Project Title: Examining the Microbial Ecology of Agricultural Soil

RFP Category: 1-B Faculty Research  Total Grant Amount Requested from FPDC: $7,976.00

Discipline: Biological and Environmental Sciences  Sub-Discipline:

Project Director:
Sarah L. Meiss, Ph.D.
Assistant Professor
Department of Biological and Environmental Sciences
California University of PA
724-938-4203
meiss@calu.edu

Faculty Status (see definitions below):

☐ Tenured  ☒ Probationary  ☐ Non-Tenure Track

Other Participants (names, departments, e-mail addresses):

IRB Status:  ☐ Approved (IRB # )  ☐ Pending  ☒ N/A

ABSTRACT:
Soil is one of the world’s most valuable resources providing agricultural with nutrients, water and support for growth. To understand agriculture better we must understand the entire soil ecosystem and what organisms are present and active in that soil. The presence of fungal and bacterial (microbial) organisms drastically affects the health of soil and therefore plant crops by playing roles in nutrient cycling, pathogen suppression, and bioremediation of pollution. This research has 3 goals: 1) Use DNA techniques to examine soil microbial ecology; 2) Use traditional methods to examine soil microbial ecology, and 3) Use the information to create a model for agricultural soil health. Completion of this project will result in significant professional development for the primary investigator and a novel way to assess soil health for researchers, farmers and extension agents.
Project Narrative

Background and Significance

Soil is one of the world’s most valuable natural resources, second only to water, and is one of the major components of the natural terrestrial ecosystem (Bruckman and Brady 1960; Abawi and Widmer 2000). It provides the energy and support needed for plant growth and therefore all life on earth. Soil is an ever-changing resource that is not only made up of minerals and rock, but organic and inorganic molecules and living organisms. Microorganisms in the soil play roles in filtering water and pollution, cycling nutrients and enabling food production (Doran and Parkin 1996; Kennedy and Smith 1995; Richards 1987; Kennedy et al. 1995). Research has shown that the presence of microorganisms, including fungi, has a great effect on soil health playing a major role in soil quality and therefore plant productivity (Hill et al. 2000; Doran et al. 1994). More research shows us that agricultural production is directly dependent on the health and soil productivity (Abawi and Widmer 2000; Magdoff 1992; Doran and Jones 1996; Pankhurst et al. 1997). Fungi and bacteria (microbes) specifically play significant and keystone roles in decomposition of organic material, decomposition of plant residues, increased nutrient availability, biological control of pests, and biodegradation of pesticides and other pollutants (Kennedy and Smith 1995; Richards 1987; Kennedy et al. 1995). Microbes have a direct effect on plants stimulating root development and increasing biological diversity (Abawi and Widmer 2000, Loy et al. 2004). Because of these roles played by microbes it is clear that there is a relationship between microbial diversity, soil and plant quality and overall ecosystem sustainability (Hill et al. 2000; Doran et al 1994, Loy et al. 2004, Sagaram et al. 2009).

Research on microorganisms has paid little attention to the community and species level changes in agricultural soil (Hill et al. 2000, Sagaram et al. 2009, Hamady et al. 2010). Although many techniques are used to assess soil health, only microbial biomass and microbial activity in soil have been used to examine the role of microorganism in the overall health of soil. These methods do not provide researchers and scientists with the identity or species diversity found in the soil. Plate culturing and identification of soil microorganisms has been shown to identify 1% of the possible 4000 individuals living in 1g of soil (Alexander 1977, Torsvik et al 1990, Borneman et al. 1996). Understanding microbial properties of diversity is important to scientists, and they may also be used by extension personnel and farmers in devising practical measures of soil quality (Hill et al. 2000). The role of microorganisms in disease suppression and soil health requires a systematic ecological approach to find soil health indicators and provide scientists with the most data (van Bruggen and Semenov 2000, Hamady et al. 2010). Therefore, if we want to understand the ecology of the soil, then we first need to know what is in the soil. This research aims to use a three-tiered approach to address and examine soil microbial ecology: traditional culture methods, DNA methods (microarray) and chemical analysis.

