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The DOE Consortium for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems: FY 2007-2011 Five Year Science Plan

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CSiTE, the DOE Consortium for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems

FY 2007–2011 Five-Year Science Plan
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Abstract

CSiTE FY 2007-2011 Five-Year Science Plan

The DOE Consortium for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems (CSiTE) conducts multi-scale research to acquire basic knowledge for underpinning the implementation of soil carbon (C) sequestration in an environmentally acceptable and economically feasible manner. Research is based on the premise that identifying and understanding the basic mechanisms controlling sequestration across managed and unmanaged ecosystems are fundamental to developing approaches for enhancing C capture and long-term storage. The goal is to discover and characterize links among physical, chemical, and biological processes controlling soil C dynamics and storage at a mechanistic level to facilitate the enhancement of C storage in soils to restore or surpass historical levels of organic matter in managed ecosystems. Integration across scientific themes occurs by coordinating research efforts at field sites where land-use practices, experimental manipulations, or chronosequences afford opportunities to observe climate and land-use impacts on soil C and understand the associated environmental and economic consequences of implementing sequestration strategies over relevant time scales.

During 6 years of laboratory, field, and modeling research in forest, cropland, and tallgrass prairie ecosystems, CSiTE research resulted in more than 150 refereed publications. Important contributions include elucidation of controls on the mechanisms and rates of accumulation of soil organic matter, development and application of new methods to understand the role of microbial communities in soil C dynamics, identification of novel field manipulation concepts for enhancing soil C sequestration, refinement of modeling tools and their use in supporting hypothesis-driven science, understanding landscape-scale processes, and application to full greenhouse gas accounting and evaluation of economic feasibility.

Beginning in FY 2007, CSiTE will reorganize around seven scientific themes and coordinate research activities around field experiments with switchgrass (Panicum virgatum) at Milan, Tennessee, and the Fermilab site at Batavia, Illinois. The overarching hypothesis is that simultaneous biofuel production and enhancement of soil C sequestration is sustainable. The seven themes are 1) Soil Carbon Inputs, 2) Soil Structural Controls, 3) Microbial Community Function and Dynamics, 4) Humification Chemistry, 5) Intrasolum Carbon Transport, 6) Mechanistic Modeling, and 7) Integrated Evaluation. The overall goal to understand coupled physical, chemical, and biological controls over soil C sequestration at a fundamental level remains unchanged. However, by using the production of switchgrass, an important bioenergy crop, as our test bed to study carbon sequestration in an intensive, vertically integrated study, our findings will have immediate application to the successful development of an important energy technology. CSiTE is a research collaboration among Argonne National Laboratory, Oak Ridge National Laboratory, and Pacific Northwest National Laboratory including affiliated academic institutions.
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I. Introduction

As a result of the past two centuries of industrial activity, agriculture, and forestry the concentrations of atmospheric carbon dioxide (CO$_2$) and other greenhouse gases (GHG) have risen dramatically, and it is hypothesized that this is impacting climate systems on a scale that in coming decades could lead to undesirable consequences associated with global warming. This has led to investigations of approaches for GHG mitigation, including environmental sequestration of CO$_2$ (U.S. DOE 1999). Carbon moves among the atmosphere, the ocean, and terrestrial ecosystems. The atmosphere currently contains 770 petagrams (Pg = one gigaton) of C, with nearly all of it as CO$_2$. The oceans store about 40,000 Pg, and terrestrial systems contain 2000 Pg, most of which resides in the soil (1500 Pg). Approximately half of all soil C in managed ecosystems has been lost to the atmosphere as a result of cultivation and harvesting or destruction of forests. This now represents an opportunity for C storage as a near-term GHG mitigation option (Lal et al. 1998; McCarl et al. in press).

It was in this context that the Consortium for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems (CSiTE) was established by the Office of Biological and Environmental Research (BER) at the U.S. Department of Energy (DOE) in 1999. CSiTE conducts multi-scale investigations to acquire basic knowledge to underpin implementation of soil C sequestration in an environmentally acceptable and economically feasible manner. Research is based on the premise that identifying and understanding the fundamental physical, chemical, and biological mechanisms controlling sequestration across managed and unmanaged ecosystems are crucial to protecting stored C and enhancing C accrual. The central focus has been to develop a mechanistic understanding of how soil is affected by different management practices. This has been done at experimental field sites where long-term manipulations or chronosequences afford opportunities to observe climate and land-use impacts on C sequestration and the associated economics of implementation over relevant time scales. CSiTE research has also begun to explore the question of whether it is possible to restore soil organic C (SOC) to historical or greater than historical levels in different ecosystems. Detailed results of the research program to date are included in Appendix A (CSiTE Accomplishments and 2005-2006 Progress Report). In summary, CSiTE has significantly advanced our basic understanding of soil C sequestration and is poised to build on this work by addressing a set of interrelated scientific themes that address the overarching hypothesis that simultaneous biofuel production and enhancement of soil C sequestration is sustainable.

The U.S. government is committed to an ambitious expansion of the bioenergy contribution to a sustainable energy future, including a 30% replacement with renewable biomass by 2030 of current petroleum consumption for transportation fuels (U.S. DOE 2005). To achieve this ambitious goal will require development of cost-effective technologies for converting approximately one billion tons of lignocellulosic feedstock conversion to ethanol. This level of sustainable feedstock production will require the dedication of nearly 25 million ha of cropland to biofuel crops such as switchgrass (*Panicum virgatum*) (Perlack et al. 2005). Included will be very large areas of existing and idle cropland as well as marginal and degraded lands. Important questions in need of resolution center on whether removing aboveground biomass as biofuel feedstock can be sustained in a manner compatible with enhanced soil C sequestration while maintaining or enhancing related ecosystem services and benefits.
Switchgrass, a native, warm-season perennial grass, was chosen in 1991 by the Bioenergy Feedstock Development Program in DOE’s Office of Energy Efficiency and Renewable Energy (EERE) as a model biofuel crop. Different varieties can be cultivated across a large area of the midwest, southeast, Texas, and parts of the west; are adaptable to marginal and poor soils; and are compatible with domestication by breeding for improved agronomic traits. Importantly, preliminary studies suggest that switchgrass develops an extensive rooting system that could be managed for enhanced soil C sequestration (McLaughlin and Kszos 2005). Research is needed to understand how traditional and innovative management practices, different varieties, climate, and soil type impact soil processes at a mechanistic level to determine whether aboveground biomass production gains are compatible with enhanced soil C sequestration and can be sustained over decades. The work proposed in this science plan is critical because with the exception of the work by Ma et al. (2000, 2001) and Garten and Wullschleger (1999, 2000), very little is known about belowground processes associated with switchgrass production and soil C dynamics.

To address this knowledge gap, CSiTE is reorganizing its research program to focus on hypothesis-driven research across five scientific themes: Soil Carbon Inputs, Soil Structural Controls, Microbial Community Function and Dynamics, Humification Chemistry, and Intrasolum Carbon Transport. Mechanistic Modeling and Integrated Evaluation themes provide the tools that facilitate science integration while providing insight into landscape-scale processes, regional and national sequestration potential, and technical and economic feasibility. Research across all seven themes will coalesce around experimental switchgrass sites at Milan, Tennessee, and Batavia, Illinois (Section II, B) and is designed to address five overarching scientific questions:

I. What is the nature of belowground C inputs by switchgrass, and are they compatible with sustained aboveground biomass production and soil C sequestration?

II. What are the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil, and how do they interact in space and time?

III. What processes control the movement and distribution of C through the soil profile?

IV. How are the fundamental processes controlling C distribution and movement manifested across landscapes and time?

V. How can fundamental knowledge best be used to identify and implement methods and practices for sustained enhancement of soil C in the context of biomass production for energy in an environmentally acceptable and economically feasible fashion?

This 5-year research plan lays out an integrated program of scientific research with the objective of answering these questions so that the full potential for soil C sequestration as a GHG mitigation option will be realized while playing a central role in the emergence of the national vision for an economic future sustained by renewable energy sources.
II. CSiTE FY2007-FY2011 Science and Integration Plan

A. Science Plan Objectives

The overarching objective of the CSiTE 5-Year Science Plan is to undertake an intensive, vertically integrated study on soil C sequestration combining laboratory, field, and modeling components designed to:

- Rigorously and quantitatively test our holistic understanding of soil C sequestration developed since the start of the project in late 1999 with a focus on deepening our process understanding of the roles of C inputs, soil structural controls, microbial community function and dynamics, humification chemistry, and intrasolum C transport on soil C sequestration.

- Develop and validate a mechanistic model that
  - Integrates and incorporate our process understanding of soil C sequestration
  - Enables testing of new technologies/approaches to enhance soil C sequestration
  - Facilitates regional and national forecasts of soil C sequestration

- Explore C sequestration enhancement opportunities created by large-scale production of perennial herbaceous energy crops.

Such a study will advance the science of enhancing soil C sequestration and fulfill goals of the Climate Change Technology Plan (CCTP) by consolidating our understanding of soil C sequestration and providing a much-improved capability for quantitatively and mechanistically forecasting the C sequestration benefit of multiple technologies and management practices across various crop production systems, climates, and soils. This need has been repeatedly highlighted in the draft CCTP strategic plan (http://www.climatetechnology.gov/stratplan/draft/index.htm) and is essential to predicting the impact climate change will have on soil C sequestration. This study will also give us the fundamental understanding needed to address the tradeoffs (if any) between enhancing aboveground yield for bioenergy feedstock production, sequestering C in the soils, and maintaining long-term soil sustainability. The proposed research will also lead to new approaches for enhancing C sequestration by manipulating key processes.

The proposed plan is a natural follow-on to the first 6 years of CSiTE, during which we took advantage of opportunities at numerous sites to improve our understanding of the many processes controlling soil C sequestration from the molecular to national scale (Figure 1). The first 6 years allowed us to develop a more rigorous conceptual understanding of the coupling of these processes. As noted in the December 2004 review of CSiTE, it is now time to test this understanding through a focused, intensive, hierarchically organized field and modeling study. Given the renewed interest in bioenergy, specifically cellulosic ethanol, a focus on a perennial herbaceous energy crop is timely and builds on our previous research on C sequestration under prairie soils.
B. Overview of Science Plan

Our research will be organized around seven themes depicted in Figure 2. Each theme has specific research goals described in this plan. The research of the five experimental themes, 1) Soil Carbon Inputs, 2) Soil Structural Controls, 3) Microbial Community Function and Dynamics, 4) Humification Chemistry, and 5) Intrasolum Carbon Transport, will be focused on the same soils, land use, and locations. Each experimental theme’s research will contribute to the sixth theme, Mechanistic Modeling. In turn, the seventh theme, Integrated Evaluation of Carbon Sequestration Technologies, will draw upon the Mechanistic Modeling theme for estimates of potential soil C sequestration across the wide range of soils, climate, and crops and management regimes possible in the U.S. By combining those estimates with the GHG emissions and the economic value of those crops and management regimes, the seventh theme will explore the economic consequences and GHG benefits for various strategies to enhance soil C sequestration at the national scale.

For the five experimental themes we have selected:

- An Alfisol located in western Tennessee (Milan) as our primary test soil type and a Mollisol located in northeastern Illinois (Fermilab) as our secondary test soil type. As research evolves we will explore other soils, specifically Ultisols, if resources permit.

- The production and harvesting of switchgrass for bioenergy as our test land use

- Manipulations of C input and soil conditions to affect C sequestration processes at the sites.

Figure 1. Investigating carbon sequestration processes across multiple scales.
During years 1-2, the five experimental themes will use the range of soil pH, C input, and possibly carbon quality conditions created under existing fertilizer and switchgrass cultivar experiments at the Milan, Tennessee, site to test their respective hypotheses. (See later sections for these hypotheses). We will also establish a new experiment to manipulate C sequestration processes at the northern Illinois Mollisol site in Year 1 and at the Milan, Tennessee, Alfisol site in Year 2 and use this experiment in years 3-5 to test and extend the understanding garnered in years 1 and 2 from the pre-existing experiments at Milan.

We will use the Erosion Productivity Impact Calculator (EPIC) (Izaurralde et al. 2006) as the basis of our mechanistic modeling activities. We will use the systems modeling language STELLA® or Mathcad® as a tool to engage the experimental scientists in building the conceptual and quantitative links among the five experimental themes in a way that can then be incorporated into the more multidimensional EPIC model. The Forest and Agriculture Sector Optimizing Model (FASOM) model (McCarl and Schneider 2001), which is already integrated with EPIC and depicts total U.S. agricultural and forestry activities over time incorporating GHG issues of permanence, leakage, and additionality, will form the basis for our regional- to national-scale analysis of developed and potential soil C sequestration enhancement opportunities (Figure 3).
a. Rationale for Field Experiments

1. Selection of an Alfisol in western Tennessee and a Mollisol in northern Illinois (Fermilab) as our test soils

We chose an Alfisol in Tennessee as our primary test soil for several reasons. Alfisols cover large areas of the U.S. and are commonly used for agriculture (Figure 4). Because of their agricultural history, many Alfisols show C depletion and thus present an opportunity for C sequestration. Furthermore, previous analyses have shown that western Tennessee is a desirable (economically competitive) location for switchgrass production (Ugarte et al. 2003). In western Tennessee, the Alfisols are of a loess origin and highly erodible, thus there is the potential to use switchgrass production for bioenergy to sequester C by increasing the amount in the existing soils and reducing the loss caused by erosion. With their deep lower profiles, Alfisols also have high potential to sequester C in lower horizons.

We chose a northern Illinois Mollisol because, like Alfisols, Mollisols also cover large areas of the U.S., are commonly used for agriculture, and are expected to be used for switchgrass production. Mollisols are developed under prairie conditions and are typically more C-rich than Alfisols and thus provide a good test for the understanding developed from studying Alfisols. We have a rich body of work on the soils at Fermilab that will be helpful in interpreting our findings at this site. Ultisols are also likely candidates for switchgrass production and have good...
2. Selection of production of switchgrass for bioenergy as our test land use

Switchgrass is a native North American tall grass prairie C₄ species (Figure 5). A warm-season grass, its natural range covers much of the U.S. east of the Rockies and extends north into Canada and south to Central America (McLaughlin et al. 1999). It is used as a forage species and was selected in 1991 as a model herbaceous energy crop for the U.S. after field trials with a variety of annual and perennial grasses and perennial legumes (McLaughlin and Kszos 2005; McLaughlin et al. 1999). It has small seeds and often doesn’t reach its full production capacity until its third growing season. If it is being managed as an energy crop, it is typically harvested in the fall soon after senescence has taken place. It is usually harvested in square or round bales leaving a 15-cm-tall stubble. Switchgrass productivity is generally enhanced with modest (50 kg N/ha/y) amounts of annual nitrogen (N) fertilizer application.

We selected the production of switchgrass for bioenergy feedstock as our test land use for three reasons. First, it is an emerging land use with the potential to cover tens of millions of hectares of land in the U.S. (Perlack et al. 2005). A prairie species, it is known to increase soil C (McLaughlin and Kszos 2005). Thus as a land use, switchgrass production for bioenergy is relevant to reduction of greenhouse gas emissions because it both displaces fossil fuels and sequesters soil C. Second, a focus on switchgrass allows us to build off our previous research and

Figure 4. Soil orders in the U.S.
findings from the Fermilab Prairie chronosequence. It is a desirable perennial energy crop for research because of its short development phase (2-3 years) and spatial homogeneity (in contrast to woody crops). Finally, it is complimentary to the research proposed in DOE’s recently released roadmap focused on making cellulosic ethanol a practical alternative to gasoline (U.S. DOE 2006). Switchgrass findings should be very applicable to other proposed perennial grass crops for bioenergy (such as *Miscanthus gigantum*) and provide some insight on perennial woody energy crops such as hybrid poplar (*Populus* sp.) and willow (*Salix* sp.). Because switchgrass is actively grown as a forage crop, most research on the crop has focused on its aboveground properties and enhancing aboveground yield. Comparatively little is known about its belowground properties. Ma did some work (Ma et al. 2000, 2001) looking at the carbon allocation of switchgrass and root characteristics, while Garten and Wullschleger (1999, 2000) examined soil carbon inventories and dynamics under switchgrass stands.

3. Selection of experimental manipulations to affect C sequestration processes

We will initially use existing switchgrass fertilizer, seeding rate, and cultivar experiments at the University of Tennessee (UT) Experimental Station at Milan, Tennessee, about 80 miles northeast of Memphis. These experiments were planted in spring 2004 as part of a DOE Office of Biomass Program study to develop and test the feasibility and desirability of switchgrass production in Tennessee (Figure 6, [http://feedstockreview.ornl.gov/pdf/english/ut_switchgrass_project.pdf](http://feedstockreview.ornl.gov/pdf/english/ut_switchgrass_project.pdf)). The switchgrass experiments were established on four sites using identical plot designs. We will focus our research on the well-drained upland site, which is a Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). While we will use these experiments to test fundamental hypotheses about soil processes (as opposed to simply examining the effect of fertilizers and cultivars on C sequestration), we will benefit from the yield and operational (cost, equipment) data being collected at the site by the Office of Biomass Program study.

The UT cultivar experiment is a randomized, complete block design with four reps and four cultivars. Individual plots are ~5 m x 8 m (15 ft x 24 ft). The four cultivars include the widely used lowland ecotype cultivar “Alamo,” two new synthetic cultivars from Georgia (GA992 and GA993), and one from Oklahoma (SL-93-2). The cultivars from Georgia are lowland ecotype cultivars. GA992 was essentially derived from the “Kanlow” cultivar, while GA993 was essentially derived from the “Alamo” cultivar. Both cultivars out-yielded “Alamo” by >20% in previous field experiments (personal communication, Joe Bouton, Noble Foundation). The Oklahoma variety SL-93-2 also has an “Alamo” origin (personal communication Charlie Taliaferro, Oklahoma State University). No data on root morphology or chemical composition have been collected on any of the synthetic varieties. We will select three of the blocks for our field measurements.
The fertilizer-by-seeding-rate experiment is a randomized complete block with a split block design with the main plot as N rate and the split plot as seeding rate. There are four reps, four fertilizer rates (0, 67, 134, and 202 kg N/ha/y; 0, 60, 120, and 180 lb N/acre/y) and five seeding rates 2.8, 5.6, 8.4, 11.2, and 13.5 kg pure live seed/ha (2.5, 5.0, 7.5, 10, and 12.5 lb pure live seed/acre). The fertilizer is applied in a single application in the spring as ammonium nitrate starting at the second growing season. As with the cultivar study, we will select three of the blocks for our field measurements. Furthermore, we will only use the subplots that were planted at the recommended operational rate of 8.4 kg/ha (7.5 lb/acre) pure seed. Thus, we will only consider fertilization as a CSiTE treatment.

Soil samples were taken from all the plots prior to planting. The samples were air-dried and sieved and are available for analysis. Each fall after senescence, the plots have been harvested leaving a 15-cm stubble. The aboveground biomass has been weighed in the field, and samples were taken for biomass moisture content. These data are available to us. Dr. Don Tyler of UT is in charge of these experiments. He will be engaged in all our experimental work at Milan and will provide agronomic and soil guidance for the Fermilab experiment. We have been working with him since early spring 2006, and he hosted our May 2006 planning meeting.

At the time that the cultivar and fertilizer-by-seeding experiments were being established, additional acreage was planted to switchgrass using the standard Alamo cultivar. This acreage is being managed conventionally (a single application of fertilizer each spring at 67 kg/ha (60 lb/acre) and harvested in the fall) and is available to us for any additional manipulations or measurements we might propose in the future. Land is also available for establishing the new experiment on soils similar to those associated with the existing experiments.

We are using these existing experiments because they provide a range of C input conditions, soil pH conditions, and possibly soil chemistry conditions because of the fertilizer applications. The land use and crop management at Milan are typical of switchgrass production for bioenergy and thus are directly relevant to widespread application of this technology and thus the evaluation of this technology at regional and national scales in Theme 7.

To extend and test the process-level findings from the current Milan experiments, we will also establish in 2007 and 2008 a new experiment at the Fermilab and Milan sites, respectively. In this new experiment, we will establish six treatments with three replicates. Four of the treatments will be a factorial using two fertilizer rates (0 and 134 kg/ha/y) and two switchgrass cultivars of upland and lowland ecotypes. The fifth and sixth treatment will involve a manipulation to affect humification processes (e.g., the addition of black C prior to planting), one of the two cultivars, and one of the two fertilizer rates. The manipulations to affect humification processes will be based on the results of prior lab experiments undertaken.

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Figure 6. Switchgrass trial in Milan, Tennessee, in May 2006. This stand was planted in spring 2004. Prior to planting switchgrass the land had been in a series of corn, cotton, and soybean rotations.
in Theme 4. These six manipulations will provide a range of C sequestration conditions. If resources permit, an additional experiment may be established on an Ultisol and/or measurements collected at other sites of opportunity; however, the thrust of the proposed research will be on the four field experiments (three at Milan and one at Fermilab).

The lowland and upland ecotype cultivars to be used in the new experiments will most likely be respectively “Alamo” and “Cave-in Rock”. If, however, we can find two cultivars with contrasting rooting patterns/structure, we will use those. Lowland switchgrass cultivars are typically tetraploid, while upland cultivars are typically octoploid (Hopkins et al. 1996). Lowland cultivars have typically higher yields than upland cultivars, but upland cultivars are more cold-tolerant. The purpose behind these new field experiments is to provide a range of carbon input conditions under contrasting soils and climate conditions under which to study C sequestration processes and explore manipulating humification processes. We wish to stress that our focus is on soil processes, not on agronomic practices; we are simply using these agronomic practices as a means of altering conditions.

Dr. Tyler of UT will provide expertise on switchgrass establishment at both sites and will be responsible for the maintenance of the Milan experiment. CSiTE staff at Fermi will be responsible for the Fermi experiment. The establishment and maintenance cost of the Fermi switchgrass experiment will be borne by the Fermi National Environmental Research Park (NERP) as part of its prairie restoration and maintenance activities, while the same costs at Milan will be borne by the UT experiment station. If a switchgrass experiment can be established and maintained at Oak Ridge on an Ultisol at no cost to CSiTE, we will try to extend these manipulations to this third major soil order. There is currently some discussion of this prospect at Oak Ridge, and we will follow its development.

In the theme-specific discussion that follows, we will refer to these CSiTE field experiments as the Milan cultivar experiment, the Milan fertilizer experiment, the Milan manipulation experiment, and the Fermi manipulation experiment. In the management section, we outline the timing of the research tasks and the field experiments.

b. Rationale for the selection of EPIC and FASOM models

1. Selection of EPIC as the basis of our mechanistic model

The EPIC model was originally developed to quantify the effects of erosion on soil productivity (Williams 1995). Its first major application was in 1985 during the 2nd Resource Conservation Act appraisal to evaluate soil erosion impacts across 135 U.S. land resource regions (Putnam et al. 1988). Over the last 25 years, EPIC has evolved into a comprehensive model capable of simulating many processes in managed and unmanaged ecosystems such as plant growth, crop yield, plant competition, management operations (e.g., tillage, irrigation, fertilization, and liming), water balance, soil temperature, as well as C and N cycling (Williams 1995; Izaurralde et al. 2006).

EPIC uses the concept of light-use efficiency by which a fraction of daily photosynthetically active radiation is intercepted by the plant canopy and converted into plant biomass. Daily gains in plant biomass are affected by vapor pressure deficits and atmospheric CO₂ concentration.
Above and below plant growth as well as crop yields are also affected by environmental stresses (e.g., water, temperature, nutrients, soil compaction, temperature, and aluminum toxicity). Daily weather can be either simulated from weather parameters or read from historical records. A minimum set of soil properties (soil layer depth, texture, bulk density, and C concentration) is needed for model runs. EPIC contains algorithms that allow for a complete description of the hydrological balance at the small watershed scale (up to 100 ha). Processes calculated include snowmelt, surface runoff, infiltration, soil water content, percolation, lateral flow, water table dynamics, and evapotranspiration. Six evapotranspiration methods are available, but only one, the Penman-Monteith method, can be used in estimation of CO$_2$ fertilization effects. Wind erosion is calculated on a daily time step based on wind speed distribution and adjusted according to soil properties, surface roughness, vegetative cover, and distance across wind path. Water erosion is computed as a function of the energy in rainfall and runoff. Six equations based on the Universal Soil Loss Equation are available to the user.

EPIC has been tested under many environmental conditions worldwide. Gassman et al. (2004) provide a historical account of its development and give application examples including studies of 1) surface runoff and leaching estimates of N and phosphorus losses from fertilizer and manure applications, 2) leaching and runoff from simulated pesticide applications, 3) soil erosion losses from wind erosion, 4) climate change impacts on crop yield and erosion, and 5) C sequestration assessments.

As previously noted, the development and testing of new soil C and N subroutines in EPIC has been a major modeling activity by CSiTE investigators. Izaurralde et al. (2006) reported a new soil organic matter model in EPIC developed following concepts used in the Century model. Following the successful testing of the new EPIC model against data from the Conservation Reserve Program and a long-term experiment in Canada (Izaurralde et al. 2006), the algorithms were incorporated in the landscape version of EPIC known as APEX (Agricultural Policy Extender) (Williams and Izaurralde 2005). In both models, C and N are allocated into five pools. Carbon added to soil as plant residues, roots, or animal manure is partitioned into structural and metabolic C according to lignin and N content. The C in structural and metabolic components of litter is subsequently distributed into the various kinetic compartments of increasing turnover time (biomass, slow, and passive) or evolved as CO$_2$. Losses of C and N can occur in solid form when wind and water erosion are simulated, in soluble form during runoff and leaching events, or in gaseous form (CO$_2$). Potential transformations are based on substrate-specific rate constants, temperature, and water content. Lignin content and soil texture also affect some of these transformations (e.g., structural litter and biomass). These transformations are considered potential because they reach completion only when sufficient quantities of organic and inorganic N are available. Actual transformations are calculated based on the N supply available from each potential transformation. EPIC also calculates changes in soil bulk density as affected by changes in soil organic matter content (Izaurralde et al. 2006).

We chose EPIC because of its desirable features in the context of serving as a bridge between mechanistic experimental studies and regional to national tools for assessing soil C sequestration. EPIC employs an efficient mechanistic approach to model plant productivity as affected by atmospheric, terrain, soil, and management conditions. Variations of or interactions among these conditions may induce strong feedbacks on soil C dynamics. Because of its multiple layer treatment of belowground processes (i.e., 15 soil layers, 2-3 m soil depth), EPIC can describe
transformations and transport of soil C with depth (Izaurralde et al. 2006a). Although EPIC was initially designed to evaluate biophysical processes in managed ecosystems (e.g., crop growth, nutrient cycling, water cycling, soil erosion, etc.), it has evolved into a full-scale terrestrial ecosystem model able to describe biophysical processes under conditions of little or no management (Thomson et al. 2005). Finally, EPIC and its landscape version, APEX (Williams and Izaurralde 2005), can be run in the spatial grid of environmental, edaphic, and management regimes to facilitate regional/national analyses (Thomson et al. 2006).

2. Selection of FASOM as the basis of our regional- and national-scale evaluations of C sequestration technologies

FASOM simulates agricultural and forestry supply and demand in the U.S. It considers production of 22 traditional crops, three biofuel crops, and 29 animal products in 63 U.S. regions, plus eight forest commodities in a 100-year simulation. It does this while simultaneously accounting for environmental implications (e.g., water use), exports, imports, consumer prices, land conversion, and resource scarcity. Dr. Bruce McCarl of Texas A&M University, an author of FASOM, has refined and run FASOM as a CSiTE collaborator since CSiTE’s inception.

We chose FASOM as the basis of our analysis of large-scale application of soil C sequestration technologies and bioenergy crops because of our previous successful experience using EPIC output to inform FASOM and FASOM’s ability to a) depict C sequestration implications over time from tillage and land use change (Lee et al. 2005) and b) account for the GHG emissions associated with land-use change and products resulting from land-use change. Both agricultural and forest land use and management are considered in FASOM, along with their corresponding products. Bioenergy is also explicitly considered in FASOM. Because of these capabilities and its comparatively high degree of geographic specificity, FASOM lends itself to regional and national evaluations of the full GHG implications of strategies that include land use for bioenergy feedstock production. (McCarl et al. 2005).

Through its hook to EPIC, FASOM provides us with the ability to transfer the C sequestration, yield, water demand, and environmental results of EPIC’s mechanistic modeling into national-scale evaluations that include economic drivers as well as environmental considerations.

Finally, results from FASOM can and have been used to inform Integrated Assessment (IA) models (Gillig et al. 2004 and McCarl and Sands 2006 forthcoming). While not proposed in this science plan, using FASOM to inform IA modeling enables the direct comparison of agricultural GHG mitigation options (including soil C sequestration) with options from the energy system (e.g., carbon capture or enhanced efficiency).

Improving FASOM’s ability to account for soil C sequestration especially in the context of bioenergy is one significant step towards meeting the Science Implementation Strategy for the North American Carbon Plan, which calls for “(1) improving biophysical understanding of processes and linkages at many temporal and spatial scales, and (2) integrating and projecting realistic and consistent environmental and socio-economic scenarios that can inform decision making” (page 48, Denning 2005).
C. Theme-Specific Research

In this section we describe the specific research that will be undertaken by CSiTE. We have organized this section by the seven themes and will discuss each theme separately. Most of the CSiTE scientists will be conducting research across multiple themes

Theme 1: Soil Carbon Inputs

Purpose and Objectives

The purpose of the soil C inputs theme is to quantify belowground C inputs and root dynamics within the framework of the four CSiTE experiments. The theme plan is designed to characterize treatment (i.e., cultivar and fertilization) differences and intra-annual variation in 1) root production, 2) root mortality and decomposition, and 3) root and microbial respiration at Milan, Fermilab, and possibly ORNL and to evaluate, on that basis, proposed strategies for enhancing soil C sequestration beneath switchgrass. In addition, the soil samples taken under the auspices of this theme will be used in the other four experimental themes. This theme is focused on addressing the timing, quality, quantity, and distribution with depth of belowground C inputs beneath switchgrass. Thus it directly addresses the first overarching science question: “What is the nature of belowground C inputs by switchgrass, and are they compatible with sustained aboveground biomass production and soil C sequestration simultaneously?” Theme 1 also contributes to a better understanding of the distribution of C through the soil profile and thus also relates to the fourth overarching question: “How are the fundamental processes controlling soil C distribution and movement manifested across landscapes and time?”

Background and Science Questions

Advancements in quantifying belowground C inputs and the contribution of root production and turnover to soil C dynamics in terrestrial ecosystems are some of the grand ecological challenges of the 21st century. Currently, there is a diverse set of direct and indirect methods for measuring plant root production and mortality with no overall consensus on which method is best suited for accurate estimation of root dynamics (Vogt et al. 1998). Despite a widespread lack of agreement on which methods are best, there is universal agreement that belowground studies are labor-intensive and often carry large uncertainties about estimates of root production and mortality. Advantages and disadvantages of different methods are widely recognized and are an important consideration when selecting an overall approach to studies of plant root dynamics (Vogt et al. 1998). Root biomass is more than two-thirds of the total biomass in switchgrass plantations (Ma et al. 2001), and studies of root dynamics as they determine soil C inputs are an essential part of understanding soil C sequestration in these systems.

Depth profiles of coarse root biomass (>2 mm) for the Alamo switchgrass cultivar have been previously examined at Milan (Garten and Wullschleger 1999). Both coarse root biomass and soil organic C inventories decline in a semi-logarithmic manner with soil depth. Summation of measured and predicted amounts of biomass to a depth of 3 m at Milan indicates that >75% of the coarse root biomass resides in the top 40 cm of soil. This finding is similar to those of other investigations on the vertical distribution of switchgrass root biomass (Ma et al. 2000; Frank et al. 2004). Based on $^{13}$C natural abundance measurements, Garten and Wullschleger (2000)
estimated an input of $210 \text{ g C m}^{-2} \text{ y}^{-1}$ beneath switchgrass at Milan. The former estimate was preliminary but represents approximately one-third of the C captured aboveground by annual switchgrass production. Preliminary estimates for the turnover time for C in coarse switchgrass roots were on the order of 1 to 2 years (Garten and Wullschleger 2000). Other investigations of root dynamics beneath switchgrass indicate that coarse roots are <20% of total root biomass (Tufekcioglu et al. 1999); therefore, much remains to be learned about the distribution and dynamics of switchgrass fine roots that undoubtedly will comprise most of the belowground biomass at Milan, Fermilab, and ORNL.

While the principal source of detritus and soil organic matter under switchgrass is the root system, the rate of soil C turnover may ultimately determine the potential for soil C sequestration. Soil respiration measurements integrate the biological activity of roots and microbes that determine soil C turnover rates. It is important to separate root from microbial respiration when assessing the effects of different treatments on soil C dynamics. For example, Parkin et al. (2005) reported differences in microbial respiration between landscape positions that were correlated with organic matter and microbial biomass content; however, the effect of landscape position was masked by differences in root respiration between crops. Environmental factors, particularly temperature and water availability because they influence plant activity and organic matter decomposition, are important in controlling soil respiration. In addition, substrate quality and soil nutrients influence the rates of C turnover. In a comparison with cool-season grasses, Tufekcioglu et al. (2001) found that switchgrass had the highest live, fine-root biomass and the lowest soil respiration. Another study indicated that differences in physiology (small root turnover or low specific root respiration) possibly lead to low rates of C turnover beneath switchgrass and contribute to greater soil C accumulation (Marquez et al. 1999). Finally, management practices are known to affect soil C turnover. Mulching and adding straw have been shown to have positive effects on soil C sequestration in croplands (Rees and Chow 2005), and Ma et al. (2000) showed that soil respiration and soil C turnover were greater when switchgrass was harvested once instead of twice in a sandy loam soil. However, while there is evidence that management practices can affect soil C turnover, the effects of management practices such as nutrient amendments on soil organic matter have not been extensively studied.

This research is driven by the following science questions:

- How does C allocation and the attributes of belowground biomass (like tissue chemistry and rooting depth) influence the proclivity of different switchgrass varieties for soil C sequestration?
- How do different switchgrass management strategies, like N fertilization, impact the dynamics of belowground biomass and how are such effects translated to the accrual of soil organic C?
- What are the implications of increased stocks of soil organic matter for soil N transformations beneath switchgrass and to what extent do increased stocks of soil organic matter disrupt soil N supplies required for long-term sustainability of switchgrass plantations?
Hypotheses to Be Tested

This theme will focus on testing several general hypotheses related to the dynamics and chemistry of belowground C inputs, including both coarse (>2 mm) and fine (<2 mm) roots, and the C sequestration potential of soils within the framework of the four CSiTE experiments and possibly at ORNL.

Hypothesis 1: Cultivars with high root:shoot production ratios will result in greater soil C sequestration. The rationale for this test is based partly on work by Ma et al. (2000), who examined root characteristics of three switchgrass cultivars growing in Alabama on a Norfolk sandy loam soil. They reported significant cultivar differences in root weight density and root biomass and concluded that cultivar selection will be an important determining factor in soil C sequestration beneath switchgrass. Root:shoot ratios in switchgrass tend to decline with increasing rates of N fertilization, but this change is primarily a result of increasing aboveground production and not a decrease in root biomass (Ma et al. 2000). Although this hypothesis may appear self-evident, measured varietal differences in root yield do not necessarily correlate with aboveground production (Bransby et al. 1998), suggesting that root C inputs to soil cannot be accurately inferred from aboveground measurements or root:shoot production ratios. Rejection of this hypothesis implies that the propensity for soil C sequestration cannot be predicted on the basis of measured aboveground production.

Hypothesis 2: Cultivars with deeper roots promote greater soil C sequestration. Studies of the vertical distribution of switchgrass roots indicate very deep rooting (>1 m) (Ma et al. 2000; Frank et al. 2004) that results in significantly greater deep soil C storage under switchgrass than under conventional crops (Liebig et al. 2005). Deep-rooted grasses have the potential to sequester significant amounts of soil C (Fisher et al. 1994), partly because rates of soil C mineralization tend to decline with increasing soil depth (Accoe et al. 2002). Reduced rates of organic matter decomposition at depth likely result from lower soil temperatures and lower rates of soil microbial activity. However, switchgrass root C:N ratios also tend to increase with soil depth (Tufekcioglu et al. 2003), which means that changes in root tissue chemistry may also affect variability in decomposition through the soil profile (see Hypothesis 3). Differences among cultivars and the effects of N fertilization on deep rooting by switchgrass merit additional research.

Hypothesis 3: Cultivars and/or N fertilization that results in low root C:N ratios will accelerate rates of soil C inputs via root mortality and increase C sequestration in the soil only when protection mechanisms slow microbial activity and decomposition of soil organic matter. A recent, comprehensive review concluded that C:N ratios are a principal determinant of short-term root decomposition rates, in addition to other variables such as temperature and root Ca concentrations (Silver and Miya 2001). In grasses, the correlation (based on data from multiple studies) indicates that roots with high C:N ratios decompose slower than those with low C:N ratios. Switchgrass root N concentrations and root C:N ratios decline with N fertilization (Ma et al. 2000), and such changes in root litter chemistry could significantly impact rates of root decomposition and belowground soil C inputs. Increases in root inputs could accelerate mineralization of soil organic matter, particularly in nutrient-deficient soils, and reduce organic C storage in the soil (Fontaine et al. 2004). However, if inputs were to be incorporated into stable aggregates and protected from rapid decomposition, soil C should increase (Tisdall and Oades
Moreover, some studies indicate that higher substrate N concentrations may inhibit long-term rates of organic matter decomposition, possibly through an inhibition of lignolytic enzymes (e.g., Matocha et al. 2004). The significance of differences in tissue chemistry for switchgrass soil C dynamics merits further examination. A corollary to hypothesis 3 is that N fertilization will accelerate switchgrass root C turnover times and thereby increase soil C inputs when decomposition of soil organic matter is repressed.

**Hypothesis 4:** Maximizing belowground C inputs will increase soil N immobilization and reduce net soil N availability. Long-term sustainability is an essential element of a national, biofuel-based energy strategy. Nitrogen dynamics and processes involved in soil N transformations (such as mineralization and immobilization) will be important to long-term sustainability of switchgrass production for biofuels. Some research indicates that, unlike aboveground production, root C stocks do not change with N fertilization (Ma et al. 2001). Other studies indicate greater root dry matter at higher levels of soil N fertilization (Sanderson and Reed 2000). Hence, the impact of N fertilization on root biomass, production, and mortality is unclear. High rates of biomass production can create demands on soil N reserves that potentially jeopardize the sustainability of switchgrass plantations. The low N fertilizer use efficiency reported for switchgrass (Staley et al. 1991; Stout and Jung 1995) indicates that an understanding of naturally occurring soil N transformations will be important in managing N fertilization for maximum aboveground production. Moreover, reported changes in soil C mineralization and soil microbiology following switchgrass establishment (Ma et al. 2000) suggest that soil N transformations may change with plantation age. There is no existing research on the inter-related aspects of soil N availability; switchgrass root dynamics, and soil C sequestration.

Accelerated soil C inputs through increased production of above- and belowground switchgrass litter adds to existing stocks of labile soil organic matter and creates a substrate favorable for increased heterotrophic microbial activity. Under these circumstances, N immobilization by soil microorganisms could impose a feedback on soil N dynamics that constrains soil N availability and alters N management strategies. However, such effects have not yet been demonstrated for switchgrass and merit experimental testing. Rejection of this hypothesis would indicate that increased soil C sequestration beneath switchgrass does not adversely affect the prospect of sustaining long-term soil N availability with minimal fertilizer inputs.

**Technical Plan**

We will quantify both above- and belowground litter C inputs within the framework of the four CSiTE experiments. In addition, changes in root biomass and tissue chemistry will be measured as a function of soil depth. Likewise, at all four experiments, measurements of $^{13}$C natural abundance will be used as an independent estimate of soil C dynamics (apart from dynamic modeling). These measurements will be taken sequentially at the four CSiTE experiments. The Milan cultivar experiment will be measured in Year 1, the Milan fertilizer experiment in Year 2, the Fermi manipulation experiment in Year 3, and the Milan manipulation experiment in Year 4. Year 5 will be dedicated to analysis and collection of supporting samples at the Fermi or Milan manipulation experiments as needed.

Nitrogen-15 tracer studies will be used in the Fermi and Milan manipulation experiments in the third and fourth year, respectively, to investigate soil N transformations as they are affected by soil C inputs. Enriched $^{13}$C tracer studies will be used at the Milan fertilizer experiment and the
Milan and Fermi manipulation experiments to follow C transformations. Although highly interrelated, Theme 1 is divided into individual research tasks to facilitate research task management.

**Task 1.1 Aboveground Litter Inputs**

Aboveground litter and root mortality are the two principal soil C inputs under switchgrass plantations. Maximum removal of aboveground biomass for biofuels is expected to result in minimal aboveground litter inputs. Nevertheless, there may be some mortality of aboveground biomass before harvesting and plant stubble after harvesting that collectively contributes to aboveground litter, and hence these processes merit quantification. For example, in a comparison of corn, soybeans, cool-season grasses, and switchgrass in Iowa, Tufekcioglu et al. (2003) found that switchgrass had a slower growing-season mass loss rate that indicates a reduced propensity to organic matter decomposition.

The objective of this task is to quantify intra- and inter-annual changes in aboveground litter C inputs. The seasonal dynamics of aboveground litter C inputs will be determined from bimonthly, independent measurements of the oven-dried mass of dead plant matter in six 30- x 30-cm quadrants in each study plot (samples will be composited by plot). Collections of dead litter will be timed to capture autumn inputs associated with harvesting and the death of plant stubble. Oven-dried aboveground litter will be analyzed for total C and N concentrations using an elemental analyzer at ORNL (LECO CN-2000) and lignin, which will be submitted to a commercial laboratory for analysis. At the Milan cultivar and fertilizer experiments, 72 samples will be collected per year (72 samples/year = 4 trt x 6 sampling periods/year x 3 reps) while 108 samples will be collected at the Fermi and Milan manipulation experiments (samples/year = 6 trt x 6 sampling period/year x 3 reps). Intra- and inter-annual changes in aboveground C or N inputs (g element m$^{-2}$) will be calculated based changes over time in the dry mass litter flux (g necromass m$^{-2}$) and C or N concentrations (g element g$^{-1}$ necromass). Seasonal and annual changes in aboveground C and N inputs will be compared among cultivars and N treatments and evaluated in relation to measured belowground C and N inputs. Data from this task on the amounts, dynamics, and chemistry of aboveground litter inputs will also be used in studies of soil N transformations beneath switchgrass (see Task 1.3).

**Task 1.2 Belowground Litter Inputs**

A dynamic systems approach (Makela and Vanninen 2000) will be used to quantify coarse and fine root production and mortality within the framework of existing and proposed experiments in the science plan. The method is based on sequential soil cores, but it avoids some of the inherent limitations of sequential coring by simultaneously accounting for root growth, mortality, and necromass decomposition. Unlike simple sequential coring, the systems dynamics approach has no minimum sampling interval. Specific gross growth rates and mortality rates of switchgrass roots can be calculated from this method over any time interval on the basis of the following parameters: 1) accurate estimates of root biomass and necromass (Subtask 1.2.1), 2) the decomposition rate of root necromass (Subtask 1.2.3), and 3) the average time that dead roots remain identifiable in the soil samples (Subtask 1.2.3).
Four soil cores will be collected from each study plot prior to the growing season, just before tilling and within 3 weeks after harvest. A hydraulic soil corer (5 cm diameter) will be used for soil sampling. Each core will be divided into the following five depth increments (0-5, 5-15, 15-30, 30-60, and 60-120 cm), and identical depth increments will be pooled by plot. Soil samples needed by the other themes will be extracted from these samples as appropriate and handled as described in the theme description. While we expect most soil samples used by the other themes to originate from this sampling, not all will.

For the Milan cultivar and Milan fertilizer experiments, the triennial sampling plan will produce 180 soil samples per year (180 samples = 4 trt x 3 reps x 5 soil depths x 3 sampling periods). For the Milan and Fermi manipulation experiments, the triennial sampling plan will produce 270 soil samples per year (270 samples = 6 trt x 3 reps x 5 soil depths x 3 sampling periods). If the sample number turns out to exceed our resources for processing and analysis, we will consult with the other experimental themes as to the best approach for reducing sample numbers for analysis. Samples for carbon input analysis will be frozen until root sorting commences. Sub-sampling methods (Schroth and Kolbe 1994) will be used because large soil volumes require longer sample processing times. Roots will be separated from the soil by soaking the samples in water and gently washing the mixture through a 0.5-mm sieve. Roots will be separated into coarse (>2 mm) and fine (<2 mm) size classes and then further separated into live and dead roots based on color of the cortex and tissue elasticity (Tufekcio glu et al. 1999). Oven-dried mass (g m\(^{-2}\)) will be determined for 1) live, fine roots; 2) dead, fine roots; 3) live, coarse roots; and 4) dead, coarse roots. The dry root samples will be used for the following subtasks.

Subtask 1.2.1 – Root biomass distribution with depth

The objective of this subtask is to characterize switchgrass root dry mass (g m\(^{-2}\)) beneath different experiments at the beginning, middle, and the end of the growing season. Measurements of dry mass for the four different root categories (explained above) as a function of soil depth (0-5, 5-15, 15-30, 30-60, and 60-120 cm) will be compared among different cultivars, N fertilization treatments, and sampling times using analysis of variance. We will undertake soil sampling in a subset of study plots at each site to determine the amounts of deep root biomass (below 1 m) and, if necessary, proposed sampling protocols will be adjusted to accommodate soil depths >1 m.

Subtask 1.2.2 – Root chemistry with depth

The objective of this subtask is to quantify root C and N stocks beneath different experiments at the beginning, middle, and end of the growing season and to characterize changes in root tissue chemistry with soil depth. The four root categories from each soil sample (explained above) will be analyzed for total C, N, and lignin as described in Task 1. Measurements of root C and N stocks (g element m\(^{-2}\)) will be calculated on the basis of dry mass (dry g m\(^{-2}\)) data (Subtask 1.2.1) and measured concentrations (g element dry g\(^{-1}\)). The C and N stocks will be summarized as a function of soil depth and compared among cultivars or experimental treatments using analysis of variance. Tissue chemistry data will be used to define stocks of two broad categories of litter inputs in the EPIC model, namely structural and metabolic litter (Izaurrelde et al. 2006). Differences in root tissue chemistry (C:N and lignin:N ratios) will also be examined as a function...
of soil depth (0-5, 5-15, 15-30, 30-60, and 60-120 cm) under the existing and planned experiments.

Subtask 1.2.3 – Switchgrass root dynamics

The dynamic system approach to quantifying root production and mortality requires measurement of 1) root biomass and necromass, 2) the decomposition rate of root necromass, and 3) the average time that dead roots remain identifiable in the soil samples (Makela and Vanninen 2000). The first requirement, root biomass and necromass in coarse and fine roots, will be supplied by Subtask 1.2.1. The ratio of root necromass-to-living biomass as a function of soil depth in switchgrass has been examined in previous studies (Tufekcioglu et al. 2003). Measurements related to decomposition of root necromass and the quantification of the necromass:biomass will also be determined as part of this subtask.

An intact-core technique (Dornbush et al. 2002) will be used to estimate the decomposition rate of root necromass. Four intact-cores (5 cm diameter) will be prepared, according to methods described by Dornbush et al. (2002), and buried at two soil depths (5-15 and 30-60 cm) in the spring. Roots from the cores will be recovered at 3, 6, 12, and 18 months after burial, oven-dried, and weighed to ascertain the rate of mass loss at different soil depths. The rate of mass loss will be used to estimate the site- and treatment-specific decomposition rate of dead fine and coarse roots. The time that dead roots remain identifiable in soil samples will be measured under laboratory conditions. The incubations will involve observations over time of roots in a soil matrix sandwiched between glass plates to determine the average time that dead switchgrass roots remain identifiable.

Monte Carlo methods will be used to estimate uncertainty about predictions of root production and mortality using the dynamic system model described by Makela and Vanninen (2000). Basically, this involves estimating the uncertainty of model parameters based on field and lab measurements, performing repeated model runs by drawing parameters from a statistical distribution with set limits, and then summarizing the variability in predictions from multiple (e.g., 1000) model runs. We will compare and contrast predicted root production, mortality, and decomposition among cultivars and N fertilization regimes in the four CSiTE experiments.

Task 1.3 Measurements of Soil Respiration

The objective of this task is to study the relative importance of microbial processes to soil C sequestration potential and to better integrate Theme 1 with the microbial and the soil stabilization/aggregation themes. Prior research has found that the microbial component of soil respiration is sensitive to plant and environmental factors that are important when considering soil C sequestration potential (Hanson et al. 2000). For example, management practices such as fertilization may enhance overall C storage in soils through changes in plant activity and root mass, root chemistry, and microbial activities. However, the capacity of land management practices to enhance soil C sequestration will also be determined by environmental, edaphic (soil organic matter, microbial community, soil texture), and climatic (precipitation, temperature) factors that influence soil organic matter stability and rates of organic matter losses through microbial processes. Measurements of soil respiration can provide information on the importance of these foregoing factors.
For each switchgrass cultivar and/or N fertilization treatment at the four CSiTE experiments, we will measure soil respiration, soil temperature, and soil moisture using a vented automated soil CO\textsubscript{2} exchange system and temperature and moisture probes (ADC BioScientific Ltd.). Root and microbial respiration will be separated several times a year (three times during the growing season and twice during the dormant season) using a combination of two methods. First, we will use the regression approach described in Kucera and Kirkham (1971) and Wang et al. (2005) and recently reviewed by Kuzyakov (2006) to separate the root (plus rhizosphere) and the microbial respiration components of total soil CO\textsubscript{2} efflux. In this approach a linear regression between soil respiration and root biomass is established, and the y-intercept represents microbial respiration, while root respiration is estimated by difference between soil respiration and microbial respiration. Second, we will use the $^{13}$C natural abundance method, based on the mass balance of $^{13}$C signature of the soil respired CO\textsubscript{2} using the Keeling plot method. This method will allow estimation of the contribution of root-derived CO\textsubscript{2} according to the $^{13}$C value of the CO\textsubscript{2} evolved in the field when C\textsubscript{4} plants are grown on C\textsubscript{3} soil. Switchgrass will respire CO\textsubscript{2} of $-12\%$o ($\delta^{13}$C) versus soil microbial processes that will reflect the $^{13}$C enrichment of the soil organic matter developed under C\textsubscript{3} plants. Recent measurements show that the $\delta^{13}$C of soils at Milan and Fermilab is around $-20$ to $-22\%$o at both sites, thus there is sufficient difference between the C isotope composition of switchgrass roots and soil organic matter for the use of this method. The data will be scaled to represent annual total C losses, as well as root and microbial losses, and will be correlated to microbial biomass, soil organic matter, plant production, microbial byproducts, soil aggregation, and stabilization factors (all in conjunction with other themes). In addition, measurements of microbial respiration will contribute to a better representation of the labile, metabolizable C pool for purposes of modeling soil C dynamics.

**Task 1.4 Studies with Stable Isotopes**

**Subtask 1.4.1 – Natural abundance $^{13}$C**

Growing switchgrass, a C\textsubscript{4}-plant, in soils previously occupied by C\textsubscript{3}-vegetation gives rise to a soil organic matter labeling experiment because C\textsubscript{4} ($\approx -13\%$o) and C\textsubscript{3} (28\%) plants have different stable C isotope ratios (Balesdent et al. 1988). Consequently, the fraction of soil C derived from switchgrass can be tracked over time, and soil C inputs derived from switchgrass can be estimated on the basis of natural abundance measurements of $^{13}$C. As noted earlier, recent soil measurements under switchgrass at Milan indicate a $\delta^{13}$C signature of $-20$ to $-22\%$o with an indication of greater switchgrass C inputs in near surface soils (18 to $-19\%$o) at some sites. These findings are similar to previously reported $d^{13}$C-values at Milan where reference soils had $\delta^{13}$C-values of about 22 \%. To the extent possible, we will use changing $\delta^{13}$C-values at all four CSiTE experiments to provide a secondary check on predicted soil C inputs from the dynamics systems approach of Makela and Vanninen (2000). Garten and Wullschleger (2000) used this approach with some success in previous studies at Milan to estimate the turnover rates of soil C in particulate organic matter and mineral-associated organic matter beneath switchgrass.

**Subtask 1.4.2 – Tracer studies with enriched $^{15}$N**

The objective of enriched $^{15}$N tracer studies is to examine the fate of switchgrass root N inputs to soil and determine the degree of N fertilizer immobilization and use efficiency. Tracer studies, including end-member analysis, with $^{15}$N may also be a rapid and relatively simple approach to the quantification of root necromass:biomass ratios, because dead switchgrass roots will not
incorporate tracers that are injected into live plants. Nitrogen-15 tracer techniques for the study of N immobilization and mineralization in soils have been reviewed in greater detail elsewhere (Hart and Myrold 1996). When coupled with conventional laboratory studies of potential net N mineralization and nitrification (Hart et al. 1994) and measurements of litter C:N ratios from Tasks 1 and 2, the tracer methods will add much to our overall understanding of soil N transformations beneath switchgrass plantations and the propensity for net N immobilization under different cultivars and fertilization regimes.

As a direct test of N fertilizer immobilization and use efficiency, we will add \(^{15}\)N-labeled fertilizers to switchgrass, within the context of the planned Milan and Fermi manipulation experiments, to examine the short- and long-term fate of N additions to the soil, N immobilization in surface litter layers and soil microorganisms, and the proportion of N fertilizer additions incorporated into switchgrass biomass aboveground. These experiments will be developed in greater detail as part of the final planning of manipulation experiments at Milan and Fermilab and will be important to quantifying the overall N economy of managing switchgrass to enhance soil C sequestration. For the sake of brevity, we would simply call to the reader's attention that these methods are relatively straightforward and have been used in multiple studies to examine the fate of N inputs to soils and the short-term incorporation of N additions into non-extractable soil fractions (e.g., microbial biomass and fixation of N on clay minerals) (Hart and Myrold 1996).

Subtask 1.4.3 – Tracer studies with enriched \(^{13}\)C

The objective of enriched \(^{13}\)C tracer studies is to examine the fate of switchgrass root C inputs to soil and determine the fate of belowground C in the rhizosphere. Tracer studies for the purpose of labeling plant biomass usually involve chamber fumigations with radioactive \(^{14}\)CO\(_2\) or \(^{13}\)CO\(_2\), which has an isotopic signature distinctly different from atmospheric CO\(_2\). Fumigation methods are expensive, labor intensive, and impractical within the framework of the current science plan. As an alternative, we propose to develop innovative and easy-to-use methods for \(^{13}\)C labeling of the switchgrass rhizosphere by injecting individual plants, or clusters of individual plants, with highly enriched (99 atom %) \(^{13}\)C-labeled compounds (e.g., sucrose).

In studies comparing the efficacy of \(^{15}\)N labeling methods, Chalk et al. (2002) found that injections of \(^{15}\)N-urea into hollow-stem *Sesbania rostrata* produced the highest atom % excess \(^{15}\)N (0.1 at % ex) in roots and shoots compared to leaf and root immersion methods. Switchgrass stems are also hollow near the base of the plant and are thus ideally suited for \(^{13}\)C-labeling by stem injection methods. In other work on soybeans that used stem injection of sucrose solutions, Zhou et al. (2000) reported an uptake rate of 1.3 mL d\(^{-1}\) per plant and successfully injected as much as one-third of the plant C content. Some preliminary trials and methods development will be necessary (Year 1), but the enriched tracer approach holds considerable promise for creating a source (or substrate) pool of \(^{13}\)C-labeled roots that can be used to follow the movement over time of C from switchgrass roots into different soil fractions, aggregates, microorganisms (e.g., mycorrhizae), and drainage waters. These tracer studies will first be undertaken at Milan (Year 2) to compare the fate of belowground C inputs under four different fertilization regimes. They will then be undertaken at the Fermi manipulation experiment in Year 3 and the Milan manipulation experiment in Year 4.
**Linkages to Other Themes**

Task 1.1 on aboveground litter inputs links to multiple research themes but provides especially critical data for Theme 6 on mechanistically modeling soil C dynamics beneath switchgrass. Task 1.2 on belowground litter inputs is also strongly linked to Theme 6, but will provide important data on switchgrass root architecture (Subtask 1.2.1), chemistry (Subtask 1.2.2), and root dynamics (Subtask 1.2.3) that will benefit Theme 2 on aggregate stabilization and dynamics, Theme 3 on microbial community function and dynamics, and Theme 4 on humification chemistry. Task 1.3 on soil respiration links directly to Themes 2 and 3. Task 1.4 in Theme 1, which involves studies with stable N and C isotopes, is linked to Theme 2, Theme 3, and Theme 5 on intrasolum C and N transport because the planned use of isotopic tracers permits monitoring of the movement of $^{15}$N (or $^{13}$C) into soil aggregates, soil microorganisms, and drainage waters.

**Expected Results**

This theme will produce quantitative estimates of soil C inputs beneath switchgrass, by depth, for each treatment (e.g., fertilizer amount and switchgrass variety) in each of the planned experiments at Milan and Fermilab. It will establish relationships between organic matter inputs (largely from root turnover) and soil N availability that potentially affect switchgrass growth, rates of soil organic matter decomposition, and the function and dynamics of the soil microbial community. The studies of soil respiration will help to identify the relative importance of microbial processes to soil C sequestration under different switchgrass varieties and management regimes. The respiration studies will also provide quantitative information on the effectiveness of physicochemical stabilization (Theme 2), microbial activity and function (Theme 3), and humification rates (Theme 4). Studies with stable N and C isotopes will help place additional constraints on the process of soil C sequestration beneath switchgrass. Tracer studies will permit us to track the movement of $^{13}$C and $^{15}$N into soil aggregates, microbial biomass, and drainage waters. All of these expected results will help us to answer the overarching science question: “What is the nature of belowground C inputs by switchgrass, and are they compatible with sustained aboveground biomass production and soil C sequestration simultaneously?”

**Schedule and Milestones**

The proposed schedule is based on intensive studies of the independent effects of cultivar (Year 1) and N fertilization (Year 2) on switchgrass belowground C inputs at a single site (Milan) in the first and second year of work. Manipulation studies of interactions between cultivar and N fertilization at Milan, Fermilab, and possibly ORNL will begin in the third year. Methods development for use of enriched $^{15}$N and $^{13}$C tracers to track rhizosphere C and N dynamics will start in Year 1 and lead to an application of these methods in fertilizer trials at Milan and manipulation experiments (Milan, Fermilab, and possibly ORNL) in following years. Linkages of Theme 1 to other research themes will occur throughout the entire project.
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Theme 2: Soil Structural Controls

Purpose and Objectives

The purpose of this integrative theme is to improve understanding of soil structural controls on the transformation and stabilization of organic C inputs as soil organic matter (SOM). As the habitat for plant roots and soil organisms, soil structure affects plant growth and plays an important role in the regulation of decomposer activity. In addition, the physicochemical environment created by soil structure can influence both biotic and abiotic humification processes and can affect the transport of dissolved organic C (DOC) and other solutes through the soil profile. Thus, investigations in this theme will integrate with Theme 1 Soil Carbon Inputs, Theme 3 Microbial Community Function and Dynamics, and Theme 4 Humification Chemistry to address CSiTE’s second overarching science question: “What are the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil, and how do they interact in space and time?” This theme will also contribute to understanding near-surface processes affecting the movement and distribution of C through the soil profile (Question III) by integrating with Theme 5 Intrasolum Carbon Transport. In addition, an important objective of this theme will be to quantify the effects of switchgrass production under different edaphic and climatic conditions on the size and dynamics of SOC pools defined by the spatial organization of soil structure. This information will be used by Theme 6 Mechanistic Modeling to incorporate soil structural controls into mechanistic models of soil C dynamics and predict the soil C sequestration potential of switchgrass production systems.

Background and Science Questions

Biochemical attack of SOM is inhibited at multiple scales by the physicochemical protection afforded by soil structure. Stabilization of otherwise decomposable SOM can occur via sorption to soil surfaces, complexation with soil minerals, occlusion within aggregates, and deposition in pores inaccessible to decomposers and extracellular enzymes. The relative importance and potential saturation of these physicochemical stabilization mechanisms vary depending on soil type, the nature of C inputs, management practices, and environmental conditions.

Current conceptual models of soil C cycling consider the interactions of decomposing C inputs with soil minerals at molecular to millimeter scales and the relationship of these developing organomineral associations to the structural organization and dynamics of the soil (e.g., Oades 1984; Golchin et al. 1994; Muneer and Oades 1989; Sollins et al. 1996; Six et al. 1999; Baldock and Skjemstad 2000; Christensen 2001). The physical location of SOM within the soil structural hierarchy and the dynamics of this structure together exert significant control on potential interactions between SOM, soil minerals, and decomposers (Elliott and Coleman 1988; Golchin et al. 1994; Christensen 2001; Plante and McGill 2002; Six et al. 2004, 2006).

In our working concept of soil structural controls on C cycling (adapted from Golchin et al. 1994 and Six et al. 1999), fresh organic inputs (especially root material and associated mycorrhizal fungi) are fragmented by soil biota and colonized by microorganisms. The mucilages and other residues produced by the activities of these organisms contribute to the stabilization of soil macroaggregates (>250 μm). As these fragmented residues are further broken down into finer particles, they become encrusted with mineral particles and form the organic cores of stable
microaggregates (53-250 μm). The chances of microaggregates being formed and stabilized in this manner is thought to be greater inside stable macroaggregates, where this particulate organic matter (POM) is somewhat protected from rapid decomposition. While these organic cores are still rich in carbohydrates and chemically attractive to microorganisms, microbially produced mucilages, metabolites, and residues permeate the encrusting mineral particles and create very stable microaggregates. Once the more labile portions of the microaggregate cores are consumed, decomposition of more resistant plant structural materials proceeds more slowly. Eventually, deposition of new stabilizing microbial residues is exceeded by their decomposition, and the aggregate becomes unstable to disruptive forces such as wetting and drying, freezing and thawing, and root growth. Mineral particles and silt-sized aggregates that coated the organic cores are then freed to become associated with more labile POM.

At the next hierarchical level, microbial residues and small fragments of humified plant residues produced within microaggregates serve as nucleating sites for the formation of silt-sized aggregates. Thus, humified materials and microbial residues produced within microaggregates have a better chance of being protected long enough to become stabilized in silt-sized aggregates or finer-scale organomineral complexes than similar residues produced outside microaggregates. Along this spatially explicit decay continuum, microaggregates might be considered as “bioreactors” for the formation of new humified materials and new C-enriched silt-sized aggregates or organomineral complexes.

In this conceptual framework, factors controlling the capacity of soils to develop aggregate hierarchy and the turnover of macro- and microaggregates are important contributors to the physicochemical stabilization of SOM and to the humification processes of biochemical alteration and polymerization/condensation (Jastrow et al. in press). This paradigm is generally applied to inputs of plant detritus brought into direct physical contact with the soil matrix (i.e., root turnover or surface litter incorporation via bioturbation, physical disturbance, or mass flow) and to soils where SOM is a major aggregate binding agent. Thus, it is particularly appropriate for perennial grasses with extensive root systems, such as switchgrass, and is generally applicable to most Alfisols, Mollisols, and Ultisols that occur extensively throughout areas where switchgrass is likely to be grown.

Although the focus of our organizing concept is on the formation, stabilization, and turnover of soil aggregates, soil structure also includes the arrangement of pores within the soil. Hence, the development of aggregate hierarchy creates a parallel hierarchy of pores (Elliott and Coleman 1988; Young and Ritz 2000; Young and Crawford 2004). The pore system is where the decomposer community resides, and it also provides the environment that constrains the interactions between decomposers and their substrates (Strong et al. 2004; Ekschmitt et al. 2005). Biological factors (plant growth and turnover as well as decomposer activities) and management practices that affect soil aggregate dynamics can therefore have profound effects on soil porosity, which can then feed back to affect aggregation and plant growth.

A better understanding of these complex interactions and the factors controlling them is needed to maximize the stabilization of SOM derived from switchgrass production for biofuels while minimizing the economic and environmental costs of production. In other words, if C inputs exceed the capacity of a soil to protect and stabilize those inputs, then higher fertilization rates or other concerted efforts to increase inputs may not result in the desired or predicted enhancement
of soil C sequestration and could, in fact, result in undesirable environmental consequences such as increased emissions of N$_2$O or other greenhouse gases.

Planned research under this theme is driven by the following science questions:

- How do the interactions and feedbacks between soil structure and switchgrass production control SOC stabilization and sequestration? How are these relationships affected by edaphic properties and climate?
- Do microaggregates function as biophysical reactors that control the humification of fresh C inputs and the stabilization of SOC?
- How does the hierarchical organization of soil structure affect microbial community structure and function?

**Hypotheses to Be Tested**

**Hypothesis 1:** Switchgrass-mediated changes in aggregate stability and aggregate-size distribution will alter soil pore-size distribution with subsequent effects on C sequestration through effects on infiltration, microbial communities and their activity, the humification process, and solute transport. The strength of soil structural changes and subsequent responses will be influenced by edaphic properties and climate.

Cultivars and/or management practices that maximize root and mycorrhizal fungus density and detrital inputs should have the greatest potential to increase the C content of aggregates and feedback to improve the stability and other physical/mechanical properties of aggregates (Jastrow and Miller 1998; Blanco-Canqui et al. 2005b,c). Similarly, root growth and turnover plus the activities of the decomposer community interact to influence the size distributions of both aggregates and pores (Elliott and Coleman 1988; Jastrow and Miller 1998; De Gryze et al. 2006). We expect that the magnitude of these responses to switchgrass production and the potential for saturation of the responses will depend on edaphic factors, such as soil texture, base cation status, and clay mineralogy, and on climatic conditions. Soil pore size distribution controls the balance between water flow, water retention, and aeration; affects solute movement; and controls the activities and food web interactions of soil biota (Elliott and Coleman 1988; Young and Ritz 2000; Young and Crawford 2004). Consequently, switchgrass cultivars and/or management practices that alter aggregate- and pore-size distributions have the potential to alter SOM stabilization through effects on decomposer communities and their activities, humification processes, and DOC transport through the soil profile.

**Hypothesis 2:** An increase in the amount of C stored in microaggregates will be an early, sensitive indicator for monitoring the C sequestration potentials of switchgrass cultivars or management practices.

Although the amount of long-term protection provided by macroaggregates appears to be minimal, macroaggregates protect fresh C inputs from rapid mineralization (Plante and McGill 2002). This largely root-derived POM is believed to serve as nucleating sites (Oades and Waters 1991) for the formation and stabilization of microaggregates within the macroaggregate structure.
(Angers et al. 1997; Six et al. 2004). Indeed, Denef et al. (2001) observed increases in both the concentration of intra-microaggregate POM-C and the amount of microaggregates inside macroaggregates after only a few months in a laboratory incubation study with added plant residues. Further, Bossuyt et al. (2002) found that the distribution of $^{14}$C-labeled residues in aggregated and non-aggregated soil mirrored that of total C only 3 years after placement on the surface of no-till soil, with the highest concentration of total C and $^{14}$C found in microaggregates. Six et al. (2002) suggested that changes in the amount of microaggregate-protected POM-C could be a sensitive indicator of the effects of land use or management changes on soil C. In fact, increases in the amount and/or C contents of microaggregates isolated from inside macroaggregates accounted for much of the C accrued after several decades of afforestation or conversion to no-till practices in agricultural soils (Six et al. 2002; Del Galdo et al. 2003; Denef et al. 2004). We expect that relative changes in POM-C and especially silt-associated C in microaggregates could be used to compare the C sequestration potentials of cultivars and management practices within and between soil types and/or climatic conditions. We may also be able to use this fraction as a diagnostic tool for monitoring and identifying situations or conditions where further efforts to increase C inputs will not likely result in further increases in stable, protected C pools.

**Hypothesis 3:** Physical protection of organic matter in microaggregates will slow the decomposition of organic inputs and facilitate stabilization of plant and microbial residues in silt- and clay-sized organomineral associations.

Fractionation of SOM into POM and mineral-associated OM according to aggregate hierarchy isolates C pools tied to current conceptual understanding of mechanisms controlling the transformation of fresh C inputs to SOM (as described above). Recent work supports this concept with findings that microaggregate-associated silt- and clay-sized particles can differ from similarly sized particles that are readily dispersed—with variations in C:N ratios and hydrolyzability, lignin content and its oxidation state, and mean residence time (Plante et al. 2006; Filley et al., unpublished data; Jastrow and Six, unpublished data). Preliminary studies indicate that the Milan Alfisol exhibits aggregate hierarchy and previous studies have demonstrated aggregate hierarchy for Fermilab Mollisols (Jastrow et al. 1996). Thus, the proposed experiments at Milan and Fermilab can provide a range of inputs, soils, and climates for evaluating this hypothesis. However, studies employing fractionation schemes tied to aggregate hierarchy, while providing important insight into C cycle processes and protection mechanisms, inevitably end up with more fractions than are practical for modeling purposes (e.g., Six et al. 2002; De Gryze et al. 2004; Denef et al. 2004). Thus, more data on the cycling and turnover of C associated with fine-scale physically-isolated fractions are needed to determine how these fractions can be combined to define functionally important and measurable C pools for incorporation into SOM models.

**Hypothesis 4:** The hierarchical organization of soil structure leads to a spatial stratification of the relative abundance of functionally important microbial populations and plant/microbial residues.

Decomposer activity is limited by localized oxygen and water availability. Strong et al. (2004) demonstrated that initial rates of decomposition were most rapid in soils with large volumes of intermediate-sized pores of about 15-60 μm. In particular, decomposition was enhanced near the
gas-water interface. In larger pores (60-300 μm) oxygen was abundant, but decomposition was slower—probably because motility, diffusion, and direct contact between residues and mineral surfaces were all reduced. However, the greatest protection and slowest rates of decomposition were found in soils that had large volumes of pores with neck diameters <4 μm. Aggregate hierarchy creates a parallel hierarchy of pores that exist between and within aggregates of varying sizes. Steep declines in oxygen concentrations have been measured within small distances from large aggregate surfaces, and interactions between water films and small pore necks can lead to anaerobic patches within generally aerated aggregates (Sexstone et al. 1985; Young and Ritz 2000). Such heterogeneity of microenvironments could lead to large differences in spatially segregated microbial populations and activities. Indeed, recent studies demonstrated that bacterial communities inside microaggregates can differ from those of macroaggregates (Mummey and Stahl 2004; Mummey et al. 2006). We expect that such differences could also lead to spatial stratification in rates of decomposition and humification and, thus, the amounts of plant and microbial residues present within aggregates of varying sizes and hierarchical organization. Such information will contribute to our understanding of soil C cycling and sequestration by providing insights into the scale at which microbial activity is regulated.

**Technical Plan**

Research under this theme will use data from all four CSiTE field experiments. Data collected from these experiments will provide information on how different edaphic properties and climate regimes influence soil structural responses to varying input quantity and quality. Soil samples analyzed by this theme will be obtained in conjunction with sampling for Theme 1 to enable direct comparisons of C inputs to soil structural and C stabilization measurements, except where noted. Measurements will be made for the same depth increments identified in Theme 1 or, for some tasks, a subset of these increments depending on science questions, initial findings, and theme resources.

**Task 2.1 Switchgrass Effects on Soil Structure**

Initially, we will use the existing Milan cultivar and fertilization experiments to evaluate the effects of input quantity and quality on the size distribution (<0.053, 0.053-0.25, 0.25-1, 1-2, 2-4, 4-8 mm) and organic C content of water-stable aggregates by using standard wet-sieving methods (Kemper and Chepil 1965; Jastrow et al. 1996). We will also determine the tensile strength, density, moisture retention, and porosity of individual aggregates in the 1-2, 2-4, and 4-8 mm size classes (Blanco-Canqui et al. 2005b,c). To investigate in situ effects on soil structure, field estimates of infiltration rate and hydraulic conductivity will be made by using a tension infiltrometer with sequential measurements at 0-, 30-, 60-, and 150-mm water tensions to approximate flow associated with macro-, meso-, and micropores (Luxmoore 1981; Mohanty et al. 1996). These measurements will be coordinated with Theme 5, which will also be measuring field infiltration rate and hydraulic conductivity but only on a subset of experimental plots at the Milan fertilization experiment and the Fermi manipulation experiment. Both aggregate and in situ field measurements will also be made at the new Fermi and Milan manipulation experiments. Measurements will be taken both at the start of the manipulation experiments, before planting, and during the third year of the experiment.
**Task 2.2 Microaggregate-Associated C as an Indicator of Sequestration Potential**

We will determine if changes in microaggregate-associated C, particularly that of microaggregates within macroaggregates, can be used to assess and monitor the C sequestration potential of variations in the quantity and quality of C inputs associated with different switchgrass cultivars and fertilization rates as well as various manipulations designed to enhance humification chemistry as proposed under Theme 4. Thus, these studies will be carried out at all four CSiTE field experiments. As in Task 2.1, measurements will be taken both at the start of the manipulation experiments, before planting, and during the third year of the experiment.

Soil will be wet-sieved to separate non-aggregated litter, macroaggregates (>250 μm), free microaggregates (53-250 μm), and easily dispersed silt- and clay-sized particles. A microaggregate isolator (Six et al. 2000) will then be used to separate microaggregates from within the macroaggregate fraction. Coarse POM (>250 μm) and macroaggregate-associated silt- and clay-sized particles will also be collected in this step. Free inter-aggregate POM that isolates with the sieves used to collect both free microaggregates and microaggregates within macroaggregates will be removed by density flotation in sodium polytungstate (1.85 g cm⁻³) before analyzing the C and N content of microaggregates. Microaggregates will also be dispersed by shaking in water with glass beads, and intra-microaggregate POM will be collected on a 53-μm sieve. Differential centrifugation will be used to separate silt- and clay-sized particles isolated in the various steps of the fractionation procedure. All fractions will be analyzed for C and N by using a Carlo Erba NC2500 or LECO CN2000 elemental analyzer. Although the focus of this task is on microaggregate-associated C, isolation and quantification of all fractions is necessary for mass balance evaluation of the potential for using microaggregate-associated C as an indicator of C sequestration and for the measurements planned for Task 2.3.

**Task 2.3 Physicochemical Stabilization of SOM**

We will use $^{13}$C label added during Theme 1 at the new Milan manipulation experiment to follow the transformation of fresh C inputs to POM-C pools differentiated by aggregate hierarchy and evaluate the mean residence time of C in these pools. We will exploit natural abundance $^{13}$C at the Fermi manipulation experiment for the same purpose. We expect that POM isolated from inside microaggregates will have a longer residence time than POM located outside microaggregates. Because relatively short-term tracers cannot accurately estimate the residence time of C in mineral-associated pools with slower turnover times, we will only investigate the rate at which “new” C is incorporated into silt- and clay-sized fractions. Although C in silt-sized fractions often exhibits faster turnover times than clay-associated C, these size separates are not homogenous (e.g., Plante et al. 2006). Therefore, we will investigate whether we can reduce the heterogeneity of the silt-sized fraction by using density flotation in sodium polytungstate (2.0 g cm⁻³) to separate silt-sized POM from silt-sized aggregates and primary particles. We expect that the majority of new C entering the silt-sized fraction will initially be found in silt-sized POM. Isotopic measurements will be carried out on these silt-associated fractions and the other fractions isolated under Task 2.2. Repeated measurements over time will be necessary to quantify the C dynamics associated with the isolated fractions. To capture short-term dynamics, we will sample at 1 week, 1 month, and at least once more later in the growing season following the pulse label at the Milan manipulation experiment; annual samples will be taken after the first year. At the Fermi manipulation experiment, we will sample when the experiment is established.
and annually thereafter except during Year 4. Samples will be collected and archived for all experimental treatments, but analyses will be limited to selected fractions and treatments based on initial results and available resources.

**Task 2.4 Spatial Stratification of Microbial Populations and Residues**

We will interact with Theme 3, Microbial Processes, and Theme 4, Humification Chemistry, to investigate the spatial stratification of microbial populations and resultant effects on residues and humified materials for selected treatments from the new manipulation experiments at Milan and Fermilab. Treatments will be selected on the basis of initial results obtained by this theme and the other experimental themes. We will use the novel high-energy UV irradiation method devised by Mummey and Stahl (2004) to photo-oxidize organic matter (including microbes and their residues) on the surfaces of microaggregates isolated from within macroaggregates obtained by the methods identified in Task 2.2. Irradiated microaggregates, whole (non-irradiated) microaggregates, and macroaggregates will then be analyzed in Theme 3 to evaluate differences in microbial communities and in Theme 4 to assess differences in amounts of humified organic matter present in spatially stratified locations created by aggregate hierarchy. In addition, the spatial distribution of plant- versus microbially derived organic matter will be evaluated by using carbohydrate proxies. The ratio of galactose+mannose to arabinose+xylose in acid hydrolysis extracts will be used to examine the relative contributions of microbial- versus plant-derived organic matter (Oades 1984) in these three aggregate fractions.

**Linkages to Other Themes**

Data from all tasks will be evaluated in conjunction with and, in many cases, directly correlated with (because of common samples) results from all other experimental themes. In Task 2.1, we will assess interrelationships of soil structural changes (at both the aggregate level and field scale) with root growth and C inputs (Theme 1), microbial communities (Theme 3) and their activity (soil respiration portion of Theme 1), humification processes (Theme 4), and intrasolum transport of DOC (Theme 5). Tasks 2.2-2.4 will require data from Theme 1 to evaluate the extent to which varying inputs (quantity and quality) are stabilized as SOM. Similarly, Tasks 2.2-2.4 will interact with Theme 3 to investigate the effects of physicochemical protection and aggregate hierarchy on microbial communities through microbial measurements on selected fractions. In addition, information obtained by Theme 3 will contribute to interpretation of results obtained in Tasks 2.2-2.4. We will also work with Themes 4 and 3 to better understand the interactions between soil structure, aggregation, humification chemistry, and microbial populations to sequester SOM. For example, methods developed by Theme 4 to assess the level of humification could be applied to selected fractions from Task 2.2 or Task 2.4. Information on C stabilization in near-surface samples from Tasks 2.2-2.4 will be used to understand and interpret data on DOC transport collected by Theme 5. Lastly, data and interpretations from all tasks will be provided to Theme 6 to help with model improvement and development. Task 2.2 and 2.3 information on the size and fluxes of C pools tied to soil structural controls and protection mechanisms will be particularly important to Theme 6 modeling efforts.
**Expected Results**

Research conducted under this theme will:

- Evaluate the interrelationships between soil structure (both aggregates and pores) and C inputs, microbial communities and their activity, humification processes, and DOC transport.

- Assess the potential for using microaggregate-protected C as an early indicator of C sequestration and as a monitoring tool for predicting the capacity of a soil to protect and stabilize additional C inputs.

- Provide quantitative estimates of how functional SOC pools and their dynamics are affected by the quantity and quality of switchgrass inputs and the specific manipulations designed to enhance humification processes (developed by Theme 4) under different edaphic and climatic conditions.

- Evaluate the influence of aggregate hierarchy on microbial community structure, growth, activity, and spatial distributions.

- Provide data to Theme 6 modeling efforts on the size and fluxes of C pools functionally related to soil C cycling.

These results, along with the findings of the other experimental themes, will contribute to an integrated understanding of the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil and the transport of C through the soil profile. The findings of this theme will also help to improve the capability of mechanistic models to predict soil C dynamics and sequestration.
Schedule and Milestones

The activities of this theme will be timed to coincide with sampling led by Theme 1. Baseline measurements will be gathered at the two new manipulation experiments.

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Theme 3: Microbial Community Function and Dynamics

Purpose and Objectives

The purpose of this theme is to understand the influences of switchgrass varieties and crop management practices on soil microbial community structure and function. Identifying effects on microbial communities and soil C sequestration, together with knowledge of community function will improve understanding of fundamental mechanisms underlying maximization of the C sequestration potential. The contributions of this theme have ramifications to the other themes being presented, either directly through the quantification of microbial inputs, or indirectly through maintenance of soil structure or influence on humification. Hence, close coordination between this theme with Themes 1, 2, 4, and 6 will be necessary.

This theme will address the overarching science question of “What are the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil, and how do they interact in space and time?” The theme will further contribute to the overarching science question of “How can fundamental knowledge best be used to identify and implement methods and practices for sustained enhancement of soil C in an environmentally acceptable and economically feasible fashion?”

Background and Research Questions

Organic matter decomposition and associated nutrient cycling are regulated by the soil microbial community. Therefore, developing methods and approaches to achieve a fundamental mechanistic understanding of microbial community composition and activity is important if we are to manage C stocks in soils. One of the major issues for dramatically increasing future biofuels production is the need to identify those characteristics and functions of soil microbial communities that are necessary for maintaining soil-ecosystem function and productivity under such scenarios (U.S. DOE 2006). Genotypic versus environmental variation in switchgrass root biomass production, architecture and morphology (e.g., root hair number and length; root branching patterns), degree of dependence on mycorrhizae, root longevity and tissue chemistry, and the reciprocal effects of potential management practices have not been investigated in depth, nor has the influence of these traits on soil microbial community structure and function.

Past CSiTE research has contributed to development and application of tools and experimental methods for quantifying the relative effects of fungal and bacterial presence and activity and the role of mycorrhizal fungi in coupled C/N cycling and storage in forests, agroecosystems, and prairie restoration. These approaches will be further refined and applied to switchgrass with a continuing focus on improved fundamental understanding of the coupled physical, biological and chemical processes controlling soil C sequestration. By understanding relationships among the characteristics of switchgrass varieties and their effects on the microbial communities and soil C sequestration, we will be better able to predict and inform resource managers on the effects of current biomass production processes on soil C sequestration.
Scientific questions to be addressed by research in this theme include:

- How will variation in amounts and quality of belowground C inputs influence microbial community structure and dynamics?

- Will the influence of microbial communities on soil C and N cycling under switch grass be similar or different from what was observed in earlier CSiTE research in restored prairie, agricultural systems, and forests?

- Do specific microorganisms or groups of microorganisms exist that are predictive of soil C sequestration potential and whether a particular system is accruing or losing C?

**Hypotheses to Be Tested**

**Hypothesis 1:** Variation in belowground C allocation, rooting architecture, and root phenolic content associated with different switchgrass cultivars (genotypes) will differentially affect mycorrhizal production and microbial community structure and their influence on soil C sequestration.

We will investigate four lowland varieties of switchgrass that have been grown at the Milan site for 3 years, originally to study aboveground biomass yields under different cropping systems. We hypothesize that different varieties will have different effects on soil C via genotypic differences in 1) belowground C inputs, 2) distribution patterns of belowground C caused by differences in root architecture and mycorrhizal associations, and 3) biochemical variation in C inputs. It is known that switchgrass root systems can promote C accrual (Ma et al. 2001), and work with other fast-growing C4 grasses such as big bluestem (*Andropogon gerardii*) suggest that root architecture and mycorrhizae strongly influence soil properties (Jastrow and Miller 1993). Research with C4 grasses indicates varieties that have evolved in less-productive soils, or harsh climates often employ a strategy in which root systems rely more on mycorrhizal fungi for nutrient uptake. In contrast, plants that have evolved on more productive soils rely more heavily on fine-fibrous, high-phenolic-content roots for nutrient uptake. This observation suggests that selection of superior genotypes of switchgrass for growth on both fertile and marginal soils could be based on root morphology and architecture, as well as the mycorrhizal association.

**Hypothesis 2:** Fertilizer will have differential effects on switchgrass microbial communities; high levels of N fertilization will decrease belowground C allocation and accelerate microbial degradation of organic matter, thus decreasing sequestration.

Nitrogen fertilization affects amounts, distribution, and quality of C inputs to roots, and might also have confounding effects on pH and initiate “priming effects” on soil organic matter degradation (Kuzyakov et al. 2000). Priming effects have often been observed in other systems, presumably due to the relief of nutrient limitations in the microbial community leading to accelerated decomposition rates; however, at other times no significant changes are observed. These observed differences may be explained in part by changes in the ratio of microbial cellulase to ligninase activities (Carreiro et al. 2000; Sinsabaugh et al. 2002). Whether these changes originate from shifts in the underlying microbial community or merely the activity of the existing community is not known.
Hypothesis 3: *Variation in activity by a suite of sentinel or indicator microorganisms common to all switchgrass stands is predictive of soil C sequestration and can be identified and monitored with soil metagenomic, transcriptomic, and proteomic methods.*

Sentinel species and biomarkers have been used by ecologists as surrogates for whole-system activities. While these sentinels may be arbitrarily selected, thorough investigation of specific organisms, processes, or products can shed light on ecosystem behavior and be used to develop more mature hypotheses that overcome the initial arbitrariness. Detection of sentinels or of sentinel processes can be based on molecular fingerprinting approaches, proteomic analyses, and direct process measurements. As with the second experiment, it is not known how nitrogen will influence the longevity of inputs. It is our aim to identify soil microbial community traits that may lend themselves to identifying a soil C accruing system.

**Technical Plan**

Research in this theme area will be organized into interrelated tasks to examine switchgrass effects on root-mycorrhizal architecture and production, fertilizer effects on microbial communities, and identification of sentinel microbial consortia per the stated hypotheses above. The specific methods that will be used for addressing Hypotheses 1 and 2 will overlap considerably and will be conducted simultaneously on existing experiments at the Milan site and in later years at the Fermilab site. We therefore have combined the tasks associated with these hypotheses. Hypothesis 3 inherently addresses questions associated with multiple site comparisons that build upon the results and mechanisms identified in the above task, and are included as a separate task.

**Task 3.1 Switchgrass Varietal and N Fertilization Effects on Mycorrhizal-Root Architecture and Microbial Communities**

**Subtask 3.1.1 – Root sampling, mass, depth, and phenolic and lignin concentrations**

Paired 5-cm diameter cores will be taken to a depth of 1 m or lithic contact and divided into 0- to 5-, 5- to 15-, 15- to 30-, and subsequent 30-cm depth sections and will be used for root sampling and mycorrhizal measurements (below). Soil cores will be placed in plastic bags, transported to the laboratory, and refrigerated (4°C). Roots will be subsequently washed free of soil, with a subset frozen at -20°C, and then freeze-dried (~50°C, 80 × 10^{-3} Mbar) for 48 h. Another subset of roots will be oven-dried at 60°C for 48 h and weighed for determination of root dry matter at each depth. Subsamples from the oven-dried roots will be then used to determine root phenolic and lignin concentrations (Iiyama and Wallis 1990). At Milan, Fermilab, and potential future satellite sites to be established, samples for microbial analysis will be collected at similar stages of plant development.

**Subtask 3.1.2 – Mycorrhizal colonization, composition, and extraradical hyphal biomass**

Root length and arbuscular mycorrhizal fungus (AMF) colonization will be quantified on a subsample of the freeze-dried roots. The subsample will be rehydrated, scanned using a digital flatbed scanner with digital image capabilities (WinRhizo, Regent Instruments, Quebec), and then separated into coarse (>2.0 mm diameter) and fine (<2.0 mm diameter) categories. Colonization will be quantified using a trypan blue stain. Approximately 150-250 mg of root will
be cleared in 10% KOH solution in a 100°C oven, followed by rinsing and soaking in slightly acidified deionized water. The roots will then be submerged in lactic trypan blue stain (1:2:2 lactic acid:glycerol:deionized water by volume, with 0.6 g trypan blue per liter) for a minimum of 2 h. Stained roots will be cut into 5-cm fragments, arranged lengthwise on a slide, and mounted in polyvinyl alcohol-lactoglycerol. The proportion of root length colonized by the AMF will be quantified by the magnified-intersection method at 200× (McGonigle et al. 1990).

The extraradical mycelium (ERM) of AMF biomass will be quantified by using hyphal in-growth bags made from nylon mesh (50-µm mesh, 15 × 5 × 2 cm) that allows mycelia to grow into the bag but excludes roots. The bags will be filled with 120 cm³ of acid-washed quartz sand (0.36–2.0 mm) and sealed. The in-growth bags will be placed at a depth of 0-15 cm at the beginning of the growing season. At the end of the growing season, the bags will be removed and returned to the laboratory, where the sand will be carefully mixed and extracted for ERM. This will be accomplished by using 53-µm- and 38-µm-diameter nested sieves in sequence to collect the mycelia. The collected mycelia will be freeze-dried and weighted. A subsample of the collected ERM will be analyzed for AMF marker phospholipid 16:1w5c and saprophytic fungal marker phospholipid 18:2w6,9.

Phylospecies of AMF will be determined by using ribosomal DNA (rDNA) isolated from free-dried switchgrass roots and from ERM obtained from the mesh in-growth bags. The basic method uses a polymerase chain reaction (PCR) to amplify DNA and cloning to separate multiple sequence fragments when necessary (Helgason et al. 1999). For this study, the AMF-specific primer (AM1) Helgason et al. (1998) will be used in conjunction with the universal eukaryotic primer (NS31) (Simon et al. 1993). These primers amplify the 18S portion of the small ribosomal subunit (SSU) region of rDNA and are commonly used in AMF community studies, as they selectively target the majority of AMF species and have only a few more primitive taxa as exceptions (Redecker et al. 2000). The PCR products will be cloned separately and screened with t-restriction fragment length polymorphism (t-RFLP) analysis, with several individuals from each t-RFLP type sequenced when possible.

Subtask 3.1.3 – Phospholipid fatty acid (PFLA) and neutral lipid (NLFA) profiles

Microbial community structure is defined on the basis of parallel phospholipid fatty acid (PFLA) and neutral lipid (NLFA) profile analysis. These profiles are obtained using a root subsample that is freeze-dried (–50º C, 80 x 10⁻³ Mbar) for 48 h and then ground. Lipids will be extracted from a 30 mg in a single-phase mixture of chloroform, methanol, and phosphate buffer (pH 7.4) in a ratio of 1:2:0.8. After 3 h, water and chloroform will be added to separate the mixture into polar and nonpolar fractions, and total lipids will be extracted from the nonpolar chloroform phase. The PLFAs will be separated from other lipid classes by using silicic acid column chromatography (Vestal and White 1989; Zak et al. 1996). The PLFAs will be then methylated by using a mild-alkaline solution and the samples frozen until analysis.

Prior to analysis, PLFAs will be thawed and dissolved in a 20-ng µL⁻¹ solution of FAME 19:0 (Matreya Inc, PA) in hexane, as an internal standard. PLFA separation is by high-resolution fused-silica capillary gas chromatography (GC), using an HP 6890 GC, with an HP7683 autosampler (Agilent Technologies, Palo Alto, CA). A 3-m HP-5MS column is used, with hydrogen as the carrier gas at a constant flow rate of 4.0 mL min⁻¹. A 1 µL splitless injection will
be made for each sample, with the inlet temperature set at 230°C, and the inlet purged at 47.0 ml min\(^{-1}\), 0.75 min after injection. The oven temperature will be held at 80°C for 1 min, increased at a rate of 20°C min\(^{-1}\) to 155°C, and then increased at 5°C min\(^{-1}\) to a final temperature of 270°C and held for 5 min. Detection of PLFAs will be by flame ionization at 350°C. PLFAs will be identified by retention time in comparison to known standards, and quantified using the 19:0 internal standards.

Subtask 3.1.4 – Cellulase activity

We will measure soil cellulase activity for each sample in this study using laboratory incubations amended with high-molecular-weight carboxymethyl cellulose (Aldrich #419338) that is also treated with a microbial growth inhibitor (toluene). Low-level additions of toluene allow for the measurement native soil enzyme activity without the confounding affects of microbial growth and turnover. Reducing sugars released as a result of cellulase activity will be extracted after 24-h incubation and measured using the colorimetric method of Deng and Tabatabai (1994). Additional measurements of overall microbial biomass will be made via chloroform fumigation extraction. Specific rates of overall cellulase activity can then be calculated on a per unit biomass basis.

Subtask 3.1.5 – Soil sampling and nucleic acid extraction

Molecular biological methods for nucleic acid and protein analysis of soils are recent innovations undergoing constant modification and improvement. Because of this and because large soil volumes are required relative to other chemical, biological, and biochemical analyses, special sample collection and handling methods are required.

Four surface soil samples (30 cm deep; 30 cm apart) will be combined to yield a single composite sample at each replicate sample plot. Soils will be thoroughly mixed in a large plastic bag and immediately sieved through a 6-mm sieve. The sieved soil will be sub-sampled and either immediately frozen for genomic analysis or placed in a cooler for supporting soil process studies. After collection, soils will be stored in the lab at -80 °C until extraction and use. Nucleic acids (DNA, RNA) will be extracted using a physical disruption method according to Hurt et al. (2001) or equivalent approaches best suited to our study soils. This method has proved reliable for both sediment and soil samples from a wide variety of soils in this and other projects. This one set of extractions will serve as the template for all further nucleic acid analyses allowing for coordinated investigation of all community and population parameters.

Subtask 3.1.6 – rDNA and functional gene sequence analyses

Clone libraries will be constructed for the 16S rRNA for bacteria and 28S rDNA for fungi, the denitrification genes for nitrite reductase (nirS/K), nitrogen fixation genes (nifH), and nitrification genes (amoA), according to previously described methods (Schadt et al. 2003; Yan et al. 2003). Briefly, respective genes will be PCR-amplified from extracted DNA, cloned into plasmid vectors, transformed into \textit{E. coli}, and the inserted DNA sequenced using vector-based and internal primers on an ABI3730 sequencer. Sequences will automatically assembled using established algorithms and initial comparisons and operational taxonomic unit (OTU) assignments made using the CLASSIFIER and LIBRARY COMPARE programs of the
Ribosomal Database Project (Cole et al. 2005) and BLAST comparisons (Altschul et al. 1990). Further OTU comparisons and diversity and community description statistics will performed using DOTUR (Schloss and Handelsman 2005) and TREECLIMBER (Schloss and Handelsman 2006).

**Subtask 3.1.7 – Microbial metagenomics and fingerprinting**

A rapid fingerprinting (Automated Ribosomal Intergenic Spacer Analysis: ARISA) of each soil will be conducted for both bacteria and fungi. This may be repeated on a calendar basis; that is, early spring, mid-summer, and late summer. Fingerprints will be compared to find bands that occur consistently at each site. These may need to be constrained to dominant members, and it will likely be useful to note bands unique to each site and reserve them for possible future study.

The consistent bands will be excised and sequenced through a joint project to be proposed to the DOE Joint Genome Institute (JGI). This sequencing will expand upon the information that can be obtained from the ORNL functional gene array and allow the array to be expanded in the future. Two complementary sequencing activities will be pursued at the Fermilab plots.

1. Sequencing of metagenomic DNA: The community metagenome contains information about all of the organisms in the soil, whether active, minimally active, or dormant. To the best of our current understanding of soil microbiology, the DNA metagenome in soil should be relatively constant over the 5-year research cycle proposed, and likely beyond that. This “baseline information” will be compared with messenger RNA- (mRNA-) derived sequence as explained below.

2. Sequencing of cDNA from N-cycling and C-cycling genes: We will extract mRNA, use degenerative primer sets targeting 10 to 20 N-cycling and C-cycling gene families, and sequence the resulting cDNA. Preliminary plans are to use soils prior to switchgrass planting, and then soils collected from the different switchgrass varieties in years 1 and 2. This will provide information about the actual C- and N-cycling activities that are being conducted by the soil microbial communities under the treatment conditions. From sequence results, index organisms will be selected based on available information or absence of information.

In an evolution of t-RFLP fingerprinting conducted on soils of the Fermilab Tallgrass Prairie Restoration Chronosequence, we propose to use a faster, automated fingerprinting approach to screen the sites for bands of interest. ARISA and its non-automated counterpart (RISA) were developed to analyze soil microbial communities (Borneman and Triplett 1997; Fisher and Triplett 1999). These two approaches can be used together to provide rapid fingerprints for comparison and permit excision of target bands for sequencing and identification (or further study). We will use domain-specific primers to target the fungal and bacterial domains (Gleeson et al. 2005), and as published sequences become increasingly available, we may endeavor to develop (A)RISA primers specific for the order Glomales.

**Task 3.2 Identification of Sentinel/Indicator Microbial Signatures**

In this task we will develop and employ additional methods that build upon the molecular methods and results from the above task, in order to identify those species and/or molecular signatures that may be common indicators of C accruing systems.
Subtask 3.2.1 – Microbial functional analysis with functional gene microarrays

Microarray technology previously developed in CSiTE will be used to access changes in specific community functions across sites and management conditions. Because of low detection limits, current functional gene array (FGA) methods require large quantities (2-5 µg) of environmental DNA. The method we developed based on rolling circle amplification (Hafner et al. 2000) for isothermal, random and unbiased amplification of genomic DNA from environmental samples will be used. This technique allows direct comparison of microbial populations starting from as little as 1-10 ng of DNA (Wu et al. 2006). All amplified DNA from each sample will be used for fluorescent labeling using random primers and Klenow fragments (Invitrogen, Carlsbad, CA). The labeled target will be then hybridized with FGA arrays using the methods developed previously (Rhee et al. 2004; Wu et al. in press). Microarray scanning and initial data processing will be carried out as previously described (Wu et al. 2006).

A ScanArray® 5000 Microarray Analysis System (PerkinElmer, Wellesley, MA) will be used for scanning microarrays at a resolution of 10 µm. Scanned image displays will be saved as 16-bit TIFF files and analyzed by quantifying the pixel density (intensity) of each spot using ImaGene™ version 6.0 (Biodiscovery, Inc., Los Angeles, CA). Mean signal intensity will be determined for each spot. An integrated tool has recently been developed for data preparation and statistic analysis and will be employed here. Using this tool, the hybridization signal intensity data of the three probes of each gene is first pooled together for positive detection determination on the basis of majority rule (spots detected in at least 2 of 3 probes) and then be averaged. Outliers are then detected and removed at p<0.01. When the absolute value of a data point minus the mean is larger than 2.90 σ, this data point is determined as an outlier and removed from further analysis. Potential environmental function measured by FGA hybridization with sediment community DNA samples will be analyzed against a suite of geochemical measurements of the sites as outlined in Theme 1. Microarray hybridization data will be also used to determine microbial diversity, composition and dynamic by calculating diversity indices, cluster analysis, and gene network analysis.

Subtask 3.2.2 – Ecoproteomic approaches

To overcome both of organism plurality and misidentification of proteins in complex samples, we plan to perform a dual, very-specific simplification/enrichment of the proteome, which will include first an isoelectric point fractionation of the peptides from 3.5-4.5 pH units by using a prototype device from Agilent Technologies called off-gel electrophoresis (Heller et al. 2005), followed by a cysteine peptide isolation. Both of these techniques are highly specific (i.e., >99%), and the estimated proteome simplification is estimated to >100-fold. At the same time a significant portion of the proteome of each organism is represented by those peptides. As the likelihood of having peptides other than the ones that we have isolate (i.e., cysteine-containing peptides with a pI of <3.5-4.5), the database that we can search against can be equally decreased to contain only these peptides, leading to faster searching times and low false-discovery rates.

This approach is supported by other sources at PNNL, not CSiTE; however, CSiTE will leverage this capability by providing complex samples for the testing of the ecoproteomic approach.
**Linkages to Other Themes**

The work in this theme will be closely tied to Themes 1, 2, and 4 to comprehensively understand the relative contributions of each of these important drivers of C sequestration in soils. In particular, as aggregate fractions of particular interest are identified in Theme 2, we will investigate these fractions for any distinguishing microbial functional or structural differences. We will use the fingerprint-sequencing and microarray approaches outlined above as appropriate in these efforts. Finally, results from this theme will directly inform Theme 6 model development.

**Expected Results**

The research addressed in the Microbial Community Function and Dynamics Theme will better quantify switchgrass varietal influences on soil C sequestration by:

- Determining the proportion of microbial C inputs derived from mycorrhizal fungi vs. saprophytic microbial processes
- Determining the relationship between root inputs, root morphology, root lignin content, and microbial structure and function (Theme 1)
- Evaluating the potential for specific microbial groups or subgroups to act as sentinels of aggrading or degrading systems (Theme 6)
- Determining the relationships of changes in microbial community structure and function to physical protection of soil C and soil structure (Themes 2 and 6)
- Integrating expression of soil proteins with changes in humification processes (Theme 4)
- Establishing a system of voucher samples for future Genomics: GTL studies in C sequestration.
**Schedule and Milestones**

Research in Theme 3 will consist of parallel methods development and application to the field experiments at Milan and Fermilab. While all subtasks are shown in the table as ongoing from Year 1, the rate of progress is dependent on available funding and successful development of leveraged collaborations such as with the DOE JGI.

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**Theme 4: Humification Chemistry**

**Purpose and Objectives**

The purpose of this theme is to develop a fundamental understanding of humification chemistry to guide the selection of potential manipulations that will enhance storage of organic C in soils under switchgrass cultivation. Specific objectives include 1) identifying the key chemical factors, such as pH, Eh, black C, Ca content, and N content of inputs, that can be manipulated economically to enhance C sequestration; 2) determining the optimal levels of these chemical factors for soils under switchgrass cultivation; and 3) developing measurement protocols that can be used to rapidly assess the current status of humification; that is, whether humic fractions are aggrading or degrading in a soil. Work under this theme primarily addresses the second overarching science question—“What are the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil, and how do they interact in space and time?” By providing information about these mechanisms this theme also contributes to questions III, IV, and V, which focus on movement and distribution of C in soils as well as the identification and implementation of sustainable and economical practices to enhance soil C.

**Background and Science Questions**

Our previous work suggests that co-catalysis of humification occurs by three mechanisms involving physical stabilization of tyrosinase, direct oxidation of the monomers, and promotion of the oxidation and condensation steps by alkaline pH (Amonette et al. 2003, 2004; Palumbo et al. 2004). Although tyrosinase activity is greatest at neutral pH, the large pH dependence of the condensation step drives the overall reaction to maximum rates under alkaline conditions. Following this hypothesis, liming of soils to slightly alkaline pH should enhance net C sequestration. Raising soil pH, however, is also likely to affect the activity of enzymes other than tyrosinase, such as various hydrolases. The hydrolase enzymes promote the breakdown of organic matter, and so the relevant question becomes one of whether the balance between humification and decomposition changes as the pH is altered. Preliminary evidence from the intermediate-scale experiment at the Santee Experimental Forest in South Carolina suggests that the balance does change and that decomposition increases relative to humification as a result of raising the pH. As a consequence, we broadened our enzyme analysis capabilities to allow monitoring of a suite of enzymes including tyrosinase, peroxidase, phosphatase, sulfatase, and other hydrolases by adapting methods of Marx et al. (2001) and Sinsabaugh et al. (1992) for microplate analysis. In addition, raising pH tends to decrease sorption of DOC to soil surfaces and thereby promotes leaching of DOC into deeper portions of the soil profile where adsorption can occur under acid conditions (Theme 5). Some evidence for this effect was also observed in the Santee experiment, confirming that two possible “desequestration” mechanisms (hydrolysis and leaching) could occur as a result of raising soil pH by alkaline fly ash amendments.

In contrast to the uncertain impact of alkaline pH, aspects of our previous work (Amonette et al., 2003) suggest that the presence of incompletely burned coal in fly ash can have a significant positive impact on the humification reaction, presumably by providing an organic surface where humic monomers preferentially accumulate and consequently react. In parallel with this observation is the renewed interest in the use of wood charcoal as an amendment to increase soil fertility while at the same time sequestering C (Glaser et al. 2003; Marris 2006). Pairing these
two sets of observations, we think it likely that the physical stabilization of enzymes and humic monomers by charcoal-like materials (i.e., black C, whether from wood or coal), promotes humification. Accordingly, a strong focus of our research in this theme area will be on further understanding the role of black C on humification in a switchgrass cropping system.

The key scientific questions that will drive our research in the humification chemistry theme are:

- How do macroscopic solution-phase soil-chemical properties such as pH and redox status influence the net rate of humification?
- How do different types of soil surfaces (black C, minerals) influence humification?
- How do different qualities of soil-C inputs (e.g., form and amounts of N resulting from different switchgrass fertilization regimes) influence humification?
- How can we readily and rapidly determine whether humification is progressing or regressing in a soil at a particular point in time?

Hypotheses to Be Tested

Previous research suggests that soil pH, Eh, black-C content, Ca content, and the N content of C inputs are five key chemical variables affecting the rate and direction of humification. Several testable hypotheses arise from consideration of these variables:

**Hypothesis 1:** Within each soil horizon, optimal levels of pH and Eh can be defined for which extracellular enzyme production and activity yield a maximum net rate of humification.

**Hypothesis 2:** Within each soil profile, optimal levels of pH and Eh in the various soil horizons can be defined for which the balance between humification rate and intrasolum transport/storage of DOC yield a maximum net rate of C sequestration.

**Hypothesis 3:** High levels of black C and exchangeable Ca promote humification.

**Hypothesis 4:** High N content of C inputs promotes humification.

**Hypothesis 5:** The balance between oxidase and hydrolase enzyme activity in a soil can be used as an indicator of current humification status, with higher oxidase:hydrolase ratios indicating aggradation and lower ratios indicating degradation.

Our work may identify other variables with greater impact on humification rates. Similar testable hypotheses will be articulated and tested for these variables if and as they are identified.

**Technical Plan**

Work in this theme will be organized into four major tasks. The first task will involve bench-scale laboratory studies using soils from the field experiments and will be focused on identifying the key chemical factors and their optimal levels for enhanced C sequestration. During the second task, we will select three promising manipulations based on the key chemical factors and
test them at the field scale in the Fermilab and Milan manipulation experiments. In the third task, we will identify measurable parameters and algorithms that can be implemented in the EPIC model to predict the progress of the field manipulations. In the fourth task, we will develop and test enzyme assays and persulfate oxidation as possible ways of determining whether the humification status of a soil is aggrading or degrading. Detailed descriptions of the work to be performed in each task follow.

**Task 4.1 Bench-Scale Laboratory Studies**

The intent of this task is to identify key soil-chemical parameters that can be manipulated to enhance the net rate of humification, determine their optimum values, and then recommend up to three field manipulations for potential implementation depending on available resources. This subtask will extend for up to 3 years, and thereafter the focus of the humification chemistry task will be entirely on the field-scale manipulations, integration with the EPIC model, and measurements of humification status.

Soils will be collected from the Milan N-fertilizer experiment plots and elsewhere depending on available funding. Samples will be segregated as a function of depth per the overall Theme 1 sampling plan (0-5, 5-15, 15-30, 30-60, and 60-120 cm) and either characterized immediately or stored at 4°C until use. Parameters to be measured using standard methods (Sparks 1996) include pH, C content (organic, inorganic), exchangeable cations including Ca and Mg, cation and anion exchange capacities, texture, and N content. Additional parameters will be measured by reputable methods in the literature or developed in-house. Examples of these additional parameters (methods) include black-C content (Haumeier and Zech 1995), C quality (Task 4.4, rate of oxidation by persulfate), mineralogy (Amonette and Zelanzny et al. 1994), oxidase/hydrolase activities (Marx et al. 2001; Sinsabaugh et al. 1992), and fungal activity (Bailey et al. 2002). We expect to see a gradient in several chemical properties in response to the rate of N fertilization. These gradients will provide some experimental space in which to determine the relative importance of the chemical properties and possibly the optimum levels. Additional gradients (e.g., liming, addition of black C) may be imposed in the laboratory.

Soils will be subjected to two types of experiments to determine the impact of chemical properties on humification rates. The first experiment will focus on determining the differences in the oxidase and hydrolase enzyme activities as chemical properties vary. We will add commercially available enzymes to the soils and determine their sorptive properties and subsequent enzymatic activities. Based on the relative changes seen, we will estimate the probable effect on humification. This experiment will be used to screen the large suite of possible combinations of chemical properties and to select the most promising chemical properties for the more intensive (and realistic) test in the second type of experiment.

The second experiment will involve incubations of soil microcosms after addition of fresh C. The fresh C will be added as a solution of phenolic and amino acid monomers (as in our previous work) or, in separate treatments, as a partly digested switchgrass residue using switchgrass collected at Milan at harvest from plots receiving the standard 67 kg/ha fertilizer regime. The course of the C transformations will be followed over several weeks/months and the rate of humification estimated from changes in the quantity and quality of the C in the soil measured by the degree of oxidation in persulfate solution. Other soil properties, such as pH, total organic C,
N content, and fungal activity (Bailey et al. 2002) will be monitored as well. Full C and N balances for this set of experiments will be undertaken to ensure proper GHG accounting.

Based on the results of these two experiments, and in concert with the results from other theme areas, we will recommend soil chemical manipulation experiments to be undertaken at the field scale.

**Task 4.2 Field Manipulations**

This task will implement soil-chemical manipulations recommended in Task 4.1 at Milan and other sites as appropriate. The first year will be spent gaining familiarity with the field site(s), gathering baseline field information, and gaining an understanding of the practicality of various types of manipulations. We expect to have at least one soil-chemical manipulation recommendation from Task 4.1 after the first year of laboratory research. Thus, early in the second fiscal year, the first field manipulation will be designed and preparations made for its implementation in the spring. To the extent possible, subplots within existing switchgrass plots will be used to maximize experimental flexibility and leave room to accommodate additional manipulations in later years.

During the field manipulations, in concert with the other themes, soil samples will be collected to monitor changes in soil C quality and quantity, as well as other selected soil-chemical properties found to be important as a results or research in Task 4.1. The impact of these manipulations on switchgrass yield, stand density, and net GHG balance will also be followed.

As the manipulations have not been defined yet, it is difficult to estimate the length of this task. However, we anticipate at least two growing seasons of data collection for each recommended manipulation to determine how well the manipulation performed from a humification standpoint. One manipulation will coincide with the intensive measurement by Themes 1, 2, 3, and 5.

**Task 4.3 Module Development for EPIC**

This task is critical to organizing and extending the knowledge gained from Tasks 4.1 and 4.2 for use in other locations and regions. The intent is to determine the measurable parameters needed and to develop, in concert with Theme 6, a module in EPIC for each of the recommended soil-chemical manipulations that can simulate their impact on C sequestration.

Our approach will involve three stages for each possible manipulation. First, we will examine the existing capabilities of the EPIC code and identify the new parameters and routines that may be needed. Next, we will work with Theme 6 to construct a putative model to simulate the probable impact of the manipulation on soil C levels and other relevant properties. Then, once the manipulation has been implemented in the field, we will compare actual field results with module predictions and use these to further refine the module.
Task 4.4 Humification Status Assays

The activities in this task will attempt to fill a critical need to measure the humification status of a soil quickly, reliably, and inexpensively. We define humification status in terms of whether the soil is aggrading C or degrading C, and assume that the dynamic C status also reflects the total GHG status of the soil.

We will explore two different types of assays. The first will focus on measurements of the relative potential activities of the oxidase and hydrolase enzymes in the soil. These measurements can be done fairly quickly and inexpensively using modern titer plate instrumentation. Our approach will involve various ways of measuring the enzyme activity (extractions, \textit{in situ}, etc.). The second assay we will explore is the determination of C quality by the rate of oxidation in persulfate solution. We will try various modifications of the primary method to try and develop a rapid and robust method for fractionating soil C into labile and recalcitrant pools. If successful, these humification-status assays might find use as characterization parameters in EPIC as well as in verification of soil C for C credit transactions.

Linkages to Other Themes

Humification Chemistry links most closely to Theme 2 (Soil Structural Controls) and Theme 3 (Microbial Community Function and Dynamics), as these two themes focus directly on the physical and biological processes leading to humification. Other linkages include Theme 5 (Intrasolum Carbon Transport), because of the potential impact of soil chemical manipulations on sorption properties of DOC, Theme 1 (Soil Carbon Inputs) because of the influence of black C and nitrogenous C on the humification process, and Theme 6 (Mechanistic Modeling) because of the need to develop modules in the EPIC code to simulate the impact of soil chemical manipulations.

Expected Results

We expect work in this theme area to advance sequestration science in the following ways:

- Improved fundamental understanding of the impact of soil chemical properties on humification rates.
- Selection of potential chemical manipulations to enhance C sequestration under switchgrass.
- Improved ability to simulate impact of changes in soil chemical properties on humification rates using EPIC (Theme 6).
- Development of quick, reliable, inexpensive method(s) to determine humification status of soils (i.e., aggrading, degrading).
- Quantitative assessment of aggregate development (Theme 2) and microbial activity (Theme 3) on humification processes and enzyme activities, and adsorption/desorption potential (Theme 5).
Moreover, the results obtained will help us answer several of the overarching scientific questions listed in the introduction. Most important, we will help provide a much better understanding of the fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil (Question II). Other overarching questions to which this work will contribute include identification of the processes that control C movement and distribution (Questions III and IV) and of the methods and practices that can be used to sustainably and economically enhance C sequestration in soils (Question V).

**Schedule and Milestones**

The proposed schedule represents a phased approach to the four tasks outlined above. Milestones are listed for each task, and the duration of the subtask leading to the milestone is shaded (e.g., the identification of the first manipulation will be completed by the end of the first year of work).

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Theme 5: Intrasolum Carbon Transport

Purpose and Objectives

The purpose of this theme is to test the hypothesis that deep subsurface soils can accumulate organic C and that accumulation will be affected by soil type, C inputs, and chemical effects induced by the addition of fertilizers and elements to enhance humification at the surface. The effort involves the immediate use of the Milan fertilizer experiment in years 1-3. In years 3-5 we will also use the Fermi manipulation experiment once switchgrass has been established. Our investigations are highly interactive with the other themes of this proposal by quantifying the impact of coupled hydrologic and geochemical processes on subsoil C and N dynamics and the processes that control enhanced organic C sequestration. This experimental information will then be used in Theme 6 to develop predictive models to assess subsurface C and N processes as a function of above ground manipulations and at larger scales. The specific objectives of this theme are to:

- Quantify the magnitude of enhanced solid- and solution-phase C accumulation through soil profiles as a function of different fertilization rates, C inputs, humification additions, and soil types,

- Quantify the impact of coupled hydrologic, geochemical, and microbial (Theme 4) processes on the fate and transport of solubilized organic C and N through the soil profile.

- Quantify the chemical nature of the sequestered C and the mechanisms responsible for immobilization by the solid phase.

This theme is focused on quantifying the belowground movement and sequestration of organic C that is derived from switchgrass field manipulations and thus directly addresses CSiTE’s overarching questions II through IV, which seek to understand a) the fundamental physical, chemical, and microbial processes controlling C accrual and storage in soil, b) the processes that control C movement and distribution through the soil profile, and c) how these fundamental processes control soil C dissemination across the landscape as a function of time.

Background and Science Questions

As noted previously, widespread, highly developed mature soils such as Alfisols and Mollisols, which will be used in the proposed research, have deep soil profiles that have a tremendous capacity to sequester organic C. The physical and chemical properties of the lower horizons (B-horizons) within these soils are ideal for maximizing organic C sorption to the solid phase (Sibanda and Young 1986; Jardine et al. 1989a,b, 1990b; McCarthy et al. 1993; Benke et al. 1999). This C pool (passive C pool) is significantly less dynamic than the C in upper soil horizons because it is strongly stabilized on mineral surfaces with estimated turnover times of millennia and longer (Trumbore 1997). Therefore, methods for enriching subsoil organic C can be a favorable technique to sequester appreciable quantities of C.
Subsurface conditions that create a favorable environment for enhanced carbon sequestration are: 1) a combination of high to moderate temperatures and large amounts of precipitation that enhance organic C decomposition rates and transport through the soil profile; 2) subsoil B-horizons with suitable mineralogical components that strongly immobilize organic carbon; 3) subsoils with acidic pH and geochemical features for maximizing C sorption; 4) soils that are highly structured and have abundant microporosity that enhances solute attenuation; and 5) soils that are mature with deep profiles, thus enhancing C residence time prior to groundwater interception. These conditions are met very well in regions dominated by Alfisols, such as those at Milan, and Mollisols, such as those at Fermi.

Our goal is to test and resolve the hypothesis that deep subsurface soils can accumulate organic C as a result of near-surface manipulations such as land-use change and variations in fertilization amount. Our investigations are highly interactive with the other themes of this proposal by quantifying the impact of coupled hydrologic and geochemical processes on subsoil C and N dynamics and the processes that control enhanced organic C sequestration.

The research is driven by the following science questions:

- How do different switchgrass management strategies (e.g., fertilization levels) influence the fate, transport, and sequestration of dissolved and solid-phase organic C through the soil profile?

- In what capacity do the different soil horizons act as sources or sinks for DOC as it moves through the profile?

- How does preferential flow and diffusion into the soil microporosity influence the transport and sequestration of DOC through the soil profile?

- How do the chemical nature and form of the DOC change as it moves through the soil profile, and how do these changes influence sorption and microbial decomposition processes and thus sequestration?

- How bioavailable are the dissolved and surface-bound organic phases in the different soil horizons, and what are the rates and mechanisms associated with the degradation (Theme 4)?

- How significant are C, N, and P losses through the soil profile as a result of fertilization for the purposes of switchgrass crop productivity and non-sustainability?

- Does the addition of fertilizer (e.g., N and P) drive DOC deeper into a soil profile, and is this DOC sequestered by the solid phase as a passive C pool?
Hypotheses to Be Tested

Proposed research within this theme is driven by the following hypotheses:

**Hypothesis 1:** Organic C solubilization in the near surface (i.e., DOC) will be transported vertically through the soil profile and sequestered by clay and Fe-oxide rich subsoils.

**Hypothesis 2:** Hydraulic and concentration gradients will drive DOC preferentially into micropores where it will be physically protected from microbes that cannot access this pore regime.

**Hypothesis 3:** Preferential flow during large storm events will diminish the potential for C sequestration in the subsoil because it will significantly bypass the soil matrix and because it will decrease C resident times in the soil profile.

**Hypothesis 4:** The significance of denitrification versus dilution and the movement of old versus new C and N through a soil profile can be quantitatively assessed through evaluation of multiple stable isotopes.

Technical Plan

Work within this theme will be organized into three major tasks. During the first task, plots will be selected at the Milan and Fermi sites for instrumentation and characterization. The second task will quantify the hydrologic and geochemical processes influencing subsurface C and N dynamics. During the third task, we will enhance the mechanistic rigor and predictive capacity of the EPIC model.

**Task 5.1 Plot Selection, Instrumentation, and Characterization**

Our approach involves multi-porosity sampling of key subsurface solutes coupled with a nonreactive tracer to quantify the movement of indigenous dissolved organic C and N through the various soil profiles within six plots at the Milan fertilizer experiment and six plots at the Fermi manipulation experiment. Within the Milan fertilizer experiment, three fertilization rates will be considered: 0, 67, and 213 kg N/ha (0, 60, and 180 lb N/acre). Because of resource constraints, only two of the three blocks will be sampled (two reps per treatment). The Milan plots will be instrumented and characterized in early spring 2007 and intensively monitored that year and in Year 2 (simultaneously with the intensive monitoring of themes 1-4) and less intensively monitored in years 3-4. Early in the spring of Year 3 we will instrument and characterize six of the Fermi plots; again doing three treatments with two reps. The three treatments we choose will depend in part on the humification treatments that are selected within Theme 4. We will pick one humification treatment and its corresponding control and the opposing cultivar treatment of that control. For example, if the humification treatment was lowland cultivar, 134 kg N/ha, and the addition of black-C N, we would also instrument the lowland cultivar, 134 kg N/ha, and no black-C treatment and the upland cultivar, 134 kg N/ha, and no black-C treatment. We will intensively monitor the Fermi plots in years 3 and 4 and less intensively in Year 5.
Each of the 12 plots (six at Milan and six at Fermi) will be instrumented with four tension lysimeters and four tension-free lysimeters. Two of each type will be placed within the A- and B-horizons of the soil profiles (eight samplers per plot). Tension-free lysimeters provide a measure of solute fluxes through macro-and mesopores, while tension lysimeters provide a measure of solute fluxes through the microporosity of the media. Using a multi-porosity porewater extraction approach will quantify 1) rapid, preferential C and N movement through the profile; and 2) the importance of matrix storage and release from the soil microporosity. Tensiometers and time domain reflectometer rods will also be installed to quantify matrix potential and water content as a function of depth.

We will characterize the plots by measuring horizon-specific parameters, such as bulk density, water retention functions, C and N transformation rates, soil temperature, solid-phase pH, carbon content, Fe-oxide content, and particle size, using standard methods. These horizon-specific samples will come from the sampling efforts within Theme 1. We will also perform tension infiltrometer measurements to assess the infiltration rates of the various soils as a function of tension. This information will provide a portion of the hydraulic conductivity that is useful for quantifying macro-, meso-, and micropore flow. Such measurements of in situ hydraulic conductivity will assist in modeling the flux of solutes and C.

**Task 5.2 Quantifying Hydrologic and Geochemical Processes Influencing Subsurface C and N Dynamics**

During the first year after lysimeter installation at Milan (spring 2007), dilute nonreactive Br tracer will be evenly applied over each of the instrumented areas using a backpack sprayer (i.e., distributed initially to the soil matrix porosity) in an effort to quantify the hydrodynamics of each site during storm events. The same application will be performed at Fermi in FY 2009. During the intensive years of monitoring (years 1 and 2 at Milan and years 3 and 4 at Fermi) solution samplers will be monitored during all storm events and analyzed for Br, DOC, N, P, inorganic anions and cations, and pH. Monitoring frequency during the less-intensive monitoring years (years 3 and 4 at Milan and Year 5 at Fermi) will depend on what we find during the intensive monitoring years. A variety of chemical and physical characterization techniques will be used to quantify the chemical nature and form of the solution-phase DOC (e.g., size fractionation, hydrophobicity, aromaticity).

Numerous selected samples will also be analyzed for stable isotopes ($^{15}$N/$^{14}$N, $^{13}$C/$^{12}$C, $^{18}$O/$^{16}$O) to quantify denitrification rates and the movement of old versus new C and N through a soil profile. If correlations between $\delta^{15}$N and $\delta^{18}$O values and nitrate-N concentrations are absent or positive, then denitrification is not a likely contributor to nitrate attenuation because denitrification leaves residual nitrate enriched in $^{15}$N and $^{18}$O. The former associations may indicate dilution as the process reducing nitrate levels in the subsurface. When denitrification is a significant process of nitrate attenuation, both $\delta^{15}$N and $\delta^{18}$O values will be negatively correlated with nitrate concentrations with a well-defined trajectory in the $\delta^{15}$N-$\delta^{18}$O diagram, reflecting an increasing abundance of $^{15}$N and $^{18}$O in residual nitrate as a function of time or distance from the source (Spalding et al. 1993; Kendall 1998). $\delta^{15}$N of soil N$_2$ also progressively deviates from that of atmospheric N$_2$ (0‰) with an increasing contribution from denitrification. Likewise, $\delta^{13}$C-values increase with soil depth along a continuum of organic matter decomposition. Old soil C
adsorbed to silt and clay is more enriched in $^{13}$C than recently added C. Thus, using the isotope approach we will be able to track old versus new C that has resulted from varying treatments.

If resources permit, we will consider subsurface soil gas monitoring systems using a passive diffusion approach for assessing pore space CO$_2$, N$_2$, and N$_2$O that are formed as a result of denitrification and C assimilation. This will involve standard gas chromatographic analysis. Characterization of selected soil hydraulic, physical, chemical, and mineralogical parameters as related to C and N dynamics and for use in mechanistic models will also be performed in this task. Bulk soil samples from each plot will also be characterized for organic C sorption isotherms as a function of depth.

**Task 5.3 Enhancing the Mechanistic Rigor and Predictive Capability of the EPIC Model**

In concert with Theme 6 (Task 6.6), a systems modeling software package such as STELLA® or MathLab® will be used to capture our process-level understanding of subsurface C and N dynamics and to configure the various coupled hydrologic and geochemical processes that control organic C and N fate and transport. The importance of preferential flow, matrix diffusion, mass transfer kinetics, sorption, degradation, etc. will be considered for both the A and B horizons of these systems and their interaction on the system as a whole. The new conceptual framework outlined in the systems model will be incorporated into the EPIC model. Also, physical and chemical parameters derived from research in this theme will be used to parameterize EPIC. Horizon-specific parameters, such as bulk density, water retention functions, C and N transformation rates, soil temperature, solid-phase pH, carbon content, Fe-oxide content, and particle size, will also be incorporated into the EPIC model in an effort to enhance the predictive capability of the model for assessing various manipulation strategies on C storage, regional effects, and other predictive methods of interest. Both site-specific and generic data from previous regional-scale research will be available for this task.

**Linkages to Other Themes**

Theme 1 is essential to this theme in that the rates and mechanisms of C and N inputs must be known to assess fate and transport processes through the soil profile. Coupling these two themes provides a needed mass balance for C and N dynamics within the soil solum. Theme 2 complements this theme by providing quantitative information on the stability of near-surface aggregates and how this impacts C and N dynamics through the entire soil profile. Coupling the two themes may provide knowledge as to how one builds deeper and denser solid-phase organic pools.

Theme 3 will benefit significantly from the approaches offered by this theme in that belowground processes are rigorously followed and thus the impact of humification manipulations on enhanced C sequestration can be quantitatively assessed. This theme requires the research endeavors of Theme 4 to quantify the bioavailability of solution and solid-phase C and N through the soil profile.

Theme 5 will provide essential hydrologic, physical, and chemical parameters needed to mechanistically model C and N dynamics through the soil profile as will be done in Theme 6. Water balance, solute mass balance, reaction chemistry, hydrologic and transport properties, and
microbial assimilation rates and mechanisms will be provided by this theme for use in the Theme 6 modeling endeavor.

**Expected Results**

Theme 5 will provide a quantitative understanding of coupled hydrological and geochemical subsurface processes of preferential flow, matrix diffusion, mass transfer kinetics, sorption, and degradation - useful for improving physical and chemical parameters in EPIC (Theme 6). It will also integrate the results of organic matter input measurements (Theme 1) and microbial assimilation rates (Theme 3) to quantify relationships between inputs, microbial communities, and DOC as affected by soil hydrology and chemical properties. The integrated theme strategy will not only provide an improved understanding and predictive capability of processes controlling C movement and storage in soil profiles, it will provide a fundamental understanding of how these processes control soil C dissemination across the landscape as a function of time.

**Schedule and Milestones**

The proposed schedule is designed to have the intensive measurement of solute transport and chemistry coincide with the intensive field measurements of the other four experimental themes. We will focus on the Milan fertilizer experiment in years 1-2 and on the Fermi manipulation experiment in years 3-4. Characterization of Milan soil structure will occur in Year 1 and Fermi soils structure in Year 3.

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<td>5.3 Support of EPIC (especially Task 6.6)</td>
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Theme 6: Mechanistic Modeling

Purpose and Objectives

The purpose of the mechanistic modeling theme is to improve our capability to mechanistically model/forecast soil organic C dynamics at local, regional, and national scales; facilitate the research of the five experimental themes; and enable evaluation of soil C sequestration technologies and the tradeoffs and complementarities of bioenergy and soil C sequestration. We will achieve this through improvements to and applications of the EPIC model. Our most significant improvement is that we will replace the current SOC decomposition submodel, which, like all widely used SOC decomposition models, is currently based on the use of conceptual SOC pools described by first-order kinetics and does not consider physicochemical protection. While this submodel is much improved by CSiTE (Izauralde 2006 a,b) it is still based on conceptual SOC pools. We will insert a totally new SOC decomposition submodel that combines measurable pools based on aggregate size and mineral association with differential equations capturing the relationships between these entities. This transition from conceptual pools to measurable pools characterized by physicochemical protection will enable a much greater degree of mechanistic process to be captured in the model and thereby enhance our ability to forecast soil C responses to novel land management and diverse crop types and reduce our reliance on empirical field trials for projecting C sequestration.

The work under this theme is broken into seven tasks. Each modeling task is directly linked to one of the other six themes. The work in this theme serves as a tool for analysis in themes 1-5 and is therefore connected to each of the five overarching questions in the Introduction. In particular, this theme is essential to addressing questions I, IV, and V by connecting the amount and nature of organic matter inputs to C distribution and dynamics in space and time.

Background and Science Questions

As a result of a need to integrate erosion impacts on soil C (Izaurralde et al. 2006b) and soil biogeochemical dynamics of trace gas exchange with the atmosphere (McGill et al. 2004), the development of EPIC (Williams 1995; Izaurralde et al. 2006a) and its landscape version APEX (Williams and Izaurralde 2005) has led to an advanced soil C dynamics model particularly suited for soil C sequestration analyses at site and regional scales (Izaurralde et al. 2006a,b; He et al. 2006; Thomson et al. 2006). Within CSiTE, we are well positioned to continue the development and application of EPIC by including the representation of additional processes and improving model parameters involved in soil C sequestration enhancement with an increased emphasis on perennial biomass energy crops. Improvements in model representations and suitable parameter estimates depend on experimental measurements to be investigated in themes 1-5.

The integration between CSiTE experimental and modeling activities will occur from both directions. From one direction, model development will benefit from knowledge emerging from CSiTE experimental sites. From the other, modeling activities will facilitate the design of field experiments by pre-testing hypotheses and helping design data collection. This theme will enhance the collaboration among CSiTE theme elements during study design, implementation of fieldwork, model development, and model evaluation by using modeling prototyping tools such
as STELLA® or MathLab® to frame and evaluate model concepts in a way that encourages the participation of experimentalists.

The research is driven by the following science questions:

- Would a model representation of a hierarchical organization of soil aggregates improve the correspondence between measured and simulated soil C pools? Further, would such a model representation improve the simulation of SOC sequestration over that of current, conceptual pool-based models?

- Would a more refined treatment of microbial biomass dynamics in SOC models lead to improved simulations of SOC sequestration when field crops are replaced by perennial vegetation such as switchgrass? Currently, SOC models (including EPIC) contain a single microbial biomass pool that largely controls N mineralization-immobilization processes. Such models may not simulate a significant portion of the increase in SOC observed when perennial crops such as switchgrass replace field crops. Such replacements are known to induce shifts in bacteria to fungi ratios and changes in microbial conversion efficiency of POC to acid hydrolysis resistant material (lignin-like fungal residues, polymerized aliphatic materials, etc.). Nitrification and N₂O evolution during nitrification is reduced or eliminated by a shift from bacterial-dominated decomposition to fungal-dominated decomposition.

- Would incorporation into models of interactive effects of biochemical and physicochemical properties (e.g., soil pH, C content and quality, base saturation, cation and anion exchange capacities, mineralogy, texture, N content, oxidase/hydrolase activities, and fungal activity) on humification processes help explain results obtained in laboratory and field experiments?

- Does modeling N cycling for deep-rooted perennial bioenergy crops require a detailed soil-profile level understanding of root distribution, root biochemistry, and C and N transformation dynamics?

- Would an improved model representation of coupled hydrologic and geochemical processes controlling dissolved organic C and N transport and fate enhance EPIC’s predictive ability for determining C dynamics of deep soil layers? Furthermore, could such an improvement serve for assessing local and regional effects of manipulation strategies on C storage and N export?

**Technical Plan**

EPIC model refinement and new algorithm development, described in tasks 6.1-6.6, will be carried out in collaboration with ongoing CSiTE experiments described in themes 1-5. This theme will enhance the collaboration among CSiTE investigators during study design, implementation of fieldwork, model development, and model evaluation by using modeling prototyping tools such as STELLA® or MathLab® to frame and evaluate model concepts and engage experimentalists from the start of the proposed field and laboratory experiments. This will make maximum use of field measurements and investigator insights. Task 6.7 is designed to
be collaboration between this theme and Theme 7 by developing a series of regional simulations that will be required to answer the research questions of Theme 7. Details are provided in each task description.

**Task 6.1 Define/Improve the Parameters for Switchgrass and Poplar Growth**

Switchgrass and poplar are two species that have been identified as “model” species for the study and development of bioenergy crops (McLaughlin et al. 1999). EPIC (Williams 1995; Izaurralde et al. 2006) and its landscape version APEX (Williams and Izaurralde 2005) already contain parameters to simulate net primary productivity of these species as driven and controlled by weather, soil, and management variables. The plant parameters were assimilated from ALMANAC (Kiniry et al. 1992), a general weed-competition model based on EPIC. Kiniry et al. (1996) used ALMANAC to develop parameters (LAI, radiation use efficiency, growth response to temperature, and optimum nutrient concentrations) for simulating growth of “Alamo” switchgrass (*Panicum virgatum* L.). Simulated yields accounted for 79% of the variability in measured yields for one-cut and two-cut harvest systems from six diverse sites in Texas in 1993 and 1994. ALMANAC also simulated reasonably well the average switchgrass yields in Texas, Arkansas, and Louisiana but failed to account for the year-to-year variability at some locations (Kiniry et al. 2005). Sensitivity analyses showed that changes in parameters controlling the water balance (runoff curve number, stomatal conductance) had significant impacts on simulated values among the sites. Currently, EPIC, APEX, and ALMANAC lack parameters for simulating poplar growth but have the necessary infrastructure for simulating growth of woody species as demonstrated for eastern red cedar (*Juniperus virginiana* L.) and honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*).

While most of the work in the development of plant parameters has concentrated in the aboveground plant components, to predict soil C sequestration there is a need to test and, if necessary, improve the models’ ability to predict root growth and turnover of biomass crops. In EPIC and its related models, root growth is modeled as a fraction of total biomass, which decreases linearly from emergence (0.3-0.5) to maturity (0.05-0.2) according to species (Williams and Izaurralde 2005). Daily changes in root mass in each soil layer are simulated as a function of plant water use and root mass. Rooting depth is simulated as a function of heat units and potential root zone depth. Similarly, stress factors for soil strength, temperature, and aluminum toxicity are used to adjust potential root growth (Jones et al. 1991).

Current algorithms in EPIC are insufficient to capture the dynamics of deep-rooted perennial crops (e.g., switchgrass) on SOC dynamics with soil depth; thus, incorporation of knowledge on C allocation emerging from root studies under Theme 1 into EPIC will lead to an improved EPIC model capable of more realistic depictions of SOC patterns with soil depth. Modeling activities within Task 6.1 will be directly linked with the four hypotheses formulated in Theme 1 in relation to the impacts of belowground characteristics of switchgrass cultivars on soil C sequestration. The characteristics to be studied and modeled include root:shoot ratios, root depth, root C/N ratios, and interactions between belowground C inputs and N dynamics.


**Task 6.2 Incorporate Physicochemical Protections into Soil Biogeochemistry**

**Representation**

Current widely used models of SOC decomposition dynamics are based on the use of conceptual SOC pools described by first-order kinetics. This concept dates back to Olson (1963) and was first used in a multi-pool soil model by Jenkinson and Rayner (1977). A considerable amount of experience has been gained in using these models especially in regard to rate coefficient relationships with soil temperature and moisture conditions as well as in interactions with N dynamics and microbial N transformations. As noted within the Theme 2 discussion, there has been a recent increase in understanding of the relationships among particulate organic carbon (POC), mineral associated organic carbon (MOC), DOC, and soil mineral particles. These interact to form soil aggregates that physically protect POC from commutation and some types of decomposition while forming microsites for less-aerobic transformations to humic compounds that are stabilized by intimate association with mineral particles and formation of recalcitrant chemical compounds. This appears to take place in two stages in most soils—physical protection within macroaggregates and humification within microaggregates.

In this task we will address our first science question by modeling and testing the hierarchical hypothesis of Six et al. (2000), which describes the relationship between macroaggregates (>250 μm) and more stable microaggregates (53-250 μm).

Figure 6.1 is a diagrammatic representation of Six et al’s hypothesis that will form the framework for our modeling of physicochemical processes. When fresh substrate (free POC) is added to soil, macroaggregates are formed as a result of the microbial and micro-invertebrate production of easily decomposable binding agents. With initial decomposition of this free POC, fine plant residues get incorporated into microaggregates within the macroaggregates. When the macroaggregate falls apart, the microaggregates that were enclosed become part of the free microaggregate pool. In turn, when microaggregates fall apart, much of the enclosed organic matter is then transformed into CO$_2$, DOC, and humic compounds intimately associated with silt and clay-sized mineral particles.

This view of SOC dynamics introduces several features not encompassed by current soil decomposition models:

1. **Alignment of dynamics with measurable C pools** – Physical separation of organic C pools that takes into account aggregates as depicted in Figure 6.1 results in more uniform separation of C dynamical pools. The additional challenge, however, is that the formation and dissolution of micro- and macroaggregates must be explicitly modeled.

2. **Saturation behavior** – All decomposition models rely on first-order kinetics (rate of decomposition depends linearly on amount of material to be decomposed). Consequently, total SOC at equilibrium increases linearly with increased in organic matter inputs to soil. If SOC transformations to long turnover pools depend on mineral particle surface area and access to these surfaces, which is a function of aggregate dynamics, then there may be limits to the rate at which SOC can be transformed to resistant forms. This results in a saturation of the amount of C that can be stabilized in soil with increases in organic matter inputs or with time.
Figure 6.1. Diagram of a SOC model based on integrating soil aggregated dynamics and SOC kinetics, which will provide the conceptual framework for modeling physicochemical processes in soil. Two classes of aggregates, macroaggregates (>250 μm) and microaggregates (53-250 μm), along with an unaggregated fraction consisting of silt and clay particles and their interactions are depicted. Each contains two organic matter classes—POC and MOC. Each of these organic fractions will be directly extracted in the soils from our four field experiments with a combination of sieving, density flotations, and combustion as described in themes 2 and 3.

Measurements for each physical fraction of C, $^{13}$C, and estimates of tensile strength, stability, moisture retention capacity, density, with C content will be used to develop simple first-order ordinary differential equation representations of the relationships depicted in Figure 6.1. We will use a modeling language like STELLA® or MathLab® to prototype model formulations and examine consistency with measurements. Data from previous CSiTE work at Fermilab, a prairie chronosequence, and at Coshocton, comparison of till and no-till agriculture (Denef et al. 2004; Puget et al. 2000; Puget et al. 2005; Blanco-Canqui et al. 2005a) will be used to guide development of new SOC decomposition model equations that account for aggregate dynamics and their influence on humification transformations (see Task 2.5).

Measurements from switchgrass plots at Milan and Fermilab, collected as part of themes 1 and 2, will be used to answer our first science question. We will also evaluate the macroaggregate model of Plante et al. (2002), which defines several compartments of water-stable soil aggregate size fractions and describes the flows among them using first-order kinetics. While this model predicted reasonably well macroaggregate dynamics in two Canadian soils, we remain aware
of—and will try to improve on—some of the identified limitations such as the relative short residence time determined for the compartments (4-95 days) and an appropriate representation of the compartments.

**Task 6.3 Improve the Representation of Microbial Biomass in SOC Transformations and Trace Gas Metabolism**

So far, EPIC seems to capture reasonably well the dynamics of microbial biomass as affected by management and environmental conditions. However, several lines of evidence have suggested the need for a more detailed representation of the microbial biomass pool—our second science question. The first line emerges from experimental evidence indicating a shift in bacterial to fungal ratios under perennial grassland, which has been related to a greater stabilization of SOC. The second line is drawn from the fundamental connections among microbial biomass, C and N transformations, and trace gas metabolism. In particular, the demonstration that *Nitrosomonas* spp. can oxidize ammonia and reduce nitrite under O$_2$-limited conditions (Bock et al. 1995; Remde and Conrad 1990; Schmidt et al. 2002) provides a unifying way to deal with “classical” nitrification and N$_2$O evolution during nitrification.

Combining measurements from Theme 3 and developing a more complete model representation of the microbial biomass based on functional groups such as autotrophs and heterotrophs (bacteria separate from fungi) will provide us the information to evaluate our second science question. To accomplish this, simple models will be created in STELLA®, MathLab®, or some similar equation prototyping tool and used to explore the range of model formulations and parameters that are consistent with microbial measurements in Theme 3. These will guide the required changes in the EPIC model for improved representation of microbial processes. This inclusive approach will also help with the treatment of methanogenesis and methanotrophy in EPIC.

**Task 6.4 Develop Equations in EPIC to Model the Influence of Soil Physicochemical and Biochemical Manipulations on Humification Chemistry**

Current algorithms in EPIC and other models based on conceptual carbon pools and first-order kinetics are insufficient to capture the influence of soil physicochemical and biochemical manipulations on humification chemistry. Incorporating the knowledge on humification processes emerging from the manipulations and measurement of humification processes under Theme 4 into EPIC will lead to an improved EPIC model capable of more realistic depictions of the dynamics of humification processes, especially in the context of aggregate formation and dissolution.

Interactions among soil pH, C content and quality, base saturation, cation and anion exchange capacities, mineralogy, texture, N content, oxidase/hydrolase activities, and fungal activity are known to control humification processes. Experimental results from Theme 4 will be used to determine these parameters and develop necessary equations for a new module in EPIC capable of representing the process response of the humification treatments in the Fermi and Milan manipulations and simulating their impact on C humification efficiency. This capability will allow us to extend the findings to a regional or national scale.
Development of this new model formulation will take place in three stages. First, we will examine the existing capabilities of the EPIC code and identify the new parameters and routines that may be needed. Next we will work with Theme 4 to construct a prototype model to simulate the probable impact of the manipulation on soil C levels (and other relevant soil properties). Then, once the manipulation has been implemented in the field, we will compare actual field results with model predictions, and use these to further refine the new model formulations.

The aggregate structure of soil under perennial vegetation results in heterogeneous environments with varying qualities of organic matter, pH, Ca, Mg and Fe concentrations, enzyme concentrations, and water contents. The humification dynamics refined in this task to respond to these conditions can also be used to take into account the range of humification dynamics that are possible within microaggregates, between microaggregates within macroaggregates, and between macroaggregates. Measurements on C content, density, and moisture retention capacity of discrete aggregates from Theme 2 will be useful in inferring the physical and chemical environments within aggregates and making appropriate modifications to estimates of C transformation parameters developed in Task 6.1.

**Task 6.5 Improve Representation of Nutrient Cycling**

The purpose of this task is to improve our understanding of soil C sequestration in relation to plant productivity, C inputs, nutrient availability, nutrient losses (e.g., erosion, leaching), and nutrient recovery. This is very important because nutrient management is essential for controlling plant productivity, soil C sequestration, and environmental impacts.

EPIC and APEX offer a comprehensive scheme to simulate N and P cycling (Williams 1995; Williams and Izaurralde 2005). Nitrogen can be added to a system via wet deposition or fertilizers (synthetic or organic amendments). Losses of N are simulated via plant harvest, erosion (organic N in sediments, soluble N in runoff), NH$_3$ volatilization, denitrification, and leaching. Nitrogen transformations among the various N pools are coupled with C transformations. A brief explanation of these transformations follows.

The simulation of daily potential C and N transformations (e.g., structural litter to biomass, biomass to leaching, slow to passive, plant uptake—for a total of 10) in each soil layer is based on substrate-specific rate constants, which are affected by soil temperature, water content, proxy-oxygen content, and soil disturbance factors (Izaurralde et al. 2006). Lignin content and soil texture also affect some of these transformations. The transformations are considered potential because they reach completion only when enough quantities of organic and inorganic N are available. If available N exceeds the N demand, then all potential transformations proceed and the excess N goes into the mineral N pool. Otherwise, when the N demand exceeds the mineral N available, EPIC calculates a proportional reduction in the net N demand and each potential transformation leading to N immobilization, a term that includes all pool transformations except for plant uptake. The N cycling component of EPIC has been tested on many occasions with generally satisfactory results (for a review of nutrient cycling studies involving EPIC see Gassman et al. 2004).

Phosphorus cycling is also modeled in detail in EPIC and APEX (Williams and Izaurralde 2005). Phosphorus can be added via synthetic fertilizers or manure. Like N, P can be lost from the
system via plant harvest, erosion (organic P in sediments, soluble P in runoff), or leaching. Phosphorus mineralization is simulated with a two-pool model: 1) fresh organic P pool, associated with crop residue and microbial biomass, and 2) a stable organic P pool, associated with soil humus. Mineral P is modeled with three pools: labile, active mineral, and stable mineral. Fertilizer P is labile (available for plant use) at application but may be quickly transferred to the active mineral pool. Flow between the labile and active mineral pools is governed by equilibrium equations. Specific sorption P coefficients exist for calcareous soils and for non-calcareous soils with different degrees of weathering. Currently, organic P transformations are not coupled to the C transformations.

While the nutrient cycling components of EPIC have been tested on numerous occasions, there is only one test reported so far examining long-term N dynamics which used the most recent version of EPIC that incorporates CSiTE-based nutrient cycling refinements. He et al. (2006) used data from a long-term N fertilization experiment in Arlington, Wisconsin, to simulate corn yields, soil C and N dynamics, net N mineralization, soil C sequestration rates, and soil bulk density. While the refined model acceptably mimicked changes in total soil N, it reproduced the trends but underpredicted net N mineralization measured during a 280-day leaching-incubation experiment. Further tests are thus needed to determine whether the underprediction is a result of model structure or the nature of the experimental and simulated data comparison.

Modeling N cycling of deep-rooted perennial crops such as bioenergy crops requires a detailed soil-profile level understanding of root distribution, root biochemistry, and C and N transformation dynamics. Under this task we propose to use weather, plant, soil, and management data from the switchgrass experiments at Milan and Fermilab to model N dynamics in relation to plant biomass, root distribution and turnover, fertilizer N additions, and N losses via leaching and denitrification.

Improving the understanding of the allocation of C to aboveground and belowground inputs at different depths is critical. The current model shows a problem of “mining” or excessive loss of SOC at depth in the soil. This is sometimes observed in soil profile measurements for conversion to no-till (West and Post 2002) and aggrading forest (Post and Kwon 2000). It is not currently clear whether this results from excessive decomposition or insufficient inputs of SOC in deeper layers. Information from Theme 1 will be used to improve the inputs of POC from roots in different soil layers.

**Task 6.6 Quantify Impacts of Dissolved Organic C Dynamics on Soil C Sequestration**

There has been a recognition that DOC interacts strongly with soil mineral particles with the magnitude depending on particle surface area, mineralogy, soil pH, and anion and base saturation. The amount of DOC in surface soil, however, is small compared to particulate organic carbon (POC), and DOC dynamics are often ignored. This is a critical omission for understanding the potential for C transport and storage in deeper soil layers. EPIC currently uses a linear partition coefficient and soil water content to calculate sorption-modified movement of organic materials from surface litter to subsurface layers (Izaurralde et al. 2006a).

Leaching of DOC from biomass, POC, and MOC with depth is modeled as a function of flow, soil water content, the liquid–solid adsorption coefficient for microbial biomass C, SOC
concentration, soil bulk density, and soil layer thickness. No thorough test has been conducted so far of this model component. Izaurralde et al. (2006b) modeled net ecosystem C balance of three long-term watersheds at Coshocton, Ohio, and found leached C to range from 22-31 kg C ha$^{-1}$ y$^{-1}$. These values were larger than the average 4.5 kg C ha$^{-1}$ y$^{-1}$ leached from a weighing lysimeter cropped during 10 years to a corn-soybean rotation (Owens et al. 2002). Currently, EPIC does not provide insight as to the influence of DOC on deep C sequestration. Thus, the opportunity exists to improve the representation of these processes in EPIC with experimental information emerging from Theme 5, Intrasolum Carbon Transport. An improved representation of coupled hydrologic and geochemical processes controlling dissolved organic C and N transport and fate will enhance EPIC’s predictive capability for determining C dynamics of deep soil layers and utility in assessing local and regional effects of manipulation strategies on C storage and N export.

Modeling activities within this task will be synchronized with experimental activities proposed under Theme 5, Task 5.3. The model infrastructure in EPIC is such that many of the physical and chemical parameters to be collected under Theme 5 could be used to improve the representation of coupled hydrologic and geochemical processes controlling organic C and N fate and transport (e.g., preferential flow, matrix diffusion, mass transfer kinetics, sorption, and degradation).

**Task 6.7 Developing the Capabilities for Modeling the Biophysical Implications Including Soil C Sequestration of Current Land Use and the Adoption of Technologies to Enhance C Sequestration Including Bioenergy Crops**

The objective of this task is to develop a regional or national scale capacity to analyze the biophysical consequences of current land use and the future application of technologies to enhance C sequestration including bioenergy crop production.

Whereas tasks 6.1-6.6 describe using the EPIC model to aid process description of soil C mechanisms and consolidate understanding among interacting processes that are quantitatively consistent with observations, the goal of this task is to provide supporting information on environmental and edaphic conditions, management regimes, and crop types that expands the capability of the model beyond the site conditions used to develop new model components and enables regional and national applications. We will take a two-phase approach to achieving this goal.

As a first research activity we will evaluate the current regional version of EPIC. This will be accomplished by comparing EPIC-derived national maps (Thomson et al. unpublished) of 1) current soil C stocks under current land use and management, and 2) potential soil C stocks assuming adoption of land management strategies such as no-till agriculture and adoption of bioenergy crops against analogous maps derived from maps of current land use at a fine resolution (created from satellite imagery, national-level soil C inventory data, and empirically derived algorithms for predicting soil C sequestration based on data from numerous field trials; West et al. unpublished). Soil C stocks and the mean and standard deviations of soil C change with adoption of specific land management strategies will be compared and their differences analyzed in terms of data structure and model assumptions. This analysis will improve estimates of current C stocks and predicted sequestration over heterogeneous regions. It will also provide
Theme 7 with interim biophysical results useful for combining with economic data for modeling with the agricultural sector model FASOM.

A second research activity that directly links to Task 7.1 involves the further development of dynamic links between EPIC and the agricultural sector model FASOM (Adams et al. 2005b). This activity will have two components. One component will be improving the database EPIC uses to model regional- and national-scale soil C sequestration, associated crop yield, and environmental parameters such as quantity of runoff, irrigation water use, and GHG emissions. The current methodology used in EPIC to model regional- and national-scale soil C sequestration is based on defining climate-soil-management (CSM) combinations that approximately capture major trends in environmental conditions, land use, and land management practices. Each CSM defines a unique combination of climate soil and crop type and crop management practices such as no till or specific fertilizer rates. We have run EPIC with 7500 unique CSMs to characterize national agricultural conditions. For each CSM, main EPIC results were transferred to FASOM. These model results included crop yields, tillage, erosion, and soil C sequestration, among others. To model the economic and environmental trade-offs of bioenergy crops, FASOM will require EPIC-derived model results that include more crops, tillage levels (e.g., tillage changes), and management practices (e.g., fertilization levels). To accommodate this, the existing modeling database will be redesigned, and we will select a representative subset of climate and soils conditions previously used and expand the simulated management options.

The second component will be using the EPIC model version that results from tasks 6.1-6.6 and the revised set of CSMs to develop the set of EPIC runs needed in Task 7.1. These runs will enable FASOM modeling to better consider the economic and biophysical implications of widespread adoption of bioenergy crops and will ensure that the process-level understanding developed through the experimental themes is allowed to play out in our evaluation of the carbon benefits of bioenergy crops.

Linkage to Other Themes

In summary, tasks 1-5 in this theme are designed to produce an improved EPIC model capable of assisting in the evaluation of the major hypotheses presented in each of previous five themes, respectively. The tasks are designed to aid in analysis of measurements and contribute to basic science discovery and hypothesis testing for each theme. The outcome of these evaluations will lead to an advanced soil C model that may be applied more generally to a wide range of climate, edaphic, biotic and management conditions. Tasks 6 and 7 aim at developing a capability to transfer information from process–level scale scientific discoveries to the economic model FASOM (Theme 7) so that the economic potential of these discoveries can be evaluated.

Expected Results

This theme will develop and validate the mechanistic model EPIC using both information and data from previous CSiTE investigations and collaboration with the field and laboratory investigations of Themes 1 through 5.

- This model will integrate and incorporate our process understanding of the roles of C inputs, soil structural controls, microbial community function and dynamics, humification chemistry, and intrasolum C transport on soil C sequestration.
It will enable testing of new technologies/approaches to enhance soil C sequestration. In particular, these modeling activities will improve our understanding of fundamental physical, chemical, and biological mechanisms controlling C accrual and storage in soil and how these mechanisms vary in time and space thereby addressing all five overarching research questions but especially questions IV, and V which address extrapolation of fundamental knowledge.

In producing results required for Theme 7, EPIC will facilitate the production of regional and national forecasts of SOC sequestration and provide information on how specific soil C sequestration practices could be implemented in an economically competitive, environmentally acceptable fashion.

### Schedule and Milestones

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<thead>
<tr>
<th>Research Task</th>
<th>Fiscal Year</th>
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<tr>
<td></td>
<td>2007</td>
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<tr>
<td>6.1 Define and improve parameters for Switchgrass and poplar growth in EPIC</td>
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<td>6.2 Add aggregate dynamics to EPIC</td>
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<td>6.3 Improve microbial representation in EPIC</td>
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<td>6.4 Improve humification chemistry in EPIC</td>
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<td>6.5 Improve nutrient cycling in EPIC</td>
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<td>6.6 Improve DOC representation in EPIC</td>
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<td>6.7 Improve EPIC regional capabilities</td>
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<td>Map comparison</td>
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<tr>
<td>EPIC regionalization</td>
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#### 2008

**Task 6.1.**

a) Version of the EPIC model with improved parameterization to model switchgrass growth based on experimental data at Milan; b) Manuscript documenting switchgrass parameterization in EPIC and its performance against experimental data; c) Manuscript comparing estimates of current soil C stocks and predicted sequestration over heterogeneous regions.

**Task 6.2.**

b) Version of EPIC with aggregate turnover explicitly modeled and calibrated using critical data from cropland to perennial grassland long-term experiments; e) Manuscript documenting new physicochemical protection equations and performance against experimental data and application to preliminary Milan measurement.

**Task 6.7.**

c) Complete comparison of EPIC regional simulations with database approach as diagnostic evaluation of EPIC regionally. G) manuscript comparing EPIC estimates of sequestration potential for bioenergy versus empirical estimates indicating differences and evaluating their causes and resolution.
2009

Task 6.3.

d) Develop new microbial compartments for EPIC with multiple interacting microbial functional types that have different decomposition parameters and processes; b) Manuscript on impact of bacterial to fungal transition in perennial grassland establishment and implications for C sequestration under switchgrass

Task 6.5.

e) Version of the EPIC model with capabilities to model C and N transformations with soil depth; e) Manuscript documenting the improvements in the nutrient cycling components of EPIC and its performance against experimental data;

Task 6.7.

f) Documentation of the links EPIC-FASOM; e) Manuscript with regional projections of soil C sequestration, and utility in economic analysis.

2010

Task 6.3.

g) Manuscript on impact of bacterial to fungal transition in perennial grassland establishment on N cycling and N trace gas emissions and implications for switchgrass establishment.

Task 6.4.

h) Construct a prototype model to simulate the probable impact of the biochemical manipulation on humification chemistry from Theme 4 on soil C levels (and other relevant soil properties); c) manuscript where we will compare actual field results from biochemical field manipulation with model predictions, and use these to further refine the new model formulations.

2011

Task 6.6.

i) Develop an improved representation of coupled hydrologic and geochemical processes controlling dissolved organic C and N transport and fate; b) manuscript describing EPIC’s predictive capability for determining C dynamics of deep soil layers, N leaching losses by inclusion of vertical hydrologic and geochemical processes.

Task 6.7.

j) Manuscript with improved/ revised regional projections of soil C sequestration that also incorporates evaluation of environmental impacts, and economic analysis.
Theme 7: Integrated Evaluation of Carbon Sequestration Technologies

Purpose and Objectives

This research theme uses fundamental mechanistic information garnered in themes 1-6 to provide broad-based evaluations of strategies to enhance soil C sequestration with a special focus on bioenergy. The primary objectives are to 1) build on CSiTE science and process modeling (EPIC) so that soil sequestration is integrated with strategies for realizing a national bioenergy economic sector and is recognized as significant in the broader C management community, and 2) facilitate the examination of competitiveness of dedicated bioenergy crops and soil C sequestration technologies in the context of the full suite of climate adaptation and GHG mitigation strategies. This theme directly addresses science question V: “How can fundamental knowledge best be used to identify and implement methods and practices for sustained enhancement of soil C in an environmentally acceptable and economically feasible fashion?”

Background and Research Questions

Soil C sequestration implications have not been fully considered in societal energy planning, including biofuel-related consideration and GHG management decision making. The potential for soil sequestration is represented either very simplistically or not at all in the analysis of alternative climate policies or large-scale expansion of biofuels. The process understanding and data requirements for extrapolation of CSiTE results to the regional and national levels require specialized knowledge and resources beyond those available to decision makers and modelers.

This theme is directed toward integrating and assembling CSiTE science and process understanding in a way that is broadly applicable and strengthens opportunities for evaluating potential prospects for sequestration and biofuel production. Therefore, we consider activities within Theme 7 as complementary to integrated assessment activities by other groups. A principal success measure will be met when IA modeling teams and policy bodies use CSiTE analyses to expand their consideration of GHG mitigation options to include terrestrial C sequestration on an equal footing with geologic sequestration, energy efficiency, nuclear power, and other energy and GHG mitigation options along with considering the soil sequestration consequences of biofuel possibilities.

Process and sectoral modeling, using EPIC and FASOM, are central to addressing the role of C sequestration. Jointly, these models allow economic and environmental simulation of alternate C sequestration strategies, their feasibility, and regional and national potential and how such potential changes with technological alternatives. EPIC simulates crop growth and environmental interaction processes, while FASOM uses results from EPIC to parameterize the biophysical and environmental tradeoffs across land uses and land management practices. Under this theme, additional research questions emerge including:

- To what extent do soil C sequestration strategies improve the overall economics, GHG mitigation potential and environmental interactions of dedicated biofuel crops at a scale commensurate with competing energy and mitigation technologies?
- What are the indirect environmental costs, with respect to carbon stocks and net carbon emissions, of changing from food crops to bioenergy crops?
• How can we combine experimental science and mechanistic modeling to improve the effects of terrestrial sequestration and biofuel production strategies along with their adoption prospects?
• How are the abilities to sequester carbon and mitigate emissions augmented or lessened by economic influences or incentives?

This involves a mixture of appraisals of the full environmental and economic implications of proposed strategies along with analyses of prospective practices to help guide research direction. We will examine a set of practices to see if some are more or less desirable and to identify aspects of practices that if changed would enhance environmental and economic attractiveness.

**Technical Plan**

We have identified FASOM and EPIC as our primary economic and biophysical simulation tools for evaluating soil C sequestration strategies and providing information that can be used by both assessment modeling teams and those pursuing field level sequestration/biofuel feedstock production. Project efforts will pursue such analyses, further interconnecting the modeling tools and strengthening the richness of the environmental, economic, and greenhouse gas aspects of these models. Major effort will be devoted toward evaluating existing, emerging and prospective biofuel and sequestration related developments attempting to identify factors limiting adoption and dialoguing with technical scientists over possible technology refinements to alleviate bottlenecks. Activities in this theme are covered in a single task.

**Task 7.1 Strengthen Link between Mechanistic Modeling (EPIC) and Economic Methods (FASOM)**

One objective of this task is to provide direction to the EPIC regional modeling efforts by making explicit the types of information FASOM requires from EPIC and formally defining the interface between EPIC and FASOM. This activity is tightly linked to Task 6.7 of Theme 6. Another objective is to expand the types of agricultural and sequestration activities that can be jointly handled by the EPIC and FASOM models, especially those that reflect CSiTE science results.

Information must be passed from EPIC to FASOM on soil C sequestration, yield, crop irrigation water demand, non-CO$_2$ GHG emissions, and runoff quantity and quality as they evolve over time by tillage strategy (current and historic), residue recovery (e.g., the harvesting of stover for bioenergy or bedding), other management practices (rotation, cover crops), fertilization, and irrigation inputs, as a function of location. As part of this task we will formally define an interface between EPIC and FASOM. We will explore adopting the interface concept applied in object-oriented programming languages that views the interface between two computational components as a contract. Results from EPIC would be stored in a location or file that is accessible to FASOM through pre-defined operations and formats. This could take the form of a database or some other data structure.

While EPIC provides essential information on the biophysical tradeoffs across agricultural practices, a full accounting of GHG emissions requires considerations not currently available in EPIC. Some examples are the energy embodied in fertilizer, product transport, and parameters
governing emissions of non-CO\textsubscript{2} GHG. Additional information on full C and GHG accounting is needed to develop the ability to quantify changes in net emissions associated with C sequestration strategies.

We have developed this ability at a relatively coarse scale. For example, we developed weighted averages of CO\textsubscript{2} emissions from agricultural production inputs in the U.S. and used these estimates to quantify the net impact of C sequestered by the use of no-till practices (West and Marland 2002a). More recently, we have moved forward on developing CO\textsubscript{2} emissions for individual production inputs. We have also developed estimates of CO\textsubscript{2} emissions from soil amendments (e.g., agricultural lime), and developed estimates of carbon-equivalent greenhouse warming potentials due to changing albedo (i.e., surface reflectance associated with changes in land cover). The EPIC-FASOM coupling will require additional information beyond currently available weighted averages for all fertilizers, pesticides, and herbicides. We will assemble tables for energy and CO\textsubscript{2} emissions associated with hundreds of pesticides and production machines used for land management.

We also need to complete energy and CO\textsubscript{2} emissions for different fertilizer types and application methods. Fertilizer types and application methods, along with changes in albedo, are important for evaluating sequestration activities in forests as well as cropland. We will use the expanded database on energy, CO\textsubscript{2} emissions, and GHG emissions to update the C and GHG accounting in FASOM so we can project the full GHG implications of sequestration technologies and biofuels. These expanded tables will be used to complete the FASOM requirements not currently supplied by EPIC and incorporated into the object-oriented interface being considered for the model coupling.

FASOM can accommodate information across a wide variety of locations, climate conditions, planting times, and other management practices. Explorations will consider:

- The effect of crop type (switchgrass, field crops) on soil C sequestration, irrigation water use, C and other GHG emissions, water runoff and biofuel feedstock quality.

- The effect of fertilizer application on yield, irrigation water use, C sequestration, runoff, N\textsubscript{2}O and CO\textsubscript{2} emissions, residues, and biofuel feedstock production.

- The effects of tillage practices, cover crops, soil amendments, and other management practices on yield, irrigation water use, runoff, soil sequestration, GHG emissions, costs, erosion, and biofuel feedstock quality.

- The regional effects of removing agricultural and forest residue on soil C sequestration, GHG emissions, erosion, and biofuel feedstock availability.

- Tradeoffs between sequestration, residue and energy crop products, manure, biodiesel, and crop and cellulosic ethanol, in terms of environmental effects, net GHG emissions, biofuel feedstock production, and market economics.

- Development of marginal abatement cost curves for sequestration, GHG offset and biofuel strategies, that can be packaged to facilitate multisectoral assessments of strategy
adoption potential in larger settings like the Climate Change Science Program (CCSP), the Climate Change Technology Program (CCTP), and the North American Carbon Program (NACP).

These capabilities allow us to compare C sequestration strategies on scale, cost, location, timing, and permanence. We plan to exploit the modeled interrelationships between sequestration and biofuels relying on FASOM features (Adams et al. 2005a) that depict production of

- Energy crops, such as switchgrass, willow, and hybrid poplar
- Corn stover and logging residues
- Agricultural waste products, such as manure, tallow, and yellow grease
- Processing products, such as cornstarch, corn oil, and soybean oil
- Processing byproducts such as lignin and bagasse
- Grains that can be converted into alcohol, such as corn, sorghum, and sugarcane

and the conversion of these items into the bioenergy products

- Ethanol through dry and wet milling plus cellulosic conversion
- Biodiesel from plant oils, tallow, yellow grease, or waste oils
- Electricity from residues, energy crops, logs, manure, bagasse, or milling residues though 100% firing or some degree of co-firing.

**Linkage to Other Themes**

This theme links directly to other CSiTE activities through Theme 6’s Task 6.7 “Developing the capabilities for modeling the biophysical implications including soil C sequestration of current land use and the adoption of technologies to enhance C sequestration including bioenergy crops.” Although the primary flow of data is from Theme 6 to Theme 7, the analysis in Theme 7 will feed back to inform the selection of locations, crops, and management practices simulated by EPIC, and the types of outputs provided by EPIC. Promising sequestration strategies identified in Theme 7 should feed back not only to the mechanistic modeling in Theme 6, but ultimately to the selection of future CSiTE experimental sites.

**Expected Results**

We expect to obtain an improved understanding of how soil C sequestration affects the overall economics and GHG mitigation potential of biofuel crops. We also expect to have an improved understanding of where C sequestration and biofuel activities might occur under various economic conditions. Specifically, results will be generated in terms of potential management manipulations arising from science findings under the other four overarching research questions:

I. Nature of belowground C inputs by switchgrass, and compatibility with sustained biomass and soil C sequestration

II. Fundamental physical, chemical, and microbial mechanisms controlling C accrual and storage in soil

III. Processes that control movement and distribution of C through the profile
IV. Regional and temporal processes controlling soil C distribution

We expect that findings from these four science questions will be reflected in the EPIC parameters transferred to FASOM. Therefore, activities under this Theme are designed to directly address science question V: How can fundamental knowledge best be used to identify and implement methods and practices for sustained enhancement of soil C in an environmentally acceptable and economically feasible fashion?

Schedule

The first 2 years will focus on formalizing the link between EPIC and FASOM, demonstrated with the current set of EPIC capabilities. During later years, we will expand the set of activities that EPIC and FASOM can simulate jointly. We expect that these activities will reflect new capabilities in EPIC and new CSiTE science results.

<table>
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<tr>
<th>Research Task</th>
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<tbody>
<tr>
<td>7.1 Strengthen EPIC-FASOM link</td>
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III. Project, Communications, and Data Management Plan

Project Management

Overall project management will be the responsibility of Dr. Blaine Metting, PNNL, and Dr. Robin Graham, ORNL (CSiTE PIs). Drs. Mac Post, Julie Jastrow, and Cesar Izaurralde (the CSiTE science leaders) will provide scientific and technical advice. The seven theme leaders—the three CSiTE science leaders and Drs. Ron Sands, Phil Jardine, Mike Miller, Chuck Garten, and Jim Amonette—will be responsible for implementing and coordinating the research under their theme. Dr. Don Tyler, UT, will be responsible for the maintenance and installation of the switchgrass plots at Milan and will provide technical guidance for the installation of switchgrass plots at Fermilab. Table 1 outlines theme involvement at the four experiments. These 11 individuals will make up the CSiTE leadership team.

Table 1. Experimental theme ties to the four CSiTE experimental sites.

<table>
<thead>
<tr>
<th>Experimental Site</th>
<th>Theme</th>
<th>Milan Cultivar</th>
<th>Milan Fertilizer</th>
<th>Fermi Manipulation</th>
<th>Milan Manipulation</th>
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<td>Year 2</td>
<td>Year 3</td>
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<td>4</td>
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<td>Years 3-4</td>
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<tr>
<td>5</td>
<td>Years 1-4 (6 of 12 plots)</td>
<td>Years 3-5 (6 of 18 plots)</td>
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Internal Communications

The CSiTE leadership team will participate in monthly telecons to ensure coordination of field sampling and measurements and to enable timely distribution of data. In addition, in late winter, there will be an annual meeting at which the CSiTE leadership team will discuss the upcoming year’s research activities, review the previous year’s activities, and develop needed coordination in field sampling and field and lab measurements. At that time, the datasets to be generated that year will be identified and the owner/generator of the dataset identified. Soil samples targeted for long-term archiving will also be identified. To the extent possible, metadata for the datasets will also be generated (see below). Datasets collected from the previous year will be reviewed and their archiving discussed. Dr. Tom Boden, PI of the Carbon Dioxide Information and Analysis Center (CDIAC) at ORNL, will provide technical guidance on database creation, metadata, and archiving. At the meeting, the leadership team will also discuss appropriate venues (meetings, workshops, symposia) for disseminating CSiTE findings.

In early spring, an all-hands meeting will be held at UT’s Jackson Experiment Station (associated with the Milan field site) in years 1, 2 and 4, and at ANL in Year 3 prior to the intensive measurements at the Fermi manipulation experiment and in Year 5. Table 2 shows the involvement of investigators across themes. At this meeting, researchers within each theme will review progress over the proceeding year and discuss research objectives and activities for the upcoming year and discuss collection and sharing of samples in the upcoming year. We envision having a all-hands sampling collection at the Milan cultivar experiment in Year 1, the Milan fertilizer experiment in Year 2, the Fermi manipulation experiment in Year 3, and the Milan manipulation experiment in Year 4.
Table 2. Investigator involvement across Science Plan themes.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Theme 1</th>
<th>Theme 2</th>
<th>Theme 3</th>
<th>Theme 4</th>
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To facilitate internal coordination and communication we will develop a SharePoint site accessible to the CSiTE PIs, theme leaders, and others as appropriate. We will use the calendar on this site for disseminating field schedules and arranging meetings. We will also create subdirectories for sharing data files and their associated metadata.

**External Communications**

The CSiTE website will be revamped in 2007 to reflect the new Science Plan, and Robin Graham will be responsible for its contents. Each summer after the spring all-hands meeting, ORNL will use a summer intern to update the CSiTE web contents. CSiTE presentations and publications will be made available for download off the website within the constraints of copyright laws. Links to relevant sites (e.g., CCTP plans, and bioenergy) will be established.

CSiTE will also sponsor a symposium on carbon sequestration in terrestrial ecosystems at the end of FY 2008 and FY 2011. We will seek a journal to publish the collective papers given at these symposiums.

While the focus of CSiTE is carbon sequestration, our findings will be relevant to those interested in the development of bioenergy and should be communicated to that user group. It
will be the responsibility of Robin Graham, a long-standing member of that community through her past work in the EERE Biofuels Feedstock Development Program, to coordinate the dissemination of CSiTE research findings to that community in a manner that is relevant to their needs. This will involve communication with both DOE- and USDA-sponsored researchers on bioenergy crops.

We will also seek to involve other non-CSiTE researchers at our new Milan and Fermi manipulation experiments and will archive sufficient soil during our sampling campaigns to facilitate other use of the soils. For example, we are in discussion with researchers Kim Magrini and Mark Davis at the National Renewable Energy Laboratory about their technique to quantify types of soil C through pyrolysis molecular beam mass spectroscopy coupled with multivariate analytical techniques.

Data Management

Processed data resulting from the CSiTE field experiments described in this Science Plan will be documented, archived, and disseminated by CDIAC at ORNL at no cost to the CSiTE project. Investigators will be required to submit data to CDIAC within 6 months of completion of measurement analyses. For 2 years, data access will be limited to use by fellow CSiTE investigators. After 2 years, CSiTE data will be made available to anyone without cost through CDIAC.

As noted above, CDIAC will provide data submission guidance to CSiTE investigators regarding data submission formats, file content (e.g., recommended units and naming conventions), and information needed to fully document the submitted data (i.e., metadata). For continuous measurements, CSiTE investigators will be required to submit processed data (e.g., 30-min averages) to the archive and not raw data (i.e., voltages from data loggers).

CSiTE investigators will be permitted to submit data in a variety of ways including via e-mail, direct deposit to a secure CDIAC File Transfer Protocol (FTP) server with areas dedicated to CSiTE submissions, on transfer media (e.g., CD-ROM), or by having CDIAC mirror a location at their institution. CDIAC will provide at no cost a web-based interface to allow CSiTE investigators to submit metadata to CDIAC electronically. The interface will be user-friendly (i.e., menu-driven with pick lists) and will capture the submitted metadata in a form (XML format) to permit CDIAC to build a catalog of submitted CSiTE data holdings using existing CDIAC cataloging tools (i.e., Mercury) and to enable commercial search engines to easily find the holdings as well. The metadata interface will also aid later modeling and synthesis efforts by imposing consistency for selected fields such as soil classifications, ecosystem classifications, site coordinates, and CSiTE sample site-naming conventions. Naming conventions will be discussed and resolved at the annual CSiTE meetings.

CSiTE data submitted to CDIAC will be checked by CDIAC. Data issues will be resolved with the contributing CSiTE PI, and no data will be changed or altered without permission from the investigator. At the end of the 5-year project, a final CSiTE database (with numerous granules) will be assembled and published with a citation acknowledging all CSiTE data contributors. In the interim, individual datasets will be referenced to the contributing investigator and their
published papers. The final database will be available through the CSiTE website or by direct FTP to the CDIAC server.

**Soil Management and Archiving**

Theme 1 researchers will work with the other four experimental theme researchers to ensure timely collection and distribution of soil samples at the prescribed depths, times (spring, summer, fall), and plots. We have staggered the intensive soil collection from the four experiments across 4 years. We intend to have all field researchers participate in the collection of soil samples during the intensive collection to facilitate soil sharing and distribute the burden of collection across all the themes. We are also pursuing the acquisition of a hydraulic soil probe to facilitate collections. Soil samples collected during the intensive collection will be archived both after air-drying and being freshly frozen. Samples collected at Milan will be stored at ORNL, and samples collected at Fermilab will be stored at ANL. We anticipate purchasing -80°C freezers at both ORNL and ANL for sample storage. We will archive these soils for future studies to take advantage of new genomic and measurement techniques that emerge over the project lifetime and provide future collaborators with samples that can be tied to CSiTE measurements.
IV. Summary of Expected Results

By addressing our five overarching science questions through an intensive, vertically integrated study that combines lab, field, and modeling components and the production of switchgrass for bioenergy as a testbed landuse the proposed study will:

- Support the objectives of the Climate Change Technology Plan (CCTP),
- Promote the sustainable development of bioenergy,
- Encourage the development and application of technologies to enhance soil C sequestration especially in agricultural settings by advancing an integrated understanding of the physical, chemical, and biological processes regulating the storage of C in soils.

The proposed research supports the CCTP in three ways. First, through the five experimental themes, we propose to develop field tools for evaluating the status of soil with respect to enhancing soil C sequestration. We will investigate the potential for using changes in microaggregate-protected C as an early indicator of C sequestration and the capacity of soil to protect and stabilize additional C inputs (Theme 2). We will evaluate the potential for specific microbial groups or subgroups to act as sentinels of aggrading or degrading soil C systems (Theme 4), and we propose to develop quick, reliable, inexpensive chemical methods of determining the humification status of soils (Theme 4). Second, we will develop a mechanistic model that allows a priori evaluation of proposed methods to increase soil C sequestration (Theme 6). The need for such a tool is highlighted in the CCTP. Finally we will develop methods for providing high quality science-based information on soil C sequestration potential as input to Integrated Assessment models so soil C sequestration technologies can be compared and evaluated with other technologies for mitigating or reducing greenhouse gas emissions.

The production of bioenergy has the potential to impact net CO$_2$ emissions by both reducing fossil fuel C emissions and enhancing the sequestration of C in soils. Our study will provide quantitative information on the potential for energy crops to sequester C and the trade-offs, if any, between maximizing fossil fuel reductions and maximizing C sequestration. Our models will permit quantification of the total greenhouse benefit of pursuing bioenergy and may suggest locations and crop production technologies that maximize the total benefit. Our exploration of the belowground C-N cycle through the use of isotopes will provide valuable information as to the long-term sustainability of switchgrass production and harvesting for bioenergy. Our examination of the microbial community dynamics under switchgrass and in the context of switchgrass management is responsive to DOE’s recent roadmap on bioenergy.

The efficient development and application of technologies to enhance C sequestration is dependent on understanding the processes that control C sequestration and being able to influence those processes in the direction that promotes C sequestration as well as a net decrease in greenhouse gases. Our research will examine soil C inputs, soil structural controls on the physicochemical stabilization of soil C, microbial community function and dynamics, humification chemistry, and intrasolum transport of C in the context of how those processes influence C sequestration. We will mechanistically examine how these factors are affected by the
application of technologies such as fertilization, altered root C inputs caused by cultivar differences, or the addition of factors such as black C intended to alter enzyme activities and C adsorption and desorption potential. We will capture and test our understanding through the development, validation, and application of a mechanistic model of the C dynamics of switchgrass production for bioenergy. And finally, we will capture the net GHG emissions associated with those technologies and create the modeling capability to extend our mechanistic understanding to the regional and national scale by linking the outcomes of the mechanistic model to a forest and agricultural sector optimization model.

The final result of our study will be a scientifically defensible answer to the question of whether simultaneous production of biofeedstocks for bioenergy and enhancement of soil C sequestration are sustainable.
V. Literature Cited


81


Matamala R., Jastrow JD, Miller RM, Garten CT. Temporal Variations in the Distribution of Carbon Stocks of Restored Tallgrass Prairie Vegetation on Long-term Cultivated Land in the U.S. Midwest. Submitted to *Ecological Applications*.


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Assessment. In B. McPherson and E. Sundquist (eds.) Science and Technology of Carbon Sequestration, American Geophysical Union.


Schmidt I; Sliekers O; Schmid M; Cirpus I; Strous M; Bock E; Kuenen JG and Jetten MSM. 2002. Aerobic and anaerobic ammonia oxidizing bacteria - competitors or natural partners? FEMS Microbial Ecology, 39:175-181


Appendix A

CSiTE Accomplishments and 2005-2006 Progress Report
Appendix A – CSiTE Accomplishments and 2005-2006 Progress Report*

Accomplishments

The overall CSiTE scientific approach has centered on integrated, hypothesis-driven science at multiple scales in the laboratory and the field. As illustrated in Figure 1, research progresses in an iterative manner with emerging data and scientific interpretation driving refinement of the scientific approach and feeding new information and knowledge to mechanistic and economic models. The models, in turn, inform assessments of sequestration capacity, technical feasibility, regional and national potential, and competitiveness with alternative greenhouse gas (GHG) mitigation technologies.

![Figure 1. Conceptual model of CSiTE work flow and research output](image)

A strategic decision was made when conceptualizing the CSiTE field campaign to identify “sites of opportunity” at which past land-use management decisions or changes over time (chronosequences) would act as surrogate experimental manipulations. The map in Figure 2 shows the locations of these sites, encompassing eastern hardwood and coniferous forest, western coniferous forest, various agricultural systems, grasslands and tallgrass prairie restoration. Results from 2 years of preliminary work at numerous sites was used to focus and integrate a more intense, CSiTE-wide program at four primary locations for the past 4 years: the Oak Ridge Reservation, the Fermilab prairie restoration site at Batavia, Illinois, the North Appalachian Agricultural Experiment Station at Coshocton, Ohio, and the Arid Lands Ecology

* Citations in this Appendix are included in Section V (Literature Cited) of the science plan.
Reserve at the Hanford site in eastern Washington state. Some smaller-scale projects were maintained as well with one example being DOE-NETL-sponsored research in collaboration with the USDA Forest Service at the Santee Experimental Forest at Charleston, South Carolina.

Figure 3 is a graphic representation of linkages among the original tasks showing how information from one task was dependent upon, supported another task, or both. As evidenced by more than 150 publications since 1999, our approach has resulted in greater research productivity than if the work been undertaken by individual principal investigators in isolation.
At a higher level, CSiTE research has resulted in a number of significant accomplishments. These include:

*Identification of manipulation concepts to enhance soil C sequestration.* The seminal 2004 *BioScience* paper by Post et al., “Enhancement of carbon sequestration in U.S. soils,” outlined a path from fundamental research to identification and implementation of novel concepts to enhance C sequestration. Following this approach, CSiTE researchers identified and are pursuing research to validate the feasibility of manipulation of soil physicochemical properties (e.g., pH, redox potential), urea for deep C storage, the use of soil amendments (e.g., fly ashes, black C) and land management strategies for enhancing soil C sequestration in a sustainable manner.

*Elucidation of controls on the rates and limits of accumulation of soil organic matter.* The development of new soil fractionation methods led to new insights on physicochemical controls over soil organic carbon (SOC) capture and longevity. Among other findings, the ability to partition particulate organic matter-silt-clay into non-aggregated, macroaggregated and microaggregated fractions resulted in demonstration that microaggregate protection increases longevity of clay-associated C and chemically resistant silt-associated C. Comparison of “old” vs. “new” C suggests that microaggregate fractions in 25 year prairie restoration experiments are not saturated. Mechanistic level understanding of humification chemistry has also been advanced.

*Understanding the role of microorganisms in soil C processing at the community level.* Methods were developed that improved fundamental understanding of the relative contribution to enhanced C sequestration of fungi and bacteria and the importance of mycorrhizae in long-term stabilization in minimally disturbed systems. Microarray technology for investigating microbial relationships to coupled C and N cycling processes was developed, resulting in the largest environmental nucleic acid array to date with over 20,000 genes.

*Advances in modeling tools and their application to landscape-scale processes, full greenhouse gas (GHG) accounting, and economic assessments.* CSiTE research has greatly improved our understanding of the environmental and economic consequences of land management practices to increase soil C sequestration. Enhancement and application of EPIC, APEX, and FASOM extended the basis for C accounting at the landscape level, comprehensive full GHG accounting, and incorporation of soil C in economic models for evaluation against other mitigation options. Transfer and utilization of components of the enhanced modeling tools has been made to U.S. EPA and the cross-agency CCTP.

**2005-2006 Progress Report**

The following progress report is organized by original task as proposed in 1999 and by which CSiTE has been organized and administered to date. Beginning in FY 2007, CSiTE will be re-organized by theme, as detailed in Section III.

**Task 1. Carbon Allocation and Carbon Sequestration Pathways.**

Ecosystem and landscape-scale understanding of soil C and N storage and dynamics are important to strategies for enhancing C sequestration in terrestrial ecosystems because land-cover management and land-use change present the best near-term options for enhancing soil C
The goal of Task 1.1 (Ecosystem and Landscape Scale Studies) has been to discover how land-use changes and complex land-management systems influence C and N cycling in vegetation and soil at the landscape scale. Results from observational and experimental field research in this task have and continue to be used to improve modeling efforts in Task 2.3 (described below) with the goal of achieving a quantitative understanding of soil C sequestration based on analysis of parameters such as plant growth, water balance, nutrient cycling and soil erosion. The role of litter inputs was studied in a forest system at the Oak Ridge Reservation while land management practices in prairie restoration and in different agricultural management systems were studied, respectively, at Fermilab and the North Appalachian Experimental Watershed in Coshocton, Ohio.

The goal of Task 1.2 (Ecophysiological Scale Studies) has been to extend fundamental understanding of microbial processes and soil aggregate properties that control soil C sequestration in managed and restored ecosystems. Principal research sites were Douglas fir stands in the Pacific Northwest and loblolly pine in the southeast, the Fermilab prairie restoration site, the Palouse of eastern Washington, and the Arid Lands Ecology Reserve at the Hanford site. Research was centered on forest nutrient management, grassland restoration from cropland, cropping systems, stabilization of SOC, microbial effects and coupled C/N cycling mechanisms. The goal of Task 1.3 (Molecular and Interfacial Scale Studies) has been to develop a basic understanding of fundamental interfacial and chemical processes that control formation of humus and organomineral complexes and how they govern dissolved organic and inorganic C storage and movement in soil. Research centered on laboratory- and pedon-scale field studies and included a regional-scale assessment of C sequestration potential in deep subsurface soils.

Forest Systems (1.1.1)

At Oak Ridge, significant topographic differences in soil C partitioning were found in the absence of detectable topographic differences in whole soil C stocks. Physical methods were used to partition whole soil C stocks into two pools with relatively fast (years) or slow (decades) turnover times. There was greater partitioning of C to the slow soil pool in mesic, N-rich valleys than on xeric, N-poor ridges and south-facing slopes. Topographic differences in N availability and soil C partitioning in site-specific studies followed the same patterns measured at the landscape scale. Measurements of annual leaf litter inputs, soil respiration, and C stocks in control plots were used to parameterize a two-compartment model of forest soil C dynamics.

Predicted fast and whole soil C stocks under leaf litter exclusion and supplemental leaf litter addition (triple ambient) were in good agreement ($r = 0.95$) with field measurements during the second year of the litter manipulation experiment. The turnover time of fast soil C at a valley site was approximately half that calculated for upland forests. Topographic differences in soil N availability did not translate to differences in forest soil C storage in a way that was easily detected using measurements of whole soil C stocks. At all sites, predicted soil C accrual over the short-term (decades) was primarily due to an accumulation of fast soil C. Both field measurements and modeling indicated that in a comparison of ridge, slope, and valley forests, mesic, N-rich valley soils are a more likely environment for long-term accumulation of soil C in the event of increased soil C inputs. A manuscript was prepared based on this research and submitted to *Water, Air, and Soil Pollution*. 
Soil sampling at the litter manipulation experiment was conducted in 2001 and 2003. Simulations with a two-compartment model indicated that continuation of the experiment over a period of 6 years would produce a 6% loss and a 21 to 33% gain in whole soil C stocks, respectively, under the litter exclusion and the litter addition treatments at a ridge site. Predictions at the valley site over the same time period indicated an 11% loss in whole soil C stocks under litter exclusion and a 14 to 29% gain under supplemental leaf litter additions. At all study sites, predicted gains or losses in whole soil C could be attributed almost entirely to changing amounts of fast soil C. Annual leaf litter transfers or exclusions have been completed each year since the start of the experiment. A final soil sampling was conducted in May 2006 for the purpose of testing model predictions and the utility of the two-compartment soil C model. A manuscript (Topographic differences in forest soil C dynamics: implications for evaluating soil C sequestration potential) was submitted to *Water, Air, and Soil Pollution*.

**Grassland Restoration from Cropland (1.1.2)**

Plots in the Fermilab prairie chronosequence that were originally sampled in 1985 were resampled in 2004 to compare soil C stocks with samples from 1985, 1989, and 1999. Comparison of measured C values in 2004 to those predicted with a model based on 1985 measurements indicated that the space-for-time substitution of the chronosequence approach is reasonably accurate, although plot-level differences in rates of C accumulation were found. Carbon accrual rates in the surface 10 cm were sustained at linear rates over 19 years, with poorly drained prairies building carbon about 1.4 times faster than better-drained prairies. In contrast, a well-drained field planted with C3 Eurasian pasture grasses 3 years before the first prairie plot was restored has not gained carbon at a measurable rate since 1985. Measurement of stable isotopes at each time point indicated that C4-derived organic matter generally contributed more than C3-derived material to soil C accumulation. Although the rate of C accrual in restored prairies appears to be at least partly controlled by soil moisture, this study cannot resolve whether the difference in species composition (C3 Eurasian grass vs. the mixture of C4 and C3 species in the prairie) or differences in soil moisture or drainage conditions were responsible for the lack of C gain in the C3 grassland.

Research at the Fermilab chronosequence shows that restoration of prairie vegetation is highly effective at rebuilding SOC stocks at shallow depths at a rate of 0.33 to 1.5 Mg C ha\(^{-1}\) y\(^{-1}\), depending on soil type. It also showed that cultivation of wet mesic soils causes a depletion of SOC at the depth of plowing but it results in a redistribution of carbon to deeper depths. In the remnant prairie, 77% of the total SOC (to a depth of 1 m) was present in the surface 25 cm of the soil profile. By comparison, only 64% or 68% of total SOC was found in the surface soils of cultivated land or the oldest restored prairies, respectively. Our results showed that restoration of tallgrass prairie can rapidly restore soil organic matter (SOM) lost through cultivation and has the potential to enhance SOC at depth. A manuscript was prepared based on this research and submitted to *Ecological Applications* (Matamala et al. 2006).

Studies also showed that C inputs to SOM are dominated by root and rhizome production. Within the first 12 years of restoration, the aboveground plant mass recovers to levels typical of a remnant prairie, but the recovery of the root system is slower and takes about 52 years. We have used the EPIC model with meteorological and edaphic data collected at Fermilab to simulate the annual rate of soil C accrual for the surface 15 cm of an agricultural field converted
to a pure stand of big bluestem. The accrual rate was underestimated, mostly because the model predicted root and rhizome production inaccurately, particularly in the surface 5 cm. Other simulations are being produced to describe the mixture of C3 and C4 plants typical of the midwest tallgrass prairie ecosystem to simulate the particularities of the recovery of the vegetation and plant diversity to approximate rates of SOC accrual under restoration of native tallgrass vegetation.

A new experiment was conducted in the fall of 2005 to compare the distribution of SOC at depth in cultivated versus native lands. Five paired row crop-prairie remnant fields were sampled across Iowa, Illinois, and Wisconsin to represent wet-mesic soil types within the climate typical of the U.S. Corn Belt region. Each pair consisted on a historically known tallgrass prairie remnant and a nearby long-term-cultivated field, currently planted to corn, on the same soil type. The soils were sampled to 1 m depth, sectioned at intervals of 2.5 cm and 5 cm, and analyzed for SOC. The plots were compared by using a cumulative mass approach to account for variations in effective sampling depth and soil mass caused by cultivation-induced changes in bulk density. Preliminary results showed a decline in SOC in surface soils that accounted for a reduction of 12-32%. This decline was constrained to the depth of plowing, where decades of tillage have lowered and homogenized the concentration of carbon throughout the volume of soil mixed by tillage. However, we also found that total SOC to a depth of 1 m was significantly greater in cultivated compared to remnant soils. Preliminary data show that this increase varied from 8% to 30%, potentially accounting for as much as 4 kg C m⁻². At this time two hypotheses are being evaluated to explain these observations, either independently or working together:

(H1) Carbon gains at depth come from the mixing of surface and subsurface soil during tillage practices, which distributes soil with greater C concentrations deeper in the soil profile.

(H2) Carbon gains at depth are a result of increased downward transport of SOCs in percolating waters under cultivation.

These results suggest that the depletion of SOC in cultivated lands located on poorly drained soils in the U.S. Corn Belt has been largely overestimated. Thus, suggesting that it may be wrong to assume that the depletion of SOC at shallow depths represents past losses of C to the atmosphere. Rather, the redistribution of C to deeper soil profiles may actually enhance the sequestration potential of cultivated lands above the levels present under native vegetation, if the redistributed C can be maintained while surface concentrations are enhanced.

Cropping Systems (1.1.3)

The field experiments were conducted at the USDA North Appalachian Experimental Watershed (NAEW) in Coshocton County, Ohio. The NAEW research station was established in 1938 initially to study the effects of conventional and conservation management practices on soil erosion, runoff and water quality (Puget et al. 2005). The NAEW research station contains a series of small and large watersheds delineated by natural boundaries and artificial berms. These watersheds have historical records of environmental conditions, soil characteristics and distribution, crop productivity, management operations, and, in some cases, surface runoff and soil sediment yield. Puget et al. (2005) selected five distinctly managed watersheds to study the
turnover rate and distribution of soil C in aggregate-size fractions and attempted to relate these to land use changes and soil management. The treatments selected were: 1) secondary forest (mixed white and red oaks with yellow poplars woodland), 2) meadow of orchard grass converted from no-till corn in 1988, 3) no-till (NT) continuous corn since 1970, 4) NT corn-soybean rotation with rye grass as cover crop practiced since 1984, and 5) conventionally (moldboard) plowed (PT) continuous corn since 1984. In two other studies, Blanco-Canqui et al. (2005a,b) used seven long-term NAEW watersheds to study the influence of soil C content and management on soil strength and mechanical properties of soil aggregates. The seven treatments were categorized by degree of soil disturbance and use of organic amendments: 1) PT, chisel plow, disk + manure, NT + manure, NT, pasture, and forest.

Puget et al. (2005) observed large differences in SOC concentration among the treatments studied. In the top 5-cm depth, SOC concentration (g C kg$^{-1}$) was 44.0 in forest, 24.0 in meadow, 26.1 in NT corn, 19.5 in NT corn-soybean, and 11.1 in PT corn. The fraction of total C in corn residue converted to SOC was 12% for NT corn, 11% for NT corn-soybean, and 8% for PT corn. SOC concentration decreased with reduction in aggregate size while macro-aggregates contained 15-35% more SOC concentration than micro-aggregates. In comparison with SOC stocks under forest to 30-cm depth (64.6 Mg C ha$^{-1}$), the proportion of SOC depletion was 24.0% in meadow, 19.8% in NT corn, 26.8% in NT corn-soybean, and 35.1% in PT corn. SOC sequestration averaged 280 kg C ha$^{-1}$ y$^{-1}$ when converting from PT to NT practices.

Blanco-Canqui et al. (2005a) determined cone index (CI), shear strength, bulk density, volumetric water content, and SOC concentration were determined at the summit, backslope, and footslope landscape positions at various soil depths. In general, SOC concentration was slightly higher at footslope than at summit positions in the cultivated watersheds. Soil bulk density was lower at footslope than at summit in NT + manure (1.22 vs. 1.42 Mg m$^{-3}$) and chisel (1.34 vs. 1.47 Mg m$^{-3}$) treatments. The forest treatment had the lowest CI (0.19 MPa), shear strength (6.11 kPa), and soil bulk density (0.93 Mg m$^{-3}$) and the highest SOC concentration (62.7 g C kg$^{-1}$). The opposite was true for the PT treatment. The addition of manure decreased both CI and shear strength while it increased SOC concentration. Results showed that landscape positions had small effect on soil physical properties, but management, particularly the addition of manure, had large and significant effects on soil strength and SOC concentration. In complementary work, Blanco-Canqui (2005b) found that soil macro-aggregates had the lowest tensile strength and density of the same long-term treatments of the previous study. The addition of manure had a positive impact on soil aggregation while excessive tillage had a negative impact.

**Stabilization of Soil Organic Carbon (1.2.2)**

In a collaborative study with J. McCarthy at the University of Tennessee, processes underlying the sequestration of organic matter in soil microaggregates were studied at the submicron scale by using ultra-small-angle x-ray scattering (USAXS) at Argonne’s Advanced Photon Source to evaluate the total porosity and organic-matter-filled porosity within microaggregates. The distribution of nano- and micropores (1 nm to 5 μm) in microaggregates was measured before and after removal of organic matter by combustion at 350°C. Long-term cultivated soils, restored prairies of increasing ages, and a remnant prairie at Fermilab exhibited differences in the proportion of organic-matter-filled pores. The dominant process affecting the accumulation of organic matter in microaggregates appeared to be protection in pores that became entirely
filled with organic matter. The data suggest that physical protection of organic matter may occur via both spatial and kinetic limitations. The pool of organic matter in filled pores that is available to microbes may be restricted spatially to the small area at the throats of these pores. The efficiency of extracellular enzyme-mediated degradation may also be limited because of restricted diffusion of enzymes to organic matter inside filled pores. These barriers could also protect organic matter in pores large enough for microbes to enter if the large pores were “walled off” from microbes and their enzymes by an outer periphery of inaccessible pores filled with organic matter.

**Microbial Effects (1.2.3) and Coupled C/N Cycling (1.2.4)**

Cessation of agriculture and reconstruction of prairie at the Fermilab site increases total microbial biomass and increases the abundance of fungi, particularly arbuscular mycorrhizal fungi, relative to bacteria. We suggest 1) that this observation is caused primarily to reduced disturbance when tillage ceases, and 2) that early changes are reversed later in succession. Vegetation characters also appear to be important; high ratios of microbial cyclopropyl phospholipid to precursors indicate that gram-negative bacterial communities are under stress (i.e., in stationary growth) in agricultural but not prairie soils, probably because C inputs are low relative to N inputs. Although the strongest gradient is the response to cessation of agriculture, a secondary gradient related to successional time is more strongly tied to soil characters, particularly soil bulk density, SOC, and soil organic N. Although the ratio of fungi to bacteria increases with SOC in agricultural soils, this ratio decreases with SOC and with successional time in prairie soils. As a result, improved metabolic efficiency resulting from increased relative abundances of fungi is unlikely to be a mechanism enhancing C storage in these soils. Instead, we suggest that fungi contribute to C sequestration through their role in soil structure and inputs of recalcitrant compounds.

We also evaluated changes in soil microbial community structure with depth in the soil column and across the landscape, along a successional gradient of native prairie grassland restorations. We found that total microbial biomass declined strongly with depth and that the decline was largely attributable to changes in soil C, N, or both. Community composition shifted with depth and age; the relative abundance of sulfate-reducing bacteria increased with both depth and age, while gram-negative bacteria declined with depth. A large component of the depth-induced change in microbial community composition was undetermined, but it might be caused by anoxia lower in the soil column. By simultaneously examining shifts in microbial community structure in two dimensions (successional time and depth), we were able to decouple variables that are strongly correlated in surface soils and reveal indirect rather than direct impacts of soil C on microbial community composition in this system. The ratio of cyclopropyl phospholipids to their precursors increased to a depth of 50 cm and then declined. We suggest that this decline reflects changes in microbial species composition, rather than a decline in stress low in the soil column. We found similar patterns of change across the landscape, regardless of whether shallow soil or an integrated soil column was used in the analysis. This observation suggests that changes in composition of microbial communities across the landscape can be determined adequately from surface soils.
Sequestration Potential in Deep Subsurface Soils (1.3.1)

The area of each series in the STATSGO database was calculated. An area-weighted sample was selected from the list of STATSGO series and identified the most recently characterized pedons in the National Soil Survey Characterization (NSSC) database. The chemistry of the selected series was calculated for data for all series by great group to ensure that selected series were representative. Requests were made to USDA state and county agents to acquire samples from approximately 100 soil series from around the country to perform C sorption isotherms. Thus far, hundreds of horizons from 20 soil series have been obtained, and C sorption and soil characterization has been completed. Because of the magnitude of the request, a memorandum of understanding between DOE/ORNL and USDA was established, and cost estimates were solicited for obtaining additional soils. Because of the cost of obtaining additional soils, we have temporarily suspended additional requests for soils.

Results indicate that Alfisol, Ultisol, and Mollisol B horizons have good sorption capacity for sequestering organic C. This is likely because of their large clay content that is often coated with Fe-oxides. Their slightly to highly acidic pH condition also enhances the sequestration potential of these soils. Ultisols are extensive in the southeast and Alfisols are extensive in the Midwest. Both soil types are dominant east of the Mississippi River. Mollisols are dominant east of the Rocky Mountains and west of the Mississippi River. Thus, the decision was made to consolidate the effort and focus on these three soil orders. The non-trivial task of how to estimate missing bulk density data within the NSSC database was determined and published (Heuscher et al. 2005).

Manipulations to Enhance Subsurface Organic C Pools (1.3.2)

Our goal is to test and resolve the hypothesis that deep subsurface soils can accumulate organic C as a result of near surface manipulations. The effort involves the use of two highly instrumented in situ soil blocks on contrasting soil types and quantifies the impact of coupled hydrological, geochemical, and microbial processes on enhanced subsoil organic C sequestration.

In December 2004, and January 2005, shallow lysimeters were installed at depths of 5, 10, and 15 cm at both Melton and Walker Branch Soil Blocks. A litter pan lysimeter was also installed to monitor the C leaching in the Oa-Oe horizons of the soil. Samples were collected from the soil blocks after rain events and were brought back to the lab for analysis. As in previous years we ran the samples for C, volume data collected, precipitation, pH, bromide, chloride, sulfate, and nitrate. In April 2005, approximately 45 soil cores were taken from both soil blocks. The cores were taken at the same depths as the water sampling ports. These samples were given to Chuck Garten for CHN and $^{13}$C/$^{14}$C analysis. This information would allow background information to be established prior to setting up a tracer study at the soil blocks in 2006.

Laboratory tracer studies were conducted on two intact soil cores were taken from the Walker Branch Soil Block area. The idea was to take two soil cores in an area near the soil block that would be representative of the soil in soil block area. Two intact cores were taken and brought back to the lab to be carved and placed into columns. Urea and bromide were used as tracers.
Data have been analyzed and allow us to determine controls for conducting the tracer study at the Melton and Walker Branch Soil Blocks in Summer 2006.

Storm driven transport of organic C through an Ultisol and Inceptisol suggested that both physical and geochemical processes control fate and transport of C through the soil profiles. The highly fractured Inceptisol exhibited the highest C flux during storm events, which is consistent with its more rapid flow and transport characteristics and lower organic C retention capacity relative to the Ultisol. Mesopore domains along dipping bedding planes served as conduits for organic C movement through the profile. Variability in organic C sorption was a function of solid phase pH, indigenous sorbed organic C, and clay content. Both aromaticity and hydrophobicity measurements suggested that larger organic C molecules were being preferentially adsorbed by the solid phase during movement through the profile (Jardine et al. 2006). These results provide quantitative information on the significance of C credits in deep soil profiles.

Humification Chemistry (1.3.3)

Previous work in this task suggested that co-catalysis of humification occurs by three mechanisms involving physical stabilization of tyrosinase, direct oxidation of the monomers, and promotion of the oxidation and condensation steps by alkaline pH. Although tyrosinase activity is greatest at neutral pH, the large pH dependence of the condensation step drives the overall reaction to maximum rates under alkaline conditions. Following this hypothesis, liming of soils to slightly alkaline pH should enhance net C sequestration. Raising soil pH, however, also is likely to affect the activity of enzymes other than tyrosinase, such as various hydrolases. Hydrolase enzymes promote the breakdown of organic matter, and so the relevant question becomes one of whether the balance between humification and decomposition changes as the pH is altered. Preliminary evidence from the intermediate-scale experiment at the Santee suggests that the balance does change and that decomposition increases relative to humification as a result of raising the pH. As a consequence analytical capabilities were broadened to allow monitoring of a suite of enzymes including tyrosinase, peroxidase, phosphatase, sulfatase, and other hydrolases. In addition, raising pH tends to decrease sorption of DOC to soil surfaces and thereby promotes leaching of DOC into deeper portions of the soil profile. Some evidence for this effect was also observed in the Santee experiment, confirming that two possible “desequestration” mechanisms (hydrolysis and leaching) could occur as a result of raising soil pH by alkaline fly ash amendments.

Given the uncertain gain from the use of alkaline fly ash, and the beneficial results we observed for an amendment with a high-C ash in a calcareous soil, the research focus shifted our focus to the role of unburned C (including charcoal and high-C fly ash). A collaboration was initiated with The Energy Institute at Pennsylvania State University to supply four eastern fly ashes having high unburned C contents (as high as 50%) and moderate acidities (as opposed to alkalinity). The first round of experiments examined the sorption of tyrosinase enzyme to a collection of alkaline and acidic fly ashes, and the impact of this sorption on its activity. The results of these experiments clearly showed that unburned C has a strong sorption affinity for tyrosinase. A collaboration was also developed with the Eprida Corporation located in Athens, GA to test charcoal generated during their innovative hydrogen production process.
Ongoing experimental efforts are focused in two areas. First, we are completing the characterization of the samples from the intermediate-scale experiment at the Santee. This characterization specifically involves analysis of the activity of the suite of enzymes identified above as a function of treatment and depth. Second, we are conducting sorption experiments with a subset of the high-C fly ashes and charcoal samples obtained from our collaborators to determine the effect of the unburned C on the activity of both phenol oxidase and hydrolase enzymes. The results of this work will be used to design a series of experiments involving small (ca. 100-mL) mesocosms as were used for wetting/drying and oxidation/reduction cycle experiments in earlier years of the project.

**Task 2. Carbon Sequestration Assessment.**

Assessment of prospects for enhanced C sequestration requires a basic, integrated understanding of the environmental and economic consequences of land-use change and land management practices that is based on knowledge of fundamental mechanisms across spatial and temporal scales. The goal of Task 2.1 (Estimating Sequestration Potential) has been to use a mechanistic and holistic understanding of soil C sequestration to develop process-based models at the landscape and ecosystem levels for application both to estimating potential and informing the progress of the experimental science in Task 1.

The goal of Task 2.2 (Full Greenhouse Gas Accounting) has been to begin to understand the full range of environmental consequences of land-use and land management practices aimed at enhanced sequestration of soil C. The focus has been on non-CO\textsubscript{2} GHG (N\textsubscript{2}O, CH\textsubscript{4}) and soil erosion and using the information leading to full C and GHG accounting in economic simulations. The goal of Task 2.3 (Balance of Environmental Impacts) has been ecosystem model development, testing and improvement based on input from experimental and observational research results from Task 1.

**Estimating Sequestration Potential (2.1) and Full Greenhouse Gas Accounting (2.2)**

The purpose of this research was to increase our ability to quantify changes in C stocks and net greenhouse gas emissions associated with potential C sequestration strategies. More specifically, estimates of C stocks and GHG emissions needed to be disaggregated by region and land-use practice, production inputs needed to be updated to reflect differences in regional inputs, and a new data compilation effort was needed to update the CSiTE C sequestration database with the large number of field studies that have been published in the last five years. All new and revised data sets were compiled spatially in a geographic information system so that CSiTE research activities could be assessed at regional or national levels.

Hundreds of fertilizers, pesticides, and other production inputs have been identified, and C coefficients have been calculated for these inputs. This work was completed with the assistance of Richard Nelson (Kansas State University) who was funded through DOE NETL with the stipulation that he work with CSITE to complete this task. Some refinements are needed, and a final paper is expected to be submitted by the end of 2006. This work represents complete documentation of C emissions associated with agricultural production inputs.
Estimates of soil C sequestration have been applied regionally and, in conjunction with remote sensing data, to obtain higher spatial resolution of C sequestration potentials. This work leveraged support from CDIAC and NASA. This research is being refined, and a paper will be submitted to Ecological Applications in July 2006. This research enables estimates of C sequestration based on soil attributes, spatial location of land cover and land use, crop rotation and production inputs, and tillage intensity all at the county scale. The methodology uses existing data compiled by a number of U.S. agencies and organizations. Results are more accurate than existing methodologies because of the spatial resolution of land use and soils data, and because of the ability of our method to represent annual shifts in management practices. A formal comparison of methodologies and results may occur under the North American Carbon Program Mid-Continental Intensive Campaign.

Research on the duration of soil C sequestration (CSiTE) was combined with research on soil C capacity (Johan Six, UC Davis) and resulted in a concept paper of how these and other C cycle dynamics interact. It is theorized that regional estimates of soil C capacity could greatly help in estimating the potential to sequester C from individual sequestration strategies (West and Six 2006).

A large unknown in full C accounting was the net C emissions associated with the application of agricultural lime on croplands. A review of C dynamics was conducted and a life-cycle analysis of calcium carbonate was made from application as agricultural lime to the deposition of lime constituents in ocean margins to estimate net C emissions (West and McBride 2005). These estimates are now being used by the U.S. EPA to calculate C flux from croplands in their annual report of U.S. Greenhouse Gas Emissions and Sinks. In a collaborative project funded by the DOE Integrated Assessment Program field data compiled from CSiTE research were analyzed to estimate sequestration potentials for climate regions within North America. These potentials were used in the Integrated Science Assessment Model to estimate the impact of historical changes in climate on soil C that has been sequestered to date in U.S. and Canadian croplands (Jain et al. 2005).

Balance of Environmental Impacts (2.3)

This task focuses on ecosystem process model development, testing and synthesis activities. It integrates experimental results from all elements of Task 1 and informs the economic modeling activities (Task 2.4). Under model development, Izaurralde et al. (2006) used concepts from the Century model to improve the representation of C and N cycling in the EPIC model. EPIC is a widely used and tested model for simulating many agroecosystem processes including plant growth, crop yield, tillage, wind and water erosion, runoff, soil density, and leaching. The new C and N modules developed in EPIC now connect the simulation of soil C dynamics to N transformations, crop management, tillage methods, and erosion processes. The added C and N routines interact directly with soil moisture, temperature, erosion, tillage, soil density, leaching, and translocation functions in EPIC. The improved EPIC model has been tested against short- and long-term data from a 6-year Conservation Reserve Program experiment at five sites in three U.S. Great Plains states and a 61-year agronomic experiment near Breton, Canada (Izaurralde et al. 2006); a 34-year experiment on continuous corn and N additions (He et al. 2006); and three long-term managed watersheds at NAEW (Izaurralde et al., in press). This last study is an example of CSiTE integration between experimental and modeling activities.
This integration concept is outlined in Post et al. (2004), and some preliminary calculations for components are provided in that publication. There are two models that CSiTE has focused on for this synthetic integration—EPIC and FASOM. The process-based biogeochemistry model EPIC (Izaurralde et al. 2006; He et al. 2006; Izaurralde et al. in press) has been developed to be capable of simulating C sequestration over a wide range of edaphic and environmental conditions. Evaluation of EPIC model performance included crop yields, factors affecting crop yields, C inputs to soil, soil bulk density, soil C and N dynamics, microbial C dynamics, N mineralization, runoff, water erosion, and eroded C. For a long-term site in Canada, EPIC accounted for 69% of the variability in grain yields, 89% of the variability in C inputs, and 91% of the variability in SOC content in the top 15 cm depth (Izaurralde et al. 2006). In another simulation study using long-term data from Arlington, Wisconsin, He et al. (2006) found that EPIC captured SOC sequestration and microbial biomass dynamics. Simulated net N mineralization rates, however, were lower than those observed in laboratory incubations. While modeling with EPIC soil C erosion and sequestration at the small watershed scale, Izaurralde et al. (in press) also found good agreement between simulated (43 kg C ha\(^{-1}\) y\(^{-1}\)) and observed values (31 kg C ha\(^{-1}\) y\(^{-1}\)) of eroded C. EPIC overestimated SOC stocks by 21% in one of the three modeled watersheds. An analysis of the simulated Net Ecosystem Carbon Balance revealed that the watershed under a PT system was a source of C to the atmosphere while the watersheds currently under NT behaved as C sinks of atmospheric CO\(_2\).

Spatial data and historical climate data have been assembled for simulations under current management practices for the continental U.S. Preliminary runs have been completed for the period 1960 to 1990 (Figure 4). When this framework is completed EPIC may be used to estimate changes in soil C sequestration for this geographic domain.

![Figure 4](image)

**Figure 4.** Spatial and historical climate data for the continental U.S.
Similar calculations have been made using ISAM (Jain et al., 2006), an integrated assessment model with less mechanistic details than EPIC and by employing empirically based soil C sequestration response functions for conversion to no-till agriculture (West et al. 2004; West and Post 2002). Another manuscript in press (Post et al., in press) indicates how this integration approach can be used to develop an estimate for potential deep soil C sequestration by enhancing DOC leaching in forest ecosystems. A key ingredient in sequestration with this method is soil B horizon sorption capacity (Harrison et al. 2005). Measurements of soil properties associated with sorption capacity indicate that Southeastern US forest soils show the greatest sorption potential (Jardine et al. 2006).

**Economic Analysis of Soil Carbon Sequestration (2.4)**

The Forest and Agriculture Sector Optimizing Model for Greenhouse Gases (FASOMGHG, McCarl and Schneider 2001) has been improved by use of information from EPIC and other CSiTE activities. Improvements of sequestration cost estimates with no-till by incorporating better estimates of sequestration with crop system and land use changes, full GHG exchange calculations are examples. An economic framework, linked to key scientific findings and biophysical models, is needed. Methods are currently being developed to link backward from FASOM to EPIC, the ecosystem simulation modeling system used in CSiTE. This will bring in important spatial and temporal dimensions that allow for observed heterogeneity of GHG responses resulting from varying environmental and edaphic conditions. This approach also allows one to develop data on possible new approaches for terrestrial C sequestration.
Appendix B

2006-2007 Research Transition Plan
Appendix B – 2006-2007 Research Transition Plan

This appendix includes descriptions of activities and tasks required for completion of ongoing research in CSiTE and transition to a focus on C sequestration in switchgrass swards. All CSiTE-affiliated scientists will align their principal efforts around integrated activities at the Milan, Tennessee and Fermilab, Batavia, Illinois switchgrass plots beginning in 2007. The Appendix is organized by original CSiTE task.

Task 1 Carbon Allocation and Carbon Sequestration Pathways

Task 1.1 Ecosystem- and Landscape-Scale Studies

Forest Systems (1.1.1)

Objective: The objective has been to use a combination of landscape-level studies, site-specific measurements, field experiments, and mathematical modeling to better understand soil sequestration potential in temperate, mixed hardwood forests. A leaf litter manipulation experiment was undertaken in 2001 at three study sites on the Oak Ridge Reservation. Litter exclusion and supplemental leaf litter additions were selected to perturb input processes that determine soil C balance and to test the usefulness of a two-compartment model for predicting the accrual or loss of soil C stocks. Litter manipulation was intended to cause a rapid, measurable change in the amount of fast soil C and to determine how this perturbation cascades through the soil system.

Remaining Activities:

- Activity 1. Soil samples collected in May 2006 will be processed with the same methods used in 2001 and 2003.
  - Twelve-week, aerobic laboratory incubations will be used to measure potential net soil nitrogen (soil N) mineralization at different study sites. Soil organic matter will be physically separated into particulate organic matter and mineral-associated organic matter. Whole soils and soil fractions will be analyzed for total C and N using combustion methods. Laboratory analyses will be completed in 2006.

- Activity 2. Measured stocks in whole soils and soil fractions will be compared against predictions from the two-compartment model of forest soil C dynamics using regression analysis.
  - Modeling and model testing will be completed by March 31, 2007. A final manuscript summarizing the results from the field study and the model testing will be completed by June 30, 2007.

Grassland Restoration from Cropland (1.1.2)

Objective: The objective is to quantify the rate of SOC accrual and its distribution in the soil profile in cropland returned to grassland to assess the C sequestration potential of
grassland restoration. Long-term cultivation for crop production leads to loss of soil C, which restoration of perennial grassland has the potential to “recover.”

The chronosequence of tallgrass prairie restorations at the Fermilab National Environmental Research Park is being studied to understand soil C sequestration potentials in restored croplands of the Midwest. Our data suggest that it may be wrong to assume that SOC depletion at shallow depths represents past C losses to the atmosphere. Rather, C redistribution in deeper soil profiles may actually enhance the sequestration potential of cultivated lands above the levels present under native vegetation if the redistributed C can be maintained while surface levels are enhanced.

A manipulative study was established at Fermilab in 2005 to differentiate between the effects of drainage and plant community composition on the rate of soil C accumulation. Soil from unfertilized Eurasian grassland with a stable C isotope signature characteristic of C3 plants was homogenized and transferred to replicate restored prairie plots across a topographic gradient inside the ring in seasonally flooded and poorly drained plots and outside the ring in up-slope and down-slope locations, representing a range of soil drainage conditions. This common soil was placed into cored holes (7.5 cm diameter x 12 cm deep) in patches of exclusively C3 plants or typical prairie mixtures (C4 grasses with C3 grasses and forbs) within each of the prairie plots.

The transplanted soil is not physically separated from surrounding soil, so that roots, fungal hyphae, and soil invertebrates can freely enter it. Transplanted soil will be removed from half of the established locations after 3 years of in situ placement. The remaining soil will be harvested after another 3 years. Soil will be removed by taking smaller cores (3.8 cm diameter x 10 cm deep) from within the transplanted soil. We hypothesize that C in the transplanted soil will increase at rates determined by moisture level and species composition. Physical fractionation of the soil, combined with measurement of stable isotopes in patches with C4 plants, will be used to detect differences in the rate of C change after 3 and 6 years of field placement and used to evaluate the effects of species composition and moisture conditions on the potential for soil C stabilization.

Remaining Activities:

- Activity 1. Monitoring soil C content at Fermilab to substantiate estimated rates of SOC accrual.
  - This task will involve obtaining and processing soil samples at several restored prairie sites at Fermilab at 2-year intervals.

- Activity 2. Completing the simulations using the EPIC model to evaluate the relative importance of root inputs versus plant species composition for SOC accrual after restoration of grassland vegetation.
  - A manuscript based on this task will be completed by fall 2007.
Activity 3. Identifying processes resulting in C accumulation at depth during cultivation and mechanisms for maintaining this C during restoration practices.

- Soil will be processed for $^{14}$C measurements leading to completion of a manuscript on the corn-remnant experiment in 2006.
- Experiments will be performed with new soil materials that differ from the mesic soil to discern the extent of the phenomena.

Relationship to New Science Plan: This work relates to a number of the new themes. Theme 1, Soil Carbon Inputs, and Theme 6, Mechanistic Modeling, are addressed in the completion of the EPIC model including modifications incorporated from information gathered during Fermilab transition activities. Theme 5, Intrasolum Carbon Transport, is addressed by the completion of work elucidating mechanisms controlling C storage at depth in row cropping. Underlying assumptions for Theme 7, Integrated Evaluation of Carbon Sequestration Technologies, may be modified if results suggest that SOC depletion in cultivated systems on poorly drained soils in the U.S. cornbelt has been overestimated.

Cropping Systems (1.1.3)

Objective: The objective of this work has been to use a combination of landscape-level experiments, site-specific measurements, and mathematical modeling to better understand how land-use change and complex land-management systems influence C and N cycling in vegetation and soils. The field experiments were conducted at the USDA North Appalachian Experimental Watershed (NAEW) in Coshocton County, Ohio. Five distinctly managed watersheds were selected to study the turnover rate and distribution of soil C in aggregate-size fractions with the intent to relate results to land use changes and soil management. The treatments selected were: 1) secondary forest (mixed white and red oaks with yellow poplars woodland), 2) meadow of orchard grass converted from no-till (NT) corn in 1988, 3) NT continuous corn since 1970, 4) NT corn-soybean rotation with ryegrass as cover crop practiced since 1984, and 5) conventionally (moldboard) plowed (PT) continuous corn since 1984. Companion studies used seven long-term NAEW watersheds to study the influence of soil C content and management on soil strength and mechanical properties of soil aggregates. The seven treatments were categorized by degree of soil disturbance and use of organic amendments. Model development also is using results from other CSiTE field studies, including the Fermilab prairie restoration site.

Remaining Activities:

- Activity 1: Simulation of soil C dynamics in the long-term prairie restoration chronosequence located at Fermilab in Batavia, Illinois.
  - Simulations will be conducted of changes in net primary productivity, soil C, and soil bulk density that occur during the transition of agricultural land use to native prairie conditions. Simulations have been undertaken in the latest version of EPIC equipped with algorithms to estimate changes in soil bulk density as affected by soil C concentration. The simulations will be calibrated,
and a set of runs will simulate the difference in soils under C3 and C4 grasses. The results will be analyzed by December 31, 2006, and a manuscript discussing the simulation results will be submitted for publication by June 30, 2007.

- Activity 2: Completion and use of a national climate, soil, and management dataset to conduct national-scale simulations of soil C sequestration.
  - Regional- and national-scale simulations of soil C sequestration and current and alternative management scenarios under historical climate and climate change scenarios will be completed. Approximately 30,000 runs are necessary to produce estimates of soil C change under the baseline scenario across ~1400 U.S. watersheds. The national database will be run under a variety of management and climate scenarios (e.g., changes in tillage, crop mix, and climate (e.g., precipitation, temperature, CO$_2$ concentration)). Preliminary runs have been made, and the database is being calibrated with final runs to be made in August 2006. Documentation of the methods and results will be summarized in a manuscript in 2007.

Relationship to New Science Plan: Research conducted under all of Task 1.1 relates directly to Theme 6, Mechanistic Modeling, of the new CSiTE Science Plan in which EPIC serves as the integrative tool for all of the other six themes. The first transitional activity will tie directly into Themes 1-5 by integrating mechanistic modeling with ongoing field studies at Fermilab on grassland composition and soil C. By incorporating modeling of C4 grasses, this will contribute information to help develop research questions in Themes 1-5 and also begin preparing EPIC for modeling switchgrass at the new CSiTE field location(s). Transition activities will contribute to Theme 6 by developing and demonstrating the capacity of EPIC to simulate soil C under different grassland ecosystems, which will also improve large-scale carbon assessment capabilities under Theme 7. Once documented, the national EPIC database from the second task can be modified to produce information necessary for national-scale assessments of soil C using the FASOM model. Results will be compared with other CSiTE methodologies for national-scale assessments and therefore will provide a strong basis for future work under Theme 7.

**Task 1.2 Ecophysiological-Scale Studies**

**Forest Nutrient Management (1.2.1)**

There are no transition activities planned for this subtask. A description of the research and accomplishments is included in the accomplishments section (Section II) of the science plan narrative.

**Stabilization of Soil Organic Carbon (1.2.2)**

Objective: The objective of this task is to determine how management practices and intrinsic factors including soil type and climate affect C inputs to pools with varying residence times and the potential for enhancing long-term C storage in protected pools. A
mechanistic understanding of factors controlling organic C transformations and stabilization is required to optimize management strategies for enhancing sequestration and improving our ability to predict soil C storage potentials. Research includes efforts to determine 1) the effect of vegetation type and soil drainage conditions on soil C accrual and stabilization, and 2) the role of soil aggregates in the physicochemical protection and stabilization of organic matter.

Remaining Activities:

• **Activity 1:** Work on the time-series samples collected from several Fermilab prairie plots, and the C3 grassland in 1985, 1989, 1999, and 2004 will be completed in 2006.
  
  o Analysis of C, N, and their isotopes in soil from the 10-20 cm depth and root samples from 0-10 and 10-20 cm is currently under way. Soil moisture is being measured in each plot so that the rate of soil C accrual can be directly related to seasonal moisture levels. Two manuscripts will be submitted by April 2007.

• **Activity 2:** The influence of soil drainage conditions and plant species composition on soil C concentrations and root biomass existing at the time of transplanting the common soil will be determined.
  
  o Soil cores removed to create holes for transplanting a common soil into patches of prairie vegetation and patches of exclusively C3 plants in Fermilab prairie plots with differing drainage conditions are currently being processed.
  
  o The first harvest of transplanted common soil will be conducted in October 2007. Samples will be frozen until sufficient time and personnel are available to process, fractionate, and analyze them
  
  o Stable isotopes will be used to determine the proportions of C3-C and C4-C in roots and soils. These activities will be completed by March 2007. Soil moisture conditions at each site are being monitored periodically.

• **Activity 3:** Additional studies designed to understand the distribution of nano- and micropores (1 nm to 5 μm) in silt- and clay-sized particles are under way in collaboration with J. McCarthy at the University of Tennessee. A manuscript on changes in organic-matter-filled porosity within microaggregates as a mechanism for carbon accumulation will be submitted in 2006.

• **Activity 4:** Ongoing studies to explore potential saturation of SOM protective mechanisms are addressing the feasibility of and potential limits to soil C sequestration enhancement as well as the potential longevity of accrued soil C.
  
  o Changes in organic matter in the Fermilab chronosequence prairies associated with POM, silt, and clay will be investigated at four levels of
aggregate protection (non-aggregated, macroaggregate, microaggregate, and microaggregate-within-macroaggregate). While few pools will likely reach equilibrium levels in the time range included in this study, the chronosequence technique can be used to predict the time to achieve equilibrium C levels in different pools by using the Fermilab chronosequence prairies with the agricultural field and remnant prairie as endpoints. This work, which will be carried out by a graduate student funded with a DOE Global Change Education Program Fellowship, will be completed in 2007.

- Activity 5: This ongoing activity examines the role of organic matter protective mechanisms on soil C dynamics on decadal and longer time scales. These longer-term dynamics are critical for determining whether soil C sequestration strategies can be implemented with any success.
  
  o The Fermilab C3-grassland that appears to have reached steady-state by 1985 was established in 1972 on cultivated soil that formed under C4 vegetation. Archived soil samples collected between 1985 and 2004 from will be physically fractionated by using methods similar to those in Activity 4.

  o Natural abundance stable carbon isotopes will be used to determine the turnover rates of soil carbon pools with decadal or longer dynamics by quantifying the loss of C4-derived C and its replacement by C3-derived C.

  o Soil fractions will also be chemically characterized to determine the source, quality and level of degradation and microbial processing of organic matter in pools with varying residence times. This work will also be carried out by the graduate student funded with a DOE Global Change Education Program Fellowship and will be completed in early FY 2008.

Relationship to New Science Plan: This research will provide quantitative information on the rate and quality of soil C accumulation under native tallgrass prairie and Eurasian pasture grasses that can be used to assess the potential for soil carbon accrual under switchgrass. In particular, direct comparisons can be made for the planned switchgrass experiments conducted at Fermilab. Information on the role of soil drainage conditions on soil C accrual can be used with these comparisons to extrapolate the results of switchgrass studies at Fermilab to a broader range of soil types and to validate models. Work on understanding the role of physical and chemical protection mechanisms on soil C stabilization, accumulation, turnover rates, and the potential saturation of these mechanisms will be directly transferable to understanding the potential for soil C sequestration and its stabilization in switchgrass bioenergy production systems. As such, this information can contribute to all experimental themes with particular relevance to the aggregation, microbial, and humification chemistry themes.
Microbial Effects (1.2.3) and Coupled C/N Cycling (1.2.4)

Objective: The structure and activity of the soil microbial community influence the cycling and stabilization of freshly added organic residues. Research has focused on two questions: 1) Are certain groups of microorganisms more strongly associated with high-C soils than others? 2) Can shifts in the structure and abundance of the microbial community be detected in soils storing C? Results to date provide insight into biological indicators of enhanced C storage and might also identify organisms whose activities, if enhanced by appropriate land use management practices, could increase soil C sequestration.

Remaining Activities:

- Activity 1. Nitrogen dynamics over 2 and 5 years will provide insights to potential C sequestration in the field.
  
  o The objective of this study is to determine the fate of N that was presumably co-sequestered with C and thus incorporated into various soil C pools 2 and 5 years post-labeling.
  
  o The $^{15}$N label will be a surrogate for C dynamics to provide quantitative information on the fate of new C inputs into the system and the recalcitrant nature of these inputs.
  
  o Ninety-day incubation is under way to measure the CO$_2$ and $^{15}$N mineralized from the $^{15}$N-treated soils. An addition of 20 µg-N/g soil of $^{14}$N was applied. With this treatment, the potential priming effects of the sequestered $^{15}$N are being investigated.
  
  o The experiment should and associated data analysis and publication will be completed in 2006.

- Activity 2. Relationships between C sequestration and substrate utilization in diverse soils.
  
  o The objective of this study is to determine the relationship between initial substrate use and longer-term C storage in different soils. This analysis will provide basic information on microbial metabolism related to the transformation of C into more stable forms and could provide management strategies for increased soil C storage.
  
  o A 125-day incubation is in progress with six $^{14}$C labeled substrates. At the end of the incubation, the remaining $^{14}$C substrate will be measured and correlated with use.
  
  o The experiment should and associated data analysis will be completed in 2006 and published in 2007.
• Activity 3. Microarray detection of the expression of lignin-degrading enzymes in soils. A microarray was used to assess the expression of lignin-degrading enzymes in soils (lignin peroxidase, laccase, manganese peroxidase, and glyoxyl oxidase). The experiment demonstrated that mRNAs can be detected in soils and can be used to report the expression of selected fungal, genes rapidly and specifically.
  - Final data analyses are being conducted and a manuscript ("Microarray detection of lignin degrading gene expression in soils") will be submitted in 2006.

• Activity 4: We will continue quantifying inputs of arbuscular mycorrhizal fungi along a successional gradient of native prairie grassland restorations.
  - Fungal inputs will be quantified by (1) measuring AM hyphal lengths using the membrane filtering procedure, (2) using the AM fungal phospholipid marker 16:1ω5c, and (3) 37 μm mesh hyphal in-growth bags. The first two approaches allow determination of the equilibrium standing crop for AM hyphal biomass. The third approach determines annual hyphal production.

Relationship to New Science Plan: This research is directly related to Theme 3, Microbial Community Function & Dynamics, which deals with soil microbial processes. Transitional research has direct relevance to the new science plan in that an important aspect of the new direction is the need to understand the influence of microbial community structure and function on soil C dynamics in switchgrass swards. Moreover, in addition to inputs, residues associated with microbial production and turnover; for example, glomalin, melanin, and chitin residues also need to be quantified. Especially important will be an understanding of the trade-offs between selection for roots with fine fibrous roots and that of coarser fine roots that rely more on AM hyphae.

Task 1.3 Molecular and Interfacial Scale Studies

Assessment of Sequestration Potential in Deep Subsurface Soils (1.3.1)

Objective: The objectives of this task are to 1) quantify the relationship(s) between subsoil organic C sequestration and soil physical, hydrologic, and geochemical properties; 2) develop a geographic method for estimating the C storage capacity of subsurface soils (B-horizons) within the U.S.; and 3) identify regions and field sites that offer the greatest potential for enhanced subsurface organic C storage and thus are most deserving of manipulation or innovative management. To date, hundreds of samples from different horizons from 20 soil series have been obtained with USDA state and county agents. The original intent was to acquire samples from ~100 series.

Remaining Activities:
• Activity. Isotherm modeling is under way for the subhorizons of the 20 series that have been received.
Multiple linear regression models will be constructed in 2007 to predict the fitted asymptotic carbon sorption maximum from the soil characterization data.

Samples will be obtained from 10 to 20 additional series in Alfisols and Inceptisols. Soil characterization and isotherm measurements will be made on these soils, the isotherms modeled, and the characterization measurements will be used to estimate the sorption maximum.

Relationship to New Science Plan: This research is directly related to Theme 5, Intrasolum Carbon Transport, and Theme 7, Integrated Assessment of Carbon Sequestration Technologies, and it may also provide useful information for Theme 6, Mechanistic Modeling. The statistical models developed to predict carbon adsorption capacity from soil properties will be useful in assessing the amount of DOC that might be sorbed by subsurface soils at the two field sites (Milan and Fermi). When linked with the STATSGO soil survey database and historical soil characterization data, we can generate regional estimates of subsurface carbon sequestration capacity. This capability will help address the question of the national potential for carbon sequestration that is part of Theme 7. Finally, it may be possible to incorporate the statistical models of carbon sequestration capacity into the mechanistic models being developed in Theme 6.

Manipulations to Enhance Subsurface Organic C Pools (1.3.2)

Objective: The overall goal is to test and resolve the hypothesis that deep subsurface soils can accumulate organic C as a result of near-surface manipulations. The effort has involved two highly instrumented in situ soil blocks on contrasting soil types to quantify the impact of coupled hydrologic, geochemical, and microbial processes on enhanced subsoil organic C sequestration. Specific objectives are to 1) quantify the magnitude of enhanced organic C accumulation in deep Ultisol and Inceptisol subsoils that have been treated with amendment strategies designed to accelerate the mineralization and dissolution of surface organic matter, 2) quantify the impact of coupled hydrologic and geochemical processes on the fate and transport of solubilized organic C through the soil profile, and 3) quantify the chemical nature of the sequestered C and the mechanisms responsible for immobilization on the solid phase.

Remaining Activities:

• Activity. Continued observations of fate and transport of organic C and associated anions as a function of storm events in two well-characterized, highly instrumented field pedons on contrasting watersheds. An Ultisol pedon was chosen because this soil type has a closer relationship, in terms of hydrology and geochemistry, to the Alfisol that will be tested in the new CSiTE science plan.

  o Manipulations of the in situ soil pedon in the Ultisol at the Walker Branch Watershed (WBW) on the Oak Ridge Reservation began in July 2006 and will continue for 6 months.
Initially apply a dilute wetting solution consisting of 2 mM CaCl$_2$ to prevent sequestration of applied solutions by capillary suction into the dry matrix pores.

Manipulations will consist of solid-phase urea, urea labeled with $^{13}$C and $^{15}$N, and the nonreactive tracer bromide. Once the pedon is wetted, simulated rainfall will be paused and solid urea granules (45% N) will be uniformly broadcast on the litter layer at a rate of 550 kg/ha N. Solid-phase urea labeled with stable isotopes $^{13}$C and $^{15}$N will be simultaneously applied to provide an isotopic tracer of the applied C and N; that is, to quantify the source term. The isotopic signature of the pedon has been previously characterized and is distinct from the applied tracers. High pH conditions develop in the immediate vicinity of hydrolyzing urea granules that renders otherwise resistant humus complexes soluble and available for transport through the soil horizon. Following application of solid urea, the drip irrigation system will be replaced with solution containing the nonreactive tracer Br (as 2 mM CaBr$_2$) to determine the flowpaths and residence times of the simulated rainfall.

The irrigation system delivers simulated steady-state rainfall. Movement of C and anions is monitored within the multiporosity samplers as a function of depth in the pedons. Once the meso- and micropore domains have been activated, as evidenced by drainage through the profiles, the manipulations will be initiated. Macropore domains are expected to function only during actual or simulated storm events. With the progress of seasons, the pedon will be monitored during storm events for changes in the concentrations of applied and natural constituents.

The progress of applied Br, dissolved organic carbon (DOC), $^{13}$C, N as NH$_4^+$ and NO$_3^-$, $^{15}$N, changes in pH, and other resident anions (Cl, SO$_4$, PO$_4$) will be monitored as a function of time and depth in the pedon. Additional analyses include dissolved inorganic carbon (DIC) as an indicator of changes in soil respiration, and nitrous oxide (N$_2$O) as an indicator of urea hydrolysis and loss from the system.

The upper portions of the pedon (O and A horizons) will be monitored using the recently installed litter pan lysimeter to monitor leaching from the Oa-Oe horizons, while subsurface soil lysimeters at depths of 5, 10, and 15 cm will be used to monitor fluxes of dissolved constituents from the O and A horizons.

Two additional types of solution samplers installed directly beneath the Oe horizon and within the A-horizon will also be monitored. Leachate passing through the Oe horizon will be routed through small columns packed with XAD-8 and XAD-4 resin buried in the underlying A-horizon. The XAD-8 resin will concentrate hydrophobic DOC, and the XAD-4 resin will concentrate hydrophilic DOC. The resin columns will be periodically changed out in the field, and the carbon mass will be stripped from the resin and...
quantified. Solution sampling within the A-horizon, as a function of depth, will use Prenart super quartz soil water samplers designed for soil nutrient monitoring. Continuous tension will be applied to the samplers, and the solution will be monitored with time. Spatial and temporal monitoring of leachate moving through the Oe and A horizons will provide a known source term, thus enhancing our ability to quantify DOC movement into the B-horizons.

- Leachate in the deeper portions of the pedon (B horizon) will be monitored in spatially distributed solution samplers continuously and during storm events. The pedon has multiporosity sampling capabilities as a function of depth, which will provide a means of computing DOC and solute fluxes through the profiles in macro-, meso-, and micropore domains. Zero tension and coarse fritted glass samplers, held at 20 cm tension, will extract fast-flowing soil water moving through macropores and mesopores. Fine fritted glass samplers will be held at 250 cm tension for extraction of soil water associated with micropores (i.e., the soil “matrix”). The multiporosity sampling strategy will allow quantification of both preferential (macropore) C transport and diffusional exchange with micropores that may sequester or release C, depending on hydraulic conditions.

- Monitoring $^{13}\text{C}$ and $^{15}\text{N}$ isotope levels in the solution-phase DOC will determine the relative contributions of new and old C and N sources. The results will be compared with background DOC and N flux data from previous years.

- Analysis of results will be published in 2007 and 2008.

Relationship to New Science Plan: Results from the pedon manipulations at WBW will provide a baseline for the future work at Milan and provide formative answers to several of the science questions posed in the new research. For example, the WBW studies will determine the extent to which the various soil horizons (O, A, B) act as sources or sinks for DOC during intersolum transport. In addition, the influences of preferential flow, which may accelerate the mobility of DOC, and matrix diffusion, which may decrease the mobility of DOC and/or increase the degradation of DOC, will be assessed. Finally, the research will determine whether N fertilization is capable of mobilizing DOC into deeper regions of the soil profile. The results of the current manipulations will be distinct from the new science plan, however, because the WBW pedon is located in a forested watershed. Thus, we will also be able to determine the applicability of our results in a forested watershed toward findings from the agricultural management strategy. This has potential long-ranging applications, because manipulations of C mobility in both settings may ultimately be necessary to reduce the atmospheric C load.

Humate Formation Chemistry (1.3.3)

Objective: The primary objective has been to develop an understanding of humate formation chemistry to guide the selection of potential manipulations to enhance soil C sequestration.
Focus has been on a model humate formation reaction involving the oxidation of phenolic monomers and subsequent condensation of the quinones with amino acids to form humates. The reaction can be catalyzed by phenol oxidase enzymes and forestalled by hydrolases that break down the monomers before they can form the more recalcitrant humates. Work has included bench-scale studies of enzyme activity in the presence of various solids, as well as participation in an intermediate-scale experiment at the Santee experimental forest that tested the possible use of low-C alkaline fly ash as an amendment to promote humification. In general, experiments show that a pH increase results in less net humification in the surface horizon, likely as a result of concomitant increases in hydrolase activity and desorption of organic matter leading to higher DOC levels in the subsoil. Also noted was that presence of charcoal and similar black C substances can enhance net humification. Future work will include a larger focus on this aspect of humification chemistry.

Remaining Activities:

- Activity 1. Complete remaining analyses of samples from the Santee intermediate-scale experiment, interpret data, and publish results.
  - Isotopic analyses for $^{13}$C and repeated analysis of enzyme activities on Santee soil samples will be completed by 31 August 2006.
  - The complete dataset will be compiled, results interpreted, and a manuscript written by 15 January 2007.

- Activity 2. Complete scoping experiments involving sorption of enzymes to a variety of black-C-bearing materials (high-C fly ashes from coal and wood combustion). Determine the net effect of sorption to black C on activity of oxidases and hydrolases; refine hypotheses regarding black C and humification chemistry.
  - Collection of sorption/enzyme activity data will be completed by 15 October 2006.
  - A manuscript will be prepared by 15 April 2007.

Relationship to New Science Plan: This work flows directly into that outlined in Theme 4, Humification Chemistry. The Santee experiment represents a first attempt at manipulations to enhance C sequestration (raising pH), and the results from this experiment will guide our future selection of soil chemical manipulations. The black-C sorption experiments will lay the groundwork for follow-on experiments in the science plan focused on possible soil chemical manipulations. Both of these transition activities also inform activities in other theme areas, such as Theme 5, Intrasolum Carbon Transport, where pH changes can influence DOC transport, and Theme 3, Microbial Community Function & Dynamics, where the relationships between enzyme/fungal activity and community structure will be explored.
Task 2. Carbon Sequestration Assessment

**Estimating Sequestration Potential (Task 2.1) and Full Carbon Accounting (Task 2.2)**

Objective: The objective is to increase our ability to quantify changes in C stocks and net greenhouse gas emissions associated with different C sequestration strategies. Estimates of C stocks and greenhouse gas emissions were disaggregated by region and land-use practice and production inputs updated to reflect differences in regional inputs.

Remaining Activity:

- A new data compilation effort is under way to include large number of field studies that have been published in the last 5 years. All new and revised data sets are being compiled spatially in a geographic information system so that CSiTE research activities can be assessed at a regional or national level with respective estimates of uncertainty.
  - Research on the spatial delineation and refinement of soil C stocks, flux, and greenhouse gas emissions is near completion. Two manuscripts will be submitted in 2006.
  - Once these manuscripts are accepted, data and maps of annual C flux for the U.S. will be archived with CDIAC and made accessible via the internet.

Relationship to New Science Plan: This research is directly related to Theme 7, Integrated Evaluation of Carbon Sequestration Technologies. Once completed and reviewed, C coefficients associated with land management inputs will be transferred to that Theme for use in economic analysis. Research that has been conducted on spatially locating and refining estimates of soil C flux will be used to enhance regional estimates and full C accounting in the EPIC model. EPIC has made many improvements in dynamically modeling C and N cycling associated with changes in land management. Under the new science plan, we will work to improve the spatial heterogeneity of EPIC. Because EPIC output is used to develop scenarios for socio-economic analyses, we expect that a higher resolution of regional C modeling will increase the accuracy of regional economic analyses. Under the new science plan, an effort will be made to more tightly link full C accounting, EPIC modeling, and economic analyses of C sequestration.

**Task 2.3 Balance of Environmental Impacts**

Objective: The objective of Task 2.3 was to develop a quantitative understanding of the environmental impacts of soil C sequestration through model analysis of ecosystem processes such as plant growth, water balance, nutrient cycling, and soil erosion.

Remaining Activity: Complete regional and national scale simulations of soil C sequestration and current and alternative management scenarios under historical climate and climate change scenarios. Approximately 30,000 runs are necessary to produce estimates of soil C change under the baseline scenario across ~1,400 US watersheds. The national database will be run under a variety of management and climate scenarios (e.g., changes in
tillage, crop mix, and climate). Preliminary runs have been made and the database is being calibrated with final runs to be made by August 2006 and documentation of the methods and results will continue through January 2007.

Relationship to New Science Plan: Research conducted under this two task builds on original Task 1.1 and relates directly to Theme 6, Mechanistic Modeling, of the new science plan for which EPIC will serve as an integrative tool. Once documented, the national EPIC database can be modified to produce information necessary for national scale assessments of soil C using the FASOM model. Results will be compared with other CSiTE methodologies for national-scale assessments and therefore will provide a strong basis for future work under Theme 7, Integrated Evaluation of Carbon Sequestration Technologies. The national database will also be modified to simulate switchgrass and other biomass crops using research results from Themes 1-5 and will directly contribute to Theme 6. Spatially extensive simulations will provide key information on technically potential carbon sequestration amounts and provide a basis for estimating price per ton of carbon sequestration. This information will be required for economic analyses of Theme 7 using FASOMGHG.

Task 2.4 Economic Analysis of Soil Carbon Sequestration

RD Sands (PNNL), B McCarl (Texas A&M)

Objective: The primary objectives of this task have been to 1) develop methods for the full appraisal of the cost of sequestering C in terrestrial ecosystems; and 2) address the ways C sequestration in terrestrial ecosystems contribute, relative to other options, towards stabilization of the atmosphere. This includes an analysis of the desirability of soil sequestration relative to other agricultural and non-agricultural options. On the agricultural side this involves comparison with forest sequestration, biofuels, and livestock emissions, among others considering the role of CO$_2$ and non-CO$_2$ greenhouse gases. On the non-agricultural side this includes the desirability relative to other energy system options. This task is the only task in the CSiTE program that considers costs and how cost measures can be used to compare greenhouse gas mitigation activities across economic sectors and over time.

Remaining Activity: Previous work on methods to compare terrestrial mitigation options to other greenhouse gas options is summarized in a paper included in the forthcoming special issue of *Climatic Change* (McCarl and Sands, 2006). In addition, the FASOM model has been used to support numerous static and dynamic simulations resulting in many publications.

Relationship to New Science Plan: The objectives of the original Task 2.4 will remain and be incorporated into Theme 7 as cost competitiveness and the potential roles of dedicated bioenergy crops and agriculture remains a question, but with a change in emphasis in two fundamental ways. First, the interface between the EPIC model (Theme 6) and the FASOM model (Theme 7) will be a separate task and receive more attention than continued development of FASOM itself with the objective being to develop a system that allows bottom-up appraisal of agricultural sequestration and biofuel options that links directly to EPIC, thus building on the experimental results from Themes 1-5. Second, instead of actually
doing a top-down economic analysis, we will emphasize providing analysis that is directly relevant and useful to the full consideration of typically omitted agricultural sequestration and biofuel options in integrated assessment modeling analyses. Therefore, economic analysis activities in CSiTE should be viewed as complementary to integrated assessment activities by other groups. One measure of success would be when integrated assessment teams use CSiTE analysis to expand their set of greenhouse gas mitigation options to include terrestrial sequestration, including soil sequestration concomitant with dedicated lignocellulosic biofuel crops.
Appendix C

C-SiTE Publications (2000-2006)

2006


An introductory chapter (McCarl et al. 2006) and two papers (West and Six 2006, McCarl and Sands 2006) were contributed to a special issue of Climatic Change. These papers have been accepted for publication


2005


2004


**2003**


2002


2001


2000


Murray, BC, and BA McCarl. 2000. “U.S. Potential for increasing forest Carbon sinks above business-as-usual scenarios: an economic analysis.” Prepared for the Inter-Agency working group on land use and forest sinks, under the direction of the US Environmental Protection Agency.
Appendix D

Biosketches
Appendix D – Biosketches

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1986–1993 Research Scientist (Level II), Pacific Northwest National Laboratory

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS
Amonette, JE, SM Heald, and CK Russell. 2003 “Imaging the heterogeneity of mineral surface reactivity using Ag(I) and synchrotron X-ray microscopy.” Phys. Chem. Miner. 30:559-569.

SYNERGISTIC ACTIVITIES


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Collaborators (past 4 years): Ercan Alp (ANL-APS), Rolf Arvidson (Rice University), Barbara Balko (Lewis and Clark College), Haluk Beyenal (Montana State University), Daniel Gamelin (University of Washington), Chuck Garten (ORNL), Gil Geesey (Montana State University), Steve Heald (ANL-APS), Julie Jastrow (ANL), Jim Kirkpatrick (University of Illinois at Urbana-Champaign), Zbigniew Lewandowski (Montana State University), Andreas Luttge (Rice University), John Miao (UCLA), Tony Palumbo (ORNL), R. Lee Penn (University of Minnesota), Brent Peyton (Montana State University), W. Mac Post (ORNL), You Qiang (University of Idaho), Rajesh Sani (Washington State University), Dan Strawn (University of Idaho), Wolfgang Sturhahn (ANL-APS), Tom Toellner (ANL-APS), Paul Tratnyek (Oregon Health and Science University), Carl Trettin (USFS).
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2000-2002 Post-Doctoral Fellow, Pacific Northwest National Laboratory
1999 Research Agronomist, Alberta Agriculture Food and Rural Development

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FIVE SELECTED PUBLICATIONS (MOST RELEVANT)
Garten, Jr., CT, and PJ Hanson. 2006. Measured forest soil C stocks and estimated turnover times along an elevation gradient. Geoderma (in press).

FIVE ADDITIONAL PUBLICATIONS
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FIVE SELECTED PUBLICATIONS (MOST RELEVANT)
“Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of
a billion-ton supply.” DOE/GO-102005-2135; ORNL/TM-2005/66, Oak Ridge National
Laboratory, Oak Ridge, TN 37831.
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greenhouse warming, biomass-based energy supply systems and accelerated domestication of
energy crops.” Pgs 12-45. In Applications of Biotechnology to Mitigation of Greenhouse
Warming; Proceedings of the St. Michaels workshop, April 13-15, 2003, St. Michaels,
Maryland. NJ Rosenberg, FB Metting and RC Izaurralde (eds). Battelle Press, Columbus,
Ohio.
watershed restoration in regions of the west: What are the environmental/community
Partnerships, October 4-8, Madison, Wisconsin. Great Lakes Regional Biomass Energy
Program, Chicago, Illinois.
Graham, RL, LL Wright, and AF Turhollow. 1992. The potential for short-rotation woody crops

FIVE ADDITIONAL PUBLICATIONS
Workshop, March 17-20, Canberra, Australia. R. Gamble and G. Page (eds). University of
Toronto Press, Toronto, Canada.


SYNERGISTIC ACTIVITIES


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FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

D-9

FIVE ADDITIONAL PUBLICATIONS

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COLLABORATORS AND OTHER ASSOCIATIONS
Collaborators: JE Amonette (PNNL), JS Amthor (DOE), MA Arshad (Agric. Canada), JD Atwood (USDA), VL Bailey (PNNL), VW Benson (Univ. of Missouri), H Blanco-Canqui (Ohio St. Univ.), N Bliss (USGS), RA Brown (IPA Consulting), CA Campbell (Agric. Canada), CC Cerri (Univ. Sao Paulo), S. Drake (Univ. of Arizona), HK Fang (Univ. of Maryland), CF Fletcher (Alberta Agric.), SJ Ghan (PNNL), TW Goddard (Alberta Agric.), RF Grant (Univ. of Alberta), KH Haugen-Kozyra (Alberta Agric.), X He (JGCRI), C Hutchinson (Univ. of Arizona), JD Jastrow (ANL), PM Jardine (ORNL), NG Juma (Univ. of Alberta), JR King (Univ. of Alberta), R Lal (Ohio St. Univ.), FJ Larney (Agric. Canada), DM Legler (CLIVAR), RL Lemke (Agric. Canada), LR Leung (PNNL), SL Liang (Univ. of Maryland), CW Lindwall (Agric. Canada), LK Mann (ORNL), SE Marsh (Univ. of Arizona), BA McCarl (Texas A&M Univ.), B McConkey (Agric. Canada), WB McGill (Univ. of Northern BC), SM McGinn (Agric. Canada), MP McLaran (Univ. of Arizona), FB Metting (PNNL), JT O'Donovan (Alberta Agric.), L Mearns (NCAR), D. Ojima (Colorado St. Univ.), LB Owens (USDA), EA Paul (Colorado St. Univ.), K Paustian (Colorado St. Univ.), WM Post (ORNL), P Puget (Ohio St. Univ.), J Reilly (MIT), T Ren (China Agric. Univ.), CW Rice (Kansas St. Univ.), JC Ritchie (USDA),
NJ Rosenberg (JGCRI), C Rosenzweig (NASA), SM Ross (Univ. of Alberta), RD Sands (PNNL), MJ Scott (PNNL), JL Smith (USDA), SJ Smith (JGCRI), R Srinivasan (Texas A&M Univ.), ZX Tan (USGS), AM Thomson (JGCRI), WJD van Leeuwen (Univ. of Arizona), X Wang (Texas A&M Univ.), TL Wigley (NCAR), JR Williams (Texas A&M Univ.), J Zhou (ORNL).

**Graduate advisors:** DE Kissel (Kansas St. Univ., PhD), JA Hobbs (Kansas St. Univ., MSc).

**Advisees:** SM Ross (JGCRI, Postdoc), X He (JGCRI, Postdoc), C Prindiville (Univ. of Lund, MSc), M Bullock (Univ. of Alberta, PhD), HP Puurveen (Univ. of Alberta, MSc), SM Ross (Univ. of Alberta, MSc), C Fletcher (Univ. of Alberta, MSc), RL Lemke (Univ. of Alberta, PhD), T Ren (Univ. of Alberta, PhD), L Haderlein (Univ. of Alberta, MSc), R Pradhan (Univ. of Alberta, MSc).
EDUCATION
B.S. 1981 University of Delaware, Soil Chemistry with a minor in Chemistry. Degree with Distinction and Cum Laude.
M.S. 1983 University of Delaware, Soil Chemistry
Ph.D. 1985 Virginia Tech, Soil Chemistry/Physics

PROFESSIONAL POSITIONS
1996-present Adjunct Professor, Department of Geological Sciences, Univ. of Tennessee.

EXPERTISE AND RELEVANT EXPERIENCE
Research skills: Over 20 year experience investigation the influence of coupled processes on the fate and transport of inorganic contaminants in heterogeneous subsurface environments. Specialize in multiscale experimental and numerical quantification of nonequilibrium mass transfer processes in fractured and laminated soils and rock. Active Research: Influence of coupled hydrological and geochemical processes on the fate and transport of radionuclides beneath the Hanford tank farms. Microbially mediated immobilization of redox sensitive contaminants through in situ biostimulation. Influence of soil properties on the bioavailability of toxic metals. Regional scale assessment of enhanced organic C sequestration in deep subsoils. Investigating the influence of remedial capping on the hydrological, geochemical, and microbial processes that control subsurface contaminant migration with implications toward long-term stewardship. Other: 12 national and international scientific awards, 130+ published manuscripts, hundreds of scientific presentations, manage numerous multi-million dollar research projects for DOE and DoD.

PROFESSIONAL ACTIVITIES AND RECOGNITION
- American Society of Agronomy Outstanding Undergraduate Award, 1981
- Sigma Xi Outstanding Undergraduate Research Award, 1981
- Potash and Phosphate Institute Fellowship Award, 1982
- Sigma Xi Research Award, 1987
- Scientific Achievement Award, Environmental Sciences Division, ORNL, 1993
- Research and Development Accomplishment Award, ORNL, 1995
- Young Independent Scientist Award, Department of Energy, Office of Energy Research, 1996
- Presidential Early Career Award for Scientists and Engineers, The President of the United States of America, 1996
- Presidential Citation for Outstanding Achievement, University of Delaware, 1997
- Ten Outstanding Young Americans, United States Junior Chamber of Commerce, 1998
- M.L. Jackson Soil Science Award, Soil Science Society of America, 1998
- Highly Cited Researchers in Environmental Studies, The Institute for Scientific Information, (ISI), 2003

Select Publications (Total > 130 peer reviewed)

FIVE MOST RELEVANT

FIVE ADDITIONAL
EDUCATION
1994 Ph.D. University of Illinois at Chicago Biological Sciences
1979 M.S. University of Illinois Urbana-Champaign Agronomy
1973 B.S. University of Illinois Urbana-Champaign Agricultural Science

PROFESSIONAL APPOINTMENTS
2003–present Adjunct Professor, Department of Geography, Northern Illinois University
2002–present Terrestrial Ecologist (Grade 708), Argonne National Laboratory
1995–2002 Terrestrial Ecologist (Grade 707), Argonne National Laboratory
1984–1995 Environmental Scientist (Grade 706), Argonne National Laboratory
1979–1984 Assistant Environmental Scientist, Argonne National Laboratory
1975–1979 Scientific Assistant, Argonne National Laboratory
1973–1975 Research Assistant, University of Illinois Urbana-Champaign

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS


SYNERGISTIC ACTIVITIES


Educational: Outstanding Mentor Award, DOE Office of Science Undergraduate Research Programs (2003); DOE Global Change Education Program GREF Mentor (2001-present); Consultant to The Field Museum of Natural History “Underground Adventure” Exhibit, (1997-1998); Mentor, NSF Research Immersion Project for junior high school teachers at Argonne (1994); Mentor for >100 student interns at Argonne (1979-present).


Other: Fermilab Ecological Land Management Committee (1985-present).

COLLABORATORS & OTHER ASSOCIATIONS

Collaborators (past 4 years): Victoria Allison (Landcare Research, NZ), Steven Allison (U. California Irvine), James Amonette (PNNL), Vanessa Bailey (PNNL), James Bever (Indiana U.), Thomas Boutton (Texas A&M), Timothy Filley (Purdue U.), Lisa Gades (ANL), Charles Garten (ORNL), Julia Gaudinski (U. California Santa Cruz), Miquel Gonzalez-Meler (U. Illinois Chicago), Robin Graham (ORNL), Carla Gunderson (ORNL), Paul Hanson (ORNL), Cesar Izaurralde (PNNL), Philip Jardine (ORNL), Dev Joslin (retired), Markus Kleber (LBNL), Julia Liao (Rice U.), Roser Matamala (ANL), Bruce McCarl (Texas A&M), John McCarthy (U. Tennessee), Blaine Metting (PNNL), R. Michael Miller (ANL), Susan Miller (self employed), Richard Norby (ORNL), Clint C. Owensby (Kansas State U.), Wilfred Post (ORNL), Charles Rice (Kansas State U.), Claudia Rivetta (Stanford U.), William Schlesinger (Duke U.), Peggy Schultz (Indiana U.), Johan Six (U. California Davis), Mohamed Sultan (Western Michigan U.), Christopher Swanston (LLNL), Margaret Torn (LBNL), Susan Trumbore (U. California Irvine), Tim Tschaplnski (ORNL), Julie Whitbeck (U. New Orleans), Stan. Wullschleger (ORNL), Donald Zak (U. Michigan), Jizhong Zhou (U. Oklahoma)

Graduate advisors: David Koeppe (deceased), John Lussenhop (retired)

Postdoctoral advisees: Roser Matamala (ANL), Peggy Schultz (Indiana U.)

ROSER MATAMALA
Biosciences Division, Argonne National Laboratory
9700 South Cass Avenue, Argonne, IL 60439
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EDUCATION
1997  Ph.D.  University of Barcelona (Spain) and Biological Sciences
       Smithsonian Institution of Washington
1993  M.S.  University of Barcelona (Spain)  Plant Biology
1991  B.S.  University of Barcelona (Spain)  Biology

PROFESSIONAL APPOINTMENTS
2005-present  Terrestrial Ecologist, Argonne National Laboratory
2002-2005  Assistant Ecologist, Argonne National Laboratory
2000-2002  Research Associate, Argonne National Laboratory
1997-2000  Research Associate, Duke University
1996-1997  Graduate Fellow, Smithsonian Environmental Research Center
1993-1996  Plant Biologist, Smithsonian Environment Research Center
1993-1997  Graduate student, University of Barcelona (Spain)
1991-1993  Research Assistant, Institut de Recerca i Tecnologia Agroalimentaries (Spain)

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS


**SYNERGISTIC ACTIVITIES**

**Review and Advisory:** DOE Global Change Education Program Review Panel (2005); Participant, International networks of data sharing for FACE forest systems (started 2002); Invited keynote speaker, European COST E-38 Conference (2005).

**Educational:** Mentor to student interns, Argonne National Laboratory (2002-present); Invited guest lecturer for symposiums, seminars and workshops on environmental issues at high school and college levels.

**Other:** Occasional reviewer: *The New Phytologist, Tree Physiology, Oecologia and Plant and Soil;* Membership in the Ecological Society of America, American Association for the Advancement of Science, Soil Science Society of America.


**COLLABORATORS & OTHER ASSOCIATIONS**

**Collaborators (past 4 years):** Victoria Allison (Landcare Research, NZ), Thomas Boutton (Texas A&M), David Cook (ANL), Evan DeLucia (U. Illinois Urbana-Champaign), Paul Doskey (ANL), James Ehleringer (U. Utah), Timothy Filley (Purdue U.), Adrien Finzi (Boston U.), Charles Garten (ORNL), Miquel Gonzalez-Meler (U. Illinois Chicago), Robin Graham (ORNL), Paul Hanson (ORNL), Cesar Izaurralde (PNNL), Robert Jackson (Duke U.), Philip Jardine (ORNL), Julie Jastrow (ANL), Gabriel Katul (Duke U.), Markus Kleber (BNL), Rao Kotamarthi (ANL), John Lichter (Bowdoin College), Blaine Metting (PNNL), R. Michael Miller (ANL), Richard Norby (ORNL), Clenton Owensby (Kansas State U.), Diane Pataki (U. Utah), Mikhail Pekour (PNNL), Elise Pendall (U. Wyoming), Jeff Pippen (Duke U.), R. Siegwolf (Paul Scherrer Institute), Wilfred Post (ORNL), Ram Oren (Duke U.), Charles Rice (Kansas State U.), William Schlesinger (Duke U.), Christopher Swanson (LLNL), Margaret Torn (BNL), Susan Trumbore (U. California Irvine), Chris Van Kessel (U. California Davis), Donald Zak (U. Michigan)

**Graduate advisors:** Josep Peñuelas (U. Barcelona), Bert Drake (Smithsonian Environmental Research Center); **Postdoctoral advisors:** William Schlesinger (Duke U.), Julie Jastrow (ANL);

**Thesis research advisees:** Scott Graham (U. Illinois Chicago), Veronica Rodriguez (U. Illinois Urbana-Champaign), Elizabeth Smith (U. Illinois Chicago)
MELANIE A. MAYES  
Research Staff Scientist, Environmental Sciences Division  
Oak Ridge National Laboratory,  
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EDUCATION
2006  Ph.D.  The University of Tennessee, Geological Sciences
1999  M.S.  The University of Tennessee, Geological Sciences
1995  B.S.  The University of Missouri, Geological Sciences

EMPLOYMENT
2002-present  Environmental Science Division, Oak Ridge National Laboratory  
Research Staff Scientist, Associate
1999-2002  Oak Ridge Institute for Science and Education Research Associate
1996-1998  Department of Geological Sciences, The University of Tennessee  
Graduate Research or Teaching Assistant
1996  Oak Ridge Institute for Science and Education Undergraduate Research Assistant
1995  Department of Geological Sciences, The University of Missouri  
Laboratory and Teaching Assistant

PUBLICATIONS
Germene to this proposal
Jardine, PM, MA Mayes, PJ Mulholland, PJ Hanson, JR Tarver, RJ Luxmoore, JF McCarthy,  
and GV Wilson. 2006. Vadose zone flow and transport of dissolved organic carbon at  
multiple scales in humid regimes. Vadose Zone Journal 5:140-152.
Gwo, J-P, MA Mayes, and PM Jardine. 200_. Quantifying the physical and chemical mass  
transfer processes for the fate and transport of Co(II)EDTA in a partially-weathered  
limestone-shale saprolite. Journal of Contaminant Hydrology (accepted).
Mayes, MA, TL Mehlhorn, and PM Jardine. 2005. Coupled hydrological and geochemical  
processes influencing the transport of chelated metals in the ORNL vadose zone and  
groundwater. In: ACS Symposium Series 910: Biogeochemistry of Chelating Agents,  
Transport of multiple tracers in variably saturated humid region structured soils and semi-  
arid region laminated sediments. J. Hydrol. 275:141-161.
Gwo, JP, EF D’Azevedo, H Frenzel, MA Mayes, G-T Yeh, PM Jardine, KM Salvage, and  
FM Hoffman. 2001. HBGC123D: a high performance computer model of coupled  

Other
Pace, MN, MA Mayes, PM Jardine, LD McKay, XL Yin, TL Mehlhorn, Q Liu, and H Gurleyuk.  
200_. Unraveling the fate and transport of Sr2+ and SrEDTA2- in the Hanford vadose zone.  
Journal of Contaminant Hydrology (in revision).


SYNERGISTIC ACTIVITIES
Dr. Mayes has 10 years of expertise in coupled hydrological and geochemical transport theory and experimentation in both vadose and saturated zones. She has worked with fractured weathered sediments in the eastern US and layered porous sediments from the western US. She currently serves as principal investigator on an ERSP investigation to investigate and model coupled processes in layered Hanford sediments over multiple scales. She also receives funding from site contractors at the DOE’s Hanford Reservation (Tank Farm Vadose Zone Project through CH2M Hill Hanford Group, Inc.) to investigate the influence of sedimentary facies upon contaminant transport.

Collaborators and Co-Editors: P. Jardine (ORNL), S.E. Fendorf (Stanford University), S. Brooks (ORNL), R.J. Serne (PNNL), J.-P. Gwo (NRC), P. Mulholland (ORNL), J. McCarthy (U. Tennessee Knoxville), G.V. Wilson (USDA-ARS), M. Ginder-Vogel (Stanford U.), T. Borch (Stanford U.), B. Bjornstad (PNNL)

Graduate and Postdoctoral Advisors
Graduate and post-masters advisors: P. Jardine (ORNL), L. McKay (U. Tennessee Knoxville), E. Perfect (U. Tennessee Knoxville), J. Lee (U. Tennessee Knoxville), C. Mora (U. Tennessee Knoxville)

Advisees
none
EDUCATION
1973 Ph.D. Pennsylvania State University Management Science
1970 B.S. University of Colorado Business Statistics

PROFESSIONAL APPOINTMENTS
2006-date TAES Fellow, Texas A&M University
2002-date Regents Professor, Texas A&M University
2004-date Lead Economist Homeland Security funded Foreign and Zoonotic Animal Disease Defense Center, Texas A&M University
1985-date Professor, Agricultural Economics, Texas A&M University
1982-1985 Professor, Agricultural & Resource Economics, Oregon State University
1980 Visiting Professor, Agricultural & Resource Economics, Oregon State University
1978-1982 Associate Professor, Agricultural Economics, Purdue University
1973-1978 Assistant Professor, Agricultural Economics, Purdue University

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS
SYNERGISTIC ACTIVITIES

Service: Lead Author IPPC Mitigation Chapter, Agriculture 2005-2007; Contributing Lead Author IPPC Mitigation Chapter, Forestry 2006-2007; CASMGS Consortium member (2003-2006); Review and Advisory: Steering committee USDA/USEPA Ag and Forest Modeling Forum, Editor, Choices (Journal of American Agricultural Economics Association) 2004-date; Associate Editor Climatic Change, 2001-date; Associate Editor Water Resources Research 1989-2001; Associate Editor American Journal of Agricultural Economics 1986-1991;

Educational: University professor for 33 years, Broader Participation by Underrepresented Groups: Have advised more than 70 graduate students including 10 hispanic, 3 black and 25 women. Other: Distinguished Fellow, American Agricultural Economics Association, Distinguished Achievement awards for Research, USDA, EPA.

COLLABORATORS & OTHER ASSOCIATIONS

Collaborators (past 4 years): Elbakidze, L. (TAMU) R.D. Sands (PNNL), P. Smith (Aberdeen), D. Martino (Bolivia), Z. Cai (China), D. Gwary (Tanzania), H.H. Janzen (Ag Canada), P. Kumar (India), F. O'Mara (Ireland), C. Rice (Kansas State), B. Scholes (South Africa), O. Sirotenko (Soviet Union), T. McAllister (Canada), G. Pan (China), and V. Romanenkov (Soviet Union), W.M. Post (ORNL), W.M., R.C. Izaurralde (PNNL), J. Jastrow (PNNL), J.E. Amonette (PNNL), V.L. Bailey (PNNL), P.M. Jardine (ORNL), T.O. West (ORNL), and J. Zhou, (ORNL) Schneider, U.A. (Hamburg) F.B. Metting (PNNL), Butt, T.A. (AMEX) Jackson, R.B. (Duke), E.G. Jobbgy (Argentina), R. Avissar (Buke), D. Barrett (CSIRO), C.W. Cook (Duke), K.A. Farley (Duke), D.C. Maitre (Duke), B.C. Murray (Duke), and S.B. Roy, Lee, H-C. (Taiwan), D. Gillig (AMEX), D. Adams (Oregon State), R. Alig (USFS), K. Andrasko (EPA)

Graduate advisors: G. Kochenberger (Univ Colorado, Denver), John Dinkel (retired)

Postdoctoral advisees: William Nayda (Capital One), Ching Chang Cheng (Academy Sinica), D. Gillig (AMEX), Wen Yu (TAMU)

Thesis research advisees: Uwe Schneider (Hamburg), L. Elbakidze (TAMU), MK Kim (PNNL), C. Dillon (Kentucky), T. Spreen (Florida), D. Lambert (North Dakota), J. Apland (Minnesota), C. Chen (Taiwan), H. Lee (Taiwan), L. Villa Issa (Mexico), D. Fajardo (Nicaragua)
F. BLAINE METTING, JR.
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EDUCATION
1979 Ph.D. Washington State University Botany
1976 M.S. Washington State University Botany
1979 B.A. Whitman College Liberal Arts (Biology)

PROFESSIONAL APPOINTMENTS
2000–present Manager, Biological & Environmental Sciences Product Line
Pacific Northwest National Laboratory
1990–2000 Senior Research Scientist, Pacific Northwest National Laboratory
1989–1990 Principal Microbiologist, Enviros Corporation
1988-1989 Research Associate, Tufts University Medical School

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)
Climatic Change special issue (in press).
Metting, F.B., M.J. Scott, J.R. Benemann, E. Greenbaum, M. Seibert, A. Spormann, H. Yukawa,
greenhouse gas mitigation.” pp. 115-136 in Rosenberg, N.J., F.B. Metting and R.C.
Izaurralde (Eds.). Applications of Biotechnology to Mitigation of Greenhouse Warming.
Carbon Sequestration.” pp. 1-43 in: Carbon Sequestration in Soils: Science, Monitoring and
Beyond. N. Rosenberg and E. Malone (Eds.). Battelle Press, Columbus.
Analysis. Part 3. Microbiological and Biochemical Properties. 3rd edition. R. W. Weaver et
al. (Eds.). American Society of Agronomy, Madison, Wisconsin.
Metting, F. B. (Editor). 1993. Soil Microbial Ecology. Applications in Agricultural and

SYNERGISTIC ACTIVITIES
Service: Program Committee, World Congress of Industrial & Environmental Biotechnology
(2004-2005-2006), Contributing Organizer, St. Michaels Workshops on Greenhouse Gas
Educational: Adjunct Faculty (Soil Microbiology), Joint Graduate Center (now Washington
State University, Tri-Cities)(1982-1988). Lecturer in Microbiology, Columbia Basin

D-23
COLLABORATORS & OTHER ASSOCIATIONS

*Collaborators (past 4 years):* Peter Spencer (Oregon Health & Sciences University), Robin Graham, W. Mac Post (Oak Ridge National Laboratory), Julie Jastrwo, Michael Miller (Argonne National Laboratory).

*Graduate advisor:* William R. Rayburn (retired).
R. MICHAEL MILLER
Biosciences Division, Argonne National Laboratory
9700 South Cass Avenue, Argonne, IL 60439
Phone: 630-252-3395; Email: rmmiller@anl.gov

EDUCATION
1975  Ph.D.  Illinois State University  Mycology
1971  M.S.  Illinois State University  Biological Sciences
1969  B.S.  Colorado State University  Botany

PROFESSIONAL APPOINTMENTS
2005-present  Biosciences Division Deputy Division Director
1986-present  Terrestrial Ecology Group Leader
1997–present  Senior Terrestrial Ecologist, Argonne National Laboratory
1983–1998  Terrestrial Ecologist, Argonne National Laboratory
1977–1983  Assistant Ecologist, Argonne National Laboratory
1975–1977  Post doctoral Associate, Argonne National Laboratory
1989–present  Lecturer, Committee on Evolutionary Biology, University of Chicago

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS


SYNERGISTIC ACTIVITIES


COLLABORATORS & OTHER ASSOCIATIONS

Collaborators (past 4 years): Victoria Allison (Landcare Research, NZ), James Bever (Indiana U.), Thomas Boutton (Texas A&M), Charles Garten (ORNL), Catherine Gehring (Northern Arizona U.), Jason Hoeksema (U. California Santa Cruz), Nancy Johnson (Northern Arizona U.), John Klironomos (U. Guelph), Roger Koide (Penn State U.), John Moore (Northern Colorado U.), Richard Norby (ORNL), Clenton Owensby (Kansas State U.), Charles Rice (Kansas State U.), Claudia Rivetta (Stanford U.), Mark Schwartz (U. California Davis), Susan Simard (U. British Columbia), Bill Swenson (U. California Riverside), James Umbanhowar (U. North Carolina), Gail Wilson (Kansas State U.), Donald Zak (U. Michigan), Yunguan Zhu (Chinese Academy Sciences, Beijing), Catherine Zabinski (Montana State U.);

Graduate advisor: Anthony E. Liberta (retired)

Postdoctoral advisor: Roy Cameron (USEPA, Las Vegas)

Postdoctoral advisees: Victoria Allison (Landcare Research, NZ)

Thesis research advisees: Paul Benda (U. Illinois Urbana-Champaign); Shivcharn Dhillion (Illinois State U.); Greg Eckert (U. Georgia); Raymond Franson (U. Chicago); Michael Fitzsimons (U. Chicago); Kelly Gravier (U. Chicago); Antonio Golubski (U. Illinois Chicago); John Paul Schmit (U. Chicago); Molly Smith (DOE GREF Mentor, U. California Berkeley); Lina Taneva (U. Illinois Chicago)
WILFRED M. POST
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EDUCATION
1978 Ph.D. University Tennessee, Knoxville Ecology
1979 M.S. University of Wisconsin, Madison Botany
1973 B.S. University of Wisconsin, Madison Mathematics

PROFESSIONAL APPOINTMENTS
1997–present Senior Scientist, Oak Ridge National Laboratory
1980-1997 Research Staff Member, Oak Ridge National Laboratory
1978-1980 Post-Doctoral Associate, Oak Ridge National Laboratory
1985–present Adjunct Professor, Ecology and Evolutionary Biology, University of Tennessee, Knoxville

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS
SYNERGISTIC ACTIVITIES


COLLABORATORS & OTHER ASSOCIATIONS

Collaborators (past 4 years): James Amonette (PNNL), Vanessa Bailey (PNNL), Charles Garten (ORNL), Julia Gaudinski (U. California Santa Cruz), Robin Graham (ORNL), Carla Gunderson (ORNL), Paul Hanson (ORNL), Elizabeth Holland (NCAR), Cesar Izaurralde (PNNL), Atul Jain (U. Illinois), Philip Jardine (ORNL), Dev Joslin (retired), Markus Kleber (LBNL), Roser Matamala (ANL), Elaine Matthews (NASA-GISS), Bruce McCarl (Texas A&M), John McCarthy (U. Tennessee), Blaine Metting (PNNL), R. Michael Miller (ANL), Kendra McLaughlan (New Hampshire), Richard Norby (ORNL), Christopher Swanston (LLNL), Margaret Torn (LBNL), Susan Trumbore (U. California Irvine), Stan.Wullschleger (ORNL), Donald Zak (U. Michigan), Jizhong Zhou (U. Oklahoma).

Graduate advisors: Timothy F.H. Allen (University of Wisconsin, Madison), Herman H. Shugart (University of Virginia), Donald L. DeAngelis (University of Miami).

Postdoctoral advisees: John Pastor (University of Minnesota, Duluth), Jonathan Adams (Rutgers University), Kevin Harrison (University of Rhode Island), Ned Nikolov (Colorado State University), Qing Liu (Georgia Tech), Bai Yang (U. California Davis).

Thesis research advisees: Louis Provenche (University of Tennessee), Peter J. Taylor (Harvard University), Eric Pauley (University of Tennessee), Mark Wiltberger (Pennsylvania State University), Tadashi Fukami (University of Tennessee), Lala Chambers (University of Tennessee), Jackie Little (University of Tennessee), Gregory Witteman (University of Tennessee), Xiaojuan Yang (University of Illinois), Victoria Wittig (University of Illinois), Kendra McLaughlan (University of Minnesota), Jillian Salvatore (DOE-GCEP), Jesse Miller (DOE-GCEP), Jennifer Fraterrigio (DOE-GCEP), Kate Flick (DOE-GCEP), Erin Hanlon (DOE-GCEP), Victoria Wittig (DOE-GCEP), Holly Gibbs (DOE-GCEP).
RONALD D. SANDS
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EDUCATION
1990  Ph.D. University of Minnesota, Twin Cities  Economics
1979  B.S. University of Minnesota, Twin Cities  Economics
1978  B.E.E. University of Minnesota, Twin Cities  Electrical Engineering

PROFESSIONAL APPOINTMENTS
1996–present  Staff Scientist, Pacific Northwest National Laboratory
1990–1996  Senior Research Scientist, Pacific Northwest National Laboratory
1981-1986  Research Associate, University of Minnesota
1981-1982  Teaching Associate, University of Minnesota

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS
SYNERGISTIC ACTIVITIES


**Other:** Building capacity for economic and environmental modeling in developing countries at the following institutions: Mexican Petroleum Institute, Mexico City; Federal University of Rio de Janeiro, Brazil; Indian Institute of Management, Ahmedabad; Energy Research Institute, Beijing, China; Korea Energy Economics Institute, Seoul.

COLLABORATORS & OTHER ASSOCIATIONS

**Collaborators (past 4 years):** Mariano Bauer (Mexican Petroleum Institute), Geoff Blanford (EPRI), Hyun-Sik CHUNG (Sungkyunkwan U, South Korea), James Edmonds (PNNL), Allen Fawcett (US EPA), D. Gillig (Texas A&M U), Robin Graham (ORNL), Thomas Hertel (Purdue U), Akira HIBIKI (National Institute for Environmental Studies, Japan), Cesar Izaurralde (PNNL), Kejun JIANG (Energy Research Institute, Beijing, China), Mikiko KAINUMA (National Institute for Environmental Studies, Japan) Man-Keun KIM (PNNL), Marian Leimbach (Potsdam Institute for Climate Impact Research, Germany), Elizabeth Malone (PNNL), Bruce McCarl (Texas A&M U), Blain Metting (PNNL), Hugh Pitcher (PNNL), Wilfred Post (ORNL), Joe Roop (PNNL), Steven Rose (US EPA), Norman Rosenberg (PNNL), Miranda Schreurs (U of Maryland), Katja Schumacher (German Institute for Economic Research, Berlin), Michael Scott (PNNL), Michael Shelby (US EPA), P.R. Shukla (Indian Institute of Management, Ahmedabad), Eric Smith (US EPA), Allison Thomson (PNNL), Tristram West (ORNL)

**Graduate advisors:** John S. Chipman (U of Minnesota)

**Postdoctoral advisees:** Man-Keun KIM (PNNL)

**Thesis research advisees:** Kenneth Gillingham (Dartmouth College)
EDUCATION
2002    Ph.D.     University of Colorado     Biology
1996    B.S.     University of Washington     Botany

PROFESSIONAL POSITIONS
2005-Present     Staff Scientist, Oak Ridge National Laboratory
2003-Present     Faculty Affiliate, University of Tennessee
2003-2004     Postdoctoral Research Associate, Oak Ridge National Laboratory
2002-2003     Postdoctoral Research Associate, University of Colorado
1998-2002     Graduate Research Assistant, University of Colorado

EXPERTISE AND RELEVANT EXPERIENCE
Ecology of soil and subsurface microorganisms. Identification of the interrelationships between genomics and physiology of environmentally relevant microorganisms. Use of molecular genomic methods for inferring the functional abilities of microorganisms in the environment.

SELECT PUBLICATIONS
Hwang, C, WM Wu, TJ Gentry, J Carley, SL Carroll, C Schadt, D Watson, PM Jardine, J Zhou, RF Hickey, CS Criddle, and MW Fields. 2006. Changes in bacterial community structure correlate with initial operating conditions of a field-scale denitrifying fluidized bed reactor, Applied Microbiology and Biotechnology, OnlineFirst (DOI: 10.1007/s00253-005-0189-1)

SYNERGISTIC ACTIVITIES
Departmental Symposium Organizer, EPO Biology, Univ. of Colorado, 1998 & 1999
Representative, United Government of Graduate Students, Univ. of Colorado, 1998

COLLABORATORS & OTHER ASSOCIATIONS
Collaborators: Steven D. Brown (ORNL); Aimee Classen (ORNL) Craig Criddle (Stanford Univ.); Steve DiFazio (ORNL); Michael Himmel (Nat. Renewable Energy Lab); Mathew Fields (Miami Univ. of Ohio); Chuck Garten (ORNL); Terry J. Gentry (Texas A&M Univ); Robin L. Graham (ORNL); Jonathan Istok (Oregon State Univ.); Joel Kostka (Florida State Univ); Cheryl Kuske (LANL); David A. Lipson (San Diego State Univ.); Andy Martin (Univ. of Colorado); Allen Meyer (Univ. of Colorado); Jean Marc Moncalvo (Univ. of Toronto); Andrew P. Martin (Univ.of Colorado); Arturo Massol (Univ. of Puerto Rico, Mayaguez); Richard Norby (ORNL); Anthony V. Palumbo (ORNL); Steven K. Schmidt (Univ.of Colorado); Dorthea Thompson (ORNL); James M. Tiedje (Michigan State Univ.); Rytas Vilgalys (Duke Univ.); Stan Wullschegger (ORNL); Chaunlun Zhang (Univ. of Georgia); Jizhong Zhou (ORNL)

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EDUCATION
1976 B.S. University of California Berkeley Soils and Plant Nutrition
1980 M.S. Soils Washington State University
1983 Ph.D. Soil Biochemistry Washington State University

PROFESSIONAL APPOINTMENTS
1986 to present Soil Biochemist, USDA-ARS, Pullman, WA.
1983-1986 Specialist, Dept. Plant and Soil Biology, Univ. of California Berkeley
1978-1983 Research Assistant, Dept Agronomy and Soils, WSU.

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS

D-33
SYNERGISTIC ACTIVITIES

*Service*: Lead scientist ARS GRACEnet program, evaluating greenhouse gas emissions from agriculture; Consultant to State Department on the Kyoto Protocol; Past ARS representative to USGCRP; Member IPCC expert panel on National Greenhouse Gas Inventories, N₂O; active member of Soil Science Society of America, Ecological Society of America, American Geophysical Union, International Society of Microbial Ecology. *Review and Advisory*: Editorial board, Soil Biology and Biochemistry and Biology and Fertility of Soils; Review panel for DOE, NASA, USDA, and NSF. Ad hoc reviewer for 6 journals in 2006

COLLABORATORS & OTHER ASSOCIATIONS

*Collaborators (past 4 years)*: Jay Halvorson (USDA-ARS), Kent Keller, Richelle Allen-King, Steve Link, Dave Bezdicek, Rick Watts (Washington State University), Dave Huggins (USDA-ARS), Jen Bell (NRCS), Sarah Fansler, Harvey Bolton Jr., Vanessa Bailey, Blaine Metting, Cesar Izaurralde (PNNL), Alan Franzluebbers, Ron Follett, Jane Johnson, Mark Liebig, Tim Parkin, Steve DelGrosso, Mike Jawson and Dean Martens, Hal Collins (USDA-ARS)

*Graduate advisors*: H.H. Cheng, Brian McNeal and Gaylon Campbell (all retired)

*Thesis research advisees*: Sarah Fansler (PNNL), Jen Bell (NRCS), Angie Goodwin, Lauren Bissey, Debi Geyer, Tim White, Dan Mummey, Amy Simmons, Lonna Roberts, Mary Staben, Karen Sowers, Todd Kafta (Washington State University)
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EDUCATION
1999  M.E.M.  Duke University  Environmental Management
1997  B.A.  Carleton College  Geology

PROFESSIONAL APPOINTMENTS
2005-present  Senior Research Scientist (III), Pacific Northwest National Laboratory
2002-2004  Research Scientist (II), Pacific Northwest National Laboratory
2000-2001  Scientist (I), Pacific Northwest National Laboratory
1999-2000  Oceanographic Programs Assistant, National Oceanic and Atmospheric Administration

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)
Thomson, AM, RC Izaurralde, NJ Rosenberg, and X He. 2006. Climate change impacts on agriculture and soil carbon sequestration potential in the Huang-Hai Plain of China. Agriculture, Ecosystems and Environment 114(2-4).

FIVE ADDITIONAL PUBLICATIONS


SYNERGISTIC ACTIVITIES

Educational: Mentor for JGCRI Student Interns (2002-Present).

COLLABORATORS & OTHER ASSOCIATIONS

Collaborators (past 4 years): R. Cesar Izaurralde (PNNL), Norman Rosenberg (PNNL), Steven Smith (PNNL), Leon Clarke (PNNL), Xiaoxia He (PNNL), Ruby Leung (PNNL), Jimmy Williams (Texas A&M), Raghavan Srinivasan (Texas A&M), Jay Atwood (USDA), Steve Potter (USDA), Phil Gassman (Iowa State), Shunlin Liang (University of Maryland), Hongliang Fang (University of Maryland), Mitchell McClaran (University of Arizona), Ed DeSteuiger (University of Arizona), Stuart Marsh (University of Arizona)

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EDUCATION
1978 Ph.D. University of Kentucky Agronomy  
1975 M.S. University of Kentucky Agronomy  
1972 B.S. Murray State University Agriculture and Chemistry

PROFESSIONAL APPOINTMENTS
1991–present Professor, University of Tennessee, Department of Biosystems Engineering & Soil Science  
1983–1991 Associate Professor, University of Tennessee, Department of Biosystems Engineering & Soil Science  
1978–1983 Assistant Professor, University of Tennessee, Department of Biosystems Engineering & Soil Science

FIVE SELECTED PUBLICATIONS (MOST RELEVANT)

FIVE ADDITIONAL PUBLICATIONS


SYNERGISTIC ACTIVITIES

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EDUCATION
1999  Ph.D.  The Ohio State University  Agronomy
1996  M.S.  The Ohio State University  Natural Resources
1994  B.S.  University of Kentucky  Agriculture

PROFESSIONAL APPOINTMENTS
2002-present  Associate Research Scientist, Oak Ridge National Laboratory
1999-2002  Postdoctoral Research Associate, Oak Ridge National Laboratory
1996-1999  Graduate Research Fellow (U.S. Dept. of Defense), Ohio State University
1995-1996  Graduate Research Associate, Ohio State University
1994-1995  Graduate Teaching Associate, Ohio State University
2004-present  Adjunct Professor, Biosystems Eng. and Soil Sci. Dept., University of Tennessee

FIVE MOST RELEVANT PUBLICATIONS

FIVE ADDITIONAL PUBLICATIONS

PRIMARY RESEARCH INTERESTS
Effects of land management on biogeochemical cycling in terrestrial ecosystems
Quantitative modeling of the impacts of carbon sequestration strategies and other climate change mitigation options on net greenhouse gas emissions to the atmosphere
Use of remote sensing and geographic information systems to quantify soil attributes and biogeochemical cycling
Integrated assessment of climate change impacts and mitigation
Environmental effects of policies intended to reduce greenhouse gas emissions

PROFESSIONAL AFFILIATIONS
American Society of Agronomy
American Geophysical Union
Ecological Society of America
Soil Science Society of America

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Collaborators (past 4 years): Daniel De La Torre Ugarte (Univ. of Tenn.), Cesar Izzauralde (PNNL), Atul Jain (University of Illinois), James Larson (Univ. of Tenn.), Gregg Marland (ORNL), Bruce McCarl (Texas A&M), Richard Nelson (Kansas State Univ.), Stephen Ogle (Colorado State University), Wilfred M. Post (ORNL), Bernhard Schlamadinger (Joanneum Research Institute, Austria), Johan Six (University of California-Davis), Allison Thomson (PNNL), Don Tyler (Univ. of Tenn.), Mohan K. Wali (Ohio State University)
Graduate advisors: Gregg Marland (Oak Ridge National Laboratory), Mohan K. Wali (Ohio State University)
Undergraduate and Graduate Advisees:
2003: Brooke Ilene Chichakly (undergraduate), Allen McBride (undergraduate), Xiaojuan Yang (Ph.D. candidate); 2005: Adam Roddy (undergraduate; co-supervised with Stan Wullschleger), Maithilee Kunda (undergraduate; co-supervised with Gregg Marland); 2006: Maithilee Kunda (post-bachelor), Aarthi Sabesan (post-masters), Yonghai Qian (post-doctoral)