before and after incubation.
- 4) Pyrosequencing to measure the functional genes and populations of our research sites to integrate the chemical studies with a knowledge of community composition.

*Objective 1) Broaden field-based studies of ecosystem C and N compartments to enhance current understanding of C and N sequestration and dynamics.*

It is important to analyze our data and relate it to knowledge from interdisciplinary fields. Andren et al. (2008) investigated the concepts and methodology required to further develop studies in SOM and soil biology with special reference to the appropriate use of statistics, interpretation of data, and use of established literature recourses. Conant et al. (2011) conducted a meta-analysis of policies that encourage greenhouse-gas emitters to mitigate emissions through terrestrial carbon (C) offsets, C sequestration in soils or biomass, promote practices that reduce erosion and build soil fertility, while fostering adaptation to climate change, agricultural development, and rehabilitation of degraded soils. None of these benefits will be possible until changes in C stocks can be documented accurately and cost-effectively. This is particularly challenging when dealing with changes in SOC stocks. Precise methods for measuring C in soil samples are well established, but spatial variability in the factors that determine SOC stocks makes it difficult to document change. Widespread interest in the benefits of SOC sequestration has brought this issue to the fore in the development of US and international climate policy. We reviewed the challenges to documenting changes in SOC stocks, how policy decisions influence offset documentation requirements, and the benefits and drawbacks of different sampling strategies and extrapolation methods. Morris et al. (2011) measured the interaction of soil, plant type and Ca contents on C sequestration. Manipulation of controls on C sequestration such as species planted or amelioration of Ca deficiency could be cost effective and increase soil C levels.

*Objective 2) Improve our understanding of mechanism controlling C and N stabilization and dynamics.*

Soil organic matter (SOM) in agricultural soils comprises a significant part of the global terrestrial C pool. It has often been characterized by utilizing a combination of chemical dispersion of the soil and physical separation (Haile-Mariam et al. 2008; Paul et al. 2009). These fractions include a non aggregate protected, light fraction (LF) consisting of material with a density of less than  $1.7 \text{ g cm}^{-3}$ , a particulate organic matter fraction (POM) that is associated with sand size particles after dispersion and comprises the material most often considered labile and contributing to the active and slow pools of SOM. The SOM associated with silt and clay size particles constitute the oldest fractions. We fractionated soil samples from continuous-corn rotations at long-term sites in the Corn Belt to determine the concentration of C and N associated with soil fractions [LF, POM, silt clay and Bradford reactive soil protein (BRSP)]. We determined the field mean residence time (MRT) of the LF and POM, and identified the change in C concentration and  $\delta^{13}\text{C}$  signal of each fraction after extended laboratory incubations. Light fractions comprised 3 to 5 % of the SOC with no significant difference between conventional till

(CT) and no till (NT) treatments. They decreased ~ 70% after 800 d of incubation. The POM fraction accounted for 5 to 11% of the SOC in the >30% clay soils at Lamberton, MN and Hoytville, OH compared to 17 to 23% for the KBS, MI and Wooster, OH soils with <20 % clay. The highest proportion of SOC was found in the clay size fraction and ranged from 36% in the Wooster soil to 68% in the Lamberton soil. Measurement of the  $^{13}\text{C}$  change during long-term incubation showed that the average MRT of corn-derived C in the LF across sites was 3.5 yr. The POM fraction of the Wooster and Hoytville soils had MRT's of 6.1 to 8.2 yr; the KBS and Lamberton soils averaged ~12 yr. The LF is associated with the active fraction as defined by long-term incubation. The POM is closely associated with, but smaller than the kinetically, defined slow pool. The  $^{13}\text{C}$  changes during incubation show that both fractions consist of a mixture of active and resistant materials with movement between fractions. The BRSP has long MRTs except in the no till treatment of the Hoytville soil. Measurement of dynamics of these fractions provides a basis for C models to test impacts of land use and management on C sequestration, soil properties and agricultural sustainability.

We carried out mid-infrared (MidIR) spectral interpretation of fractionated fresh and incubated soils from the above sites analyzed by Haile –Mariam et al. (2008) to determine the functional group changes in SOM chemistry during incubation. Our results (Calderon et al. 2011) show that the LF and clay fractions have distinct spectral features regardless of site. The LF is characterized by absorbance at  $3400\text{ cm}^{-1}$ , as well as between  $1750$  and  $1350\text{ cm}^{-1}$ . The clay fraction is distinguished by absorption near  $1230\text{ cm}^{-1}$ , and absorption at  $780$ - $620\text{ cm}^{-1}$ . The POM, like the LF, absorbs at the broad peak at  $1360\text{ cm}^{-1}$ . High SOM soils are characterized by absorbance at  $1230\text{ cm}^{-1}$ . Soils from different sampling depths have specific spectral properties. A band at  $1330\text{ cm}^{-1}$  is characteristic of shallow depths. Because of their low OM content, the deeper samples are characterized by mineral bands such as quartz, clays and carbonate. Spectroscopic data indicates that the clay fraction and the LF underwent measurable chemical transformations during the 800 d incubation, but the POM and silt fraction did not. As the LF decomposes, it loses absorbance at  $3400\text{ cm}^{-1}$ ,  $1223\text{ cm}^{-1}$ ,  $2920$ - $2860\text{ cm}^{-1}$ . A band at  $1630\text{ cm}^{-1}$  increased during incubation, indicating a recalcitrant form of organic C that may be of microbial origin. The clay fraction suffered changes that were opposite to those of the LF, indicating that LF decomposition and clay decomposition follow different chemistries.

Pyrolysis molecular beam mass spectrometry (py-MBMS) provides a powerful and rapid means of assessing the biochemical composition of SOM (Plante et al. 2009). However, characterization of SOM composition alone is insufficient to predict its dynamic behavior. Chemical fractionation is frequently used to isolate more homogeneous SOM components, but the composition of fractions is frequently unknown. We characterized biochemical SOM composition in two previously studied soils from the USA (Akron, CO and Hoytville, OH) under contrasting land uses: cultivated agriculture and native vegetation. Bulk soils, as well as chemically isolated SOM fractions (humic acid, humin and non-acid hydrolysable), were analyzed using py-MBMS. Principal components analysis (PCA) showed distinct differences in the SOM composition of isolated fractions. Py-MBMS spectra and PCA loadings were dominated by low molecular weight fragments associated with peptides and other N-containing compounds. The py-MBMS spectra were similar for native whole-soil samples under different vegetation, while cultivation increased heterogeneity. An approach based on previously published data on marker signals also suggests the importance of peptides in distinguishing

samples. While the approach described here represents significant progress in the characterization of changing SOM composition, A truly quantitative analysis will only be achieved using multiple internal standards and by correcting for inorganic interference during py-MBMS analysis. We provided proof of principle that py-MBMS can be a powerful tool to understand the controls on SOM dynamics.

The Halsey, NE site with 95% sand and planted pine and cedar trees on a C<sub>3</sub>-C<sub>4</sub> native prairie with a <sup>13</sup>C signal presented excellent opportunities for determination of the controls on C and N stabilization and dynamics in a soils with little sand and clay such that microbiological and chemical controls would be most expressed. Under cedars, the upper soil layer gained C relative to the native grassland and to our astonishment 80% of the original native prairie C was retained for 80yr. The lower depths in the pine soil suffered C losses. Both Ca and N were shown by causal path analysis to be controlling factors. The turnover data based on the pools and fluxes calculated from the long-term incubation and acid hydrolysis were related to the field turnover rates determined with <sup>13</sup>C. We used the <sup>13</sup>C content of the evolved CO<sub>2</sub> to calculate the proportion of cedar and pine-derived C attacked by the microbiota in each of the respective plots. A complicating factor in our calculations was that the <sup>13</sup>C of the CO<sub>2</sub> from the native, prairie control did not stay constant during the incubation. It became more negative with time. We attributed this to preferential utilization of the carbohydrates and amino acid, early in the incubation. These have a less negative signal than the lignin and long chain aliphatics decomposed later in the incubation.

A large portion of SOM is associated with the silt and clay and has previously been shown to be old in earlier <sup>14</sup>C dates. Acid hydrolysis has served us well in defining an easily measurable, resistant pool for purposes of determining pool sizes and fluxes. Our data shows that the non hydrolysable still loses C during incubation and becomes 5000 yr old. (Paul et al.2011) We have further characterized the KBS, Hoytville, and Akron sites by: a) fractionating and carbon dating the silt, clay and the POM and LF of field soils to complement the <sup>13</sup>C studies; b) conducted a long-term incubation and isolated silt and clays followed by <sup>13</sup>C and; c) conducted py-MBMS and MiDIR on the fractions before and after incubation to determine the chemical changes and relate them to the information obtained on the turnover rates (in preparation).

Nannipieri and Paul (2009) discussed the concepts employed to interpret N mineralization-immobilization in soil, and how N turnover is related to the characteristics of organic N and the biota conducting the transformations. A survey of the period before the arrival of electronic searches became available provided access to the classical literature that can help interpret today's challenges. Classical (acid hydrolysis) and modern spectrometry and spectroscopy techniques indicate that protein N is the prevalent component of organic N in soil. The presence of heterocyclic N can indicate its abiotic partial synthesis as in fire-affected soils. Clays and pedogenic oxides can protect organic N against microbial degradation. The evidence for such protection is mostly based on in vitro studies involving pure clays, and proteins and their relevance to field conditions requires further work. Subdivision of organic N into sub-pools has helped to interpret mechanistic studies and modeling of N dynamics. Uncomplexed organic matter, obtained by physical fractionation procedures, is considered a labile pool. The interpretation of N mineralization measurements is affected by immobilization during microbial attack especially in high-C environments. Transfer of materials among particle size fractions and

changes in microbiological properties of aggregates also can occur during fractionation procedures.

The above discussion has shown that knowledge of the pools and fluxes of C and N soil components is required to interpret ecosystem functioning and improve biogeochemical models. Two former grassland soils (Sidney, NE and Akron, CO), where wheat or corn are currently growing, were studied by kinetic analysis of microbial biomass C and N changes, C and N mineralization rates, acid hydrolysis and pyrolysis. (Paul et al. 2011) Nearly twice as much C as N was mineralized during incubation. Modeling of changes during incubation demonstrated that two-pool first-order kinetics effectively described losses of microbial biomass C and N and concurrent N mineralization. Loss of microbial biomass N during incubation accounted for a significant portion of the N mineralized. Microbial biomass N content and soil N mineralization rates were strongly affected by soil type and soil management. Nitrification, but, not N mineralization, was inhibited during the latter stages of incubation in one of the soils. We believe nitrifier populations had dropped below effective levels. Non-acid hydrolysable C was increased in both amount and mean residence time by cultivation and incubation. Hydrolysis removed a large amount of N than incubation. Data after pyrolysis of soils, in argon at 550° C, closely reflected results for both C and N found after cultivation and incubation. This technique should be further investigated to identify the recalcitrant forms of C and N in soils. The dynamics of soil C and soil N, although related, are not identical, thus management can be targeted to soil C or N cycling in ecosystem functioning or to soil organic matter dynamics in global change.

*Objective 3) Investigate the interrelated role of soil temperature and organism type and activity as controlling mechanisms in SOC dynamics and sequestration.*

The uncertainty associated with how projected climate change will affect global carbon (C) cycling could have a large impact on soil C stocks. The purpose of our study was to determine whether the size and decomposition rates of active and slow SOM pools were similarly sensitive to changes in temperature. We tested this hypothesis using long-term soil incubations at three temperatures (15, 25, and 35°C) with twelve soils from six sites along a mean annual temperature (MAT) gradient of 2 to 25.6°C. (Haddix et al. 2011) We used a two pool model (active and slow) with first-order kinetics to fit our soil respiration data, along with a three pool model for comparison purposes. We found that the two and three pool models statistically fit the data equally well. The size of the active pool in these soils, calculated using the two pool model, increased with incubation temperature and ranged from 0.1 to 12.8% of initial soil organic C. Sites with an intermediate MAT and lowest C:N ratio had the largest active pool. Comparisons of  $Q_{10}$  values of the two pools across all soils showed similar  $Q_{10}$  values for the active ( $Q_{10} = 1.89 \pm 0.14$ ) and slow ( $Q_{10} = 1.90 \pm 0.08$ ) pools. There was a slight trend ( $p = 0.080$ ) for soils that have been converted from native systems to wheat or pasture to have higher  $Q_{10}$  values for the active pool versus the slow pool (active  $Q_{10} = 2.15 \pm 0.22$ , slow  $Q_{10} = 1.89 \pm 0.14$ ) compared to the native treatment for each of these soils (active  $Q_{10} = 1.64 \pm 0.09$ , slow  $Q_{10} = 1.92 \pm 0.10$ ). Pyrolysis Molecular Beam Mass Spectrometry on these soils indicated that there were chemical differences in the SOM between the land use treatments. Land use, with its subsequent impacts on soil characteristics, alter the temperature sensitivity of SOM pools, which has important implications for the interpretation and modeling of temperature effects on the C cycle because many ecosystem and C cycle models consider SOM pools to be equally sensitive to temperature changes across all soil and land use types.

Global climate change may induce accelerated soil organic matter (SOM) decomposition through increased soil temperature, and thus impact the C balance in soils. We hypothesized (Plante et al. 2009) that compartmentalization of substrates and decomposers in the soil matrix would decrease SOM sensitivity to temperature. We tested our hypothesis with three short-term laboratory incubations with differing physical protection treatments conducted at different temperatures. Overall, CO<sub>2</sub> efflux increased with temperature, but responses among physical protection treatments were not consistently different. Similar respiration quotient ( $Q_{10}$ ) values across physical protection treatments did not support our original hypothesis that the largest  $Q_{10}$  values would be observed in the treatment with the least physical protection. Compartmentalization of substrates and decomposers is known to reduce the decomposability of otherwise labile material, but the hypothesized attenuation of temperature sensitivity was not detected, and thus the sensitivity is probably driven by the thermodynamics of biochemical reactions as expressed by Arrhenius-type equations.

### *Integration*

The KBS site in MI has been well investigated with both DOE-sponsored and other funds. A synthesis chapter (Paul et al. 2012) has compared the measurement from physical fraction to those with long-term incubation and tracer and molecular structural analysis on this medium textured soil with relative high SOM turnover rates and low <sup>14</sup>C mean residence times relative to other corn belt soils such as that from Hoytville, OH. The physical fractions associated with free plant residues and aggregates that contribute to the active fraction of SOM were shown to have different MiDIR and pyrolysis characteristics than the clay associated materials that are associated with the resistant fraction of SOM. Afforestation that provides substantial C sequestration capability was shown to be affected by the vegetation type as well as by Ca cycling within soils and by inputs of atmospheric N. Extensive studies have related the microbial biomass to the dynamics of C and N as well as to the quality and quantity of available soil resources.

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In addition to the above published scientific papers, this project has accumulated the data required to write the following papers:

- Birge, H., M. Wallenstein, S. Snapp. E.A. Paul and R. Conant. The microbiology and enzymology of soils altered by long-term incubation.
- Brewer, E., E.A. Paul and S.J. Morris. The role of Ca and N in stabilizing soil organic matter in afforested soils.
- Calderon F., M.L. Haddix and E.A. Paul. The use of standards to quantify MiDIR for soil and fraction analysis.
- Calderon, F., K. Magrini, M.L. Haddix, R. Follett, and R. Conant. The molecular structure of soils and fractions that contribute to SOM stability as measured by MiDIR and py-MBMS.
- Haddix, M.L., R. Conant, K. Magrini and E.A. Paul. Quantification of soil molecular structure analysis by the application of standards in pyrolysis molecular beam mass spectrometry.
- Haddix, M.L., R. Follett, E. Preussner, R. Conant, S.J. Morris, S. Snapp and E.A. Paul. The <sup>13</sup>C content and dynamics of soil fractions from long-term incubation.
- Paul, E.A., R. Conant, M.L. Haddix, R. Follett, K. Magrini, and F. Calderon. The use of py-MBMS and MiDIR to characterizes grassland soils before and after long- term incubation.
- Paul, E.A., R.P. Robertson, R. Conant, M.L. Haddix, R. Follett and S.J. Morris. The use of <sup>14</sup>C dating to measure the dynamics of silt and clay fractions.

Although funds for the preparation and publication of the above are not now available, we will strive to get these articles into press as they constitute a major contribution to the literature on Ecosystem controls on C and N dynamics and sequestration.