Microarray technology or analysis requires a microarray reader which is only available at a few labs in the country. One lab is the Lawrence Berkeley National Laboratory (LBNL) in Berkeley, California. The research will be completed over three years, or in three Phases (Phase I summer 2009, Phase II summer 2010 and Phase III summer 2011). I applied for and received a three summer Fellowship from the Center for Science and Engineering Education (CSEE), a program run by the U.S. Department of Energy. This fellowship allowed me to create a Faculty and Student team (FaST) with two students (from California University of PA, Cal U) that traveled to Berkeley and spent 10 weeks carrying out preliminary research using microarray technology. During the 2009 summer my FaST team created a “mock” microbial community to use as baseline data and analyzed one organic soil for the presence of fungi, isolating and identifying over 20 different species. During the summer of 2010 (Phase II) my FaST team examined agricultural soil for overall genetic diversity and lipid (fat) diversity. I have completed Phase I and II and now need funding for Phase III.

Over the next summer (Phase III 2011), the FaST team will carry out microarray analysis to determine the identity of microbes in the soil while developing long term collaborations with host scientists and science teams at LBNL. CSEE provides funding for the FaST team (both students and
faculty member) to travel and live in Berkeley, California for 10 weeks, as well as access to the microarray reader and lab space, but does not provide funding for laboratory consumables (i.e. chemicals to carry out analysis). Phase III requires critical laboratory materials to conduct this research that are not provided by CSEE and the research fellowship.

Goals and Objectives
The broad, long term goal of this research is to develop a microbial biological model for healthy soil in an agricultural system. Along with this research goal, this project will also form a career long research program for the PI.

More specifically, the goals and objectives of the work at Lawrence Berkeley National Laboratory (LBNL) are to:

1) Analyze agricultural soil for their microbial species content by
   a. Identifying fungi to a species level using traditional methods.
   b. Identifying bacteria to a species level using microarray technology (phylochip).
   c. Examining baseline species in soil and analyze soil ecology.
   d. Examining chemical soil quality (nitrogen, carbon, fat composition)

2) Increase professional development of the Project Director by
   a. Publishing of the results of the research in the peer reviewed journal *Environmental Microbiology* (alternates: *International Journal of Plant Microbe Interactions, Crop Science*)
   b. Acquiring knowledge of novel techniques (microarray technology), that are not available at Cal U.
   c. Developing of a long term relationship (career long collaboration) with experts in the field at a National Laboratory for 10 weeks over the summer (over 3 summers).
   d. Incorporating the experience into the curriculum to enhance academic rigor of courses taught at California University of Pennsylvania, and
   e. Presenting of the results at the American Society of Microbiology National Conference.

Description of Project
Phase III

Phase I resulted in the Project director forming a professional relationship with three Research Faculty at LBNL and two undergraduate Cal U students completing a 10 week internship (with resulting paper and poster presentations). We established baseline microbial data that will be used over the next two summers. Phase II resulted in the Project director’s gathering more information on the agricultural soil such as lipid/fat content and microbial diversity.

Phase III will occur over the next summer (2011) at LBNL where the Project director has been invited back to work at the specialized facilities. A fellowship has been awarded to the Project director (from CSEE above) that will pay for travel and living stipends for the Project director and the two student interns (from Cal U). This grant will cover the costs of a variety of laboratory consumables that are essential for many aspects of this Phase.

This project will analyze agricultural soil for their microbial species content. The California University Student Association (SAI) has acquired a 94 acre plot of land on which I am working with a University Committee (CSI: California Sustainability Initiative) to develop a teaching/research garden (permaculture design). The garden will follow a sustainable gardening method permaculture and therefore is perfect to study not only the microbial community in the garden but the succession of the microbial community as the garden develops over the next 5-10 years.
I have also identified two agricultural fields that have been used for gardening for 5+ years, one that practices organic agriculture and one that practices modern agriculture. The organic field does not use synthetic pesticides or fertilizers, while the modern field does.

Phase III of this project will assess fungi in all varieties of the aforementioned soil using traditional methods. Traditional assessment of soil for fungi species includes growing fungi on agar plates in a laboratory, isolating genomic DNA, amplifying (by PCR, Polymerase Chain reaction) a specific region (called the ITS or Inner Terminal Repeat which is a specific sequence of DNA) of their DNA and comparing that region to known fungi in a database. Genomic DNA is the entire DNA found in a single organism. PCR is a commonly used method of multiplying or amplifying DNA for molecular experiments over 1 million times. Although this method allows the researcher to identify the fungi to a species level and is helpful, it tends to be limited in its use. Only fungi that grow well in the lab can be identified by this method, any fungi that do not grow in a laboratory setting will not be identified. Researchers have estimated that as many as 80-90% of species are missed by only identifying the ones that can grow in the laboratory. The results of this analysis will be used as a check to compare to the new proposed molecular approach.

Following traditional analysis, the researcher will then begin to identify fungi in the soil using a novel method called microarray technology which employs a microarray chip. The novel molecular technique, or microarray technology, improves on the traditional method. A microarray chip is a small chip (4 can fit on a typical microscope slide) that can hold a piece of DNA from thousands of different species whose identity is known. The DNA isolated from the unknown organisms is placed onto the chip. If any of the unknown DNA matches to one of the known species on the chip, then the unknown organism has been successfully identified. To use this technology, genomic DNA will again be isolated from the soil. A region of fungal genomic DNA (ITS region) and bacterial genomic DNA (16s rRNA, ribosomal RNA) will be amplified using PCR. These ITS regions in fungi and 16s rRNA regions in bacteria are small sections (sequences) of the genomic DNA that are like a fingerprint to each fungal and bacterial species. Allowing scientists to use the specific sequence to identify organisms to a species level.

The microarray chips are different for the fungal and bacterial species. The microarray chip that has the DNA from bacteria has over 30,000 isolates from different bacterial species and is called a Phylochip. The microarray chip that is used for fungi has over 16,000 fungal species isolates on it and is called a Mycochip. A microarray chip reader for both the Phylochip and Mycochip are available for use at LBNL. The chemicals necessary to carry out the reactions for the microarray reader are to be provided by the FaST team.

Phase III will conclude with the publication of the research in a peer reviewed journal as well as presentation of the results at a national conference (most likely American Society of Microbiology). The Cal U students who work as interns with me over the summer 2011 are also expected to present their research at LBNL and back at Cal U.
Table 1. Timeline of project

<table>
<thead>
<tr>
<th>Phase</th>
<th>Activity</th>
<th>Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>1. Establish relationship with Scientists and researchers at LBNL.</td>
<td>Summer 2009</td>
</tr>
<tr>
<td></td>
<td>2. 2 undergraduates carry out a 10 week internship</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Collect baseline data of microbial species in soil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Student poster presentations (with paper) at LBNL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Student poster presentations at AE week at Cal U</td>
<td>Fall 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COMPLETED</td>
</tr>
<tr>
<td>Phase II</td>
<td>1. Agricultural soil sampling</td>
<td>Summer 2010</td>
</tr>
<tr>
<td></td>
<td>2. Growth of fungi and bacteria (microorganisms) in the laboratory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Isolation of genomic DNA from microorganisms and amplification with PCR.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Student poster presentations (with paper) at LBNL (2 Cal U students completed a 10 week internship with me)</td>
<td></td>
</tr>
<tr>
<td>Phase III</td>
<td>1. Develop ratios of fungi and bacteria in soil</td>
<td>Summer 2011</td>
</tr>
<tr>
<td></td>
<td>2. Develop baseline data for soil health</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Identify soil health indicator organisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Student poster presentations (with paper) at LBNL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Student poster presentations at AE week at Cal U</td>
<td>Fall 2011</td>
</tr>
<tr>
<td></td>
<td>6. Publication and presentation (at a National Conference) of research by Project director.</td>
<td>Spring 2012</td>
</tr>
</tbody>
</table>

Expected Outcomes

The expected outcomes from this research include the following:

1. Enhanced student learning by providing two Cal U students with an experiential learning opportunity.
2. Professional development and growth for the Project director in practicing a variety of molecular techniques, analytical assessments and novel approaches.
3. Establishment of a long term collaboration with scientists at the Lawrence Berkeley National Laboratory.
4. Identification of a variety of fungal species in both organic agricultural soil and monoculture agricultural soil using traditional and microarray technology methods.
5. Identification of bacterial species in agricultural soil using microarray technology.
6. Development of indicator organisms for soil health and ecological standards. Soil health could possibly be identified based on the presence of specific fungi and if this is determined, farmers and extension agents could use the information to their advantage. The knowledge may help them improve soil health and therefore improve the health of our crops.
7. Dissemination of the research and results in a presentation at a national conference (American Society of Microbiology) and through a publication in a peer reviewed journal (Environmental Microbiology).
### BUDGET SUMMARY

<table>
<thead>
<tr>
<th>Project Budget</th>
<th>Proposed Grant</th>
<th>University Contribution</th>
<th>Other Revenue Sources</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries/Stipends</td>
<td>$12,500</td>
<td></td>
<td>$12,500</td>
<td></td>
</tr>
<tr>
<td>Student Wages</td>
<td>$8,500</td>
<td></td>
<td>$8,500</td>
<td></td>
</tr>
<tr>
<td>Benefits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honoraria (for consultants)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplies</td>
<td>$7,976</td>
<td></td>
<td>$7,976</td>
<td></td>
</tr>
<tr>
<td>Equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operating Expenses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Travel</td>
<td>$800</td>
<td></td>
<td>$800</td>
<td></td>
</tr>
<tr>
<td>Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTALS</td>
<td>$7,976*</td>
<td>$21,800</td>
<td>$29,776</td>
<td></td>
</tr>
</tbody>
</table>

* This figure is the total grant amount requested from the FPDC and must be listed on the title page of the proposal.

**Budget Notes**

**Supplies:** Estimated costs to purchase,
- DNA isolation kits (2 kit * $338/kit) $676
- PCR chemicals (including primers, taq polymerase, and enzymes and clean-up kits) $1,500
- Phylochips and essential chemicals to run analysis $5800.00
  *including dyes, probes, 2% agarose gels, ethidium bromide, Biotin labels, salts, buffers,

**Other Revenue/Sources:** This research is part of the Center for Science and Engineering Education (CSEE) Summer program at the Lawrence Berkeley National Laboratory (LBNL) in Berkeley, California. The CSEE summer program pays the PI a stipend to come and spend the summer at the LBNL using the equipment and expertise available there over 10 weeks in the summer. The microarray analysis (phylochip and mycochip) require a microarray reader which is only available at a few labs in the country. CSEE provides a summer stipend for the PI to live in California and work on the microarray reader found at LBNL. (Stipend: $1,250/week for 10 weeks/ $425/week for the students).

**Travel:** The CSEE Summer program will pay for travel to and from Berkeley, California for both the PI and the intern Cal U students.
References
Sarah L. Meiss


CURRICULUM VITAE
Sarah L. Meiss

Department of Biological and Environmental Science  Email: meiss@calu.edu
326 Frich Hall  Phone: 724-938-4203
250 University Avenue  Fax: 724-938-1514
California University of PA
California, PA 15419-1394

ACADEMIC BACKGROUND

Ohio University- Athens, OH
   Doctor of Philosophy in Biological Sciences  August 2000
   Department of Biology and Environmental and Plant Biology through
   Interdisciplinary Molecular and Cellular Biology Program  March 2006

   Dissertation:  “Characterization of a mutant Phaseolus vulgaris that exhibits
     strain specific restriction of nodulation.”

   Graduation:  June 2006.

Bloomsburg University, Bloomsburg, PA
   Bachelor of Science, cum laude- Microbiology  September 1994
   Minor in Chemistry through
   May 1998

   Bloomsburg University Honors Program-
   Honors project: “Naturally occurring antibiotics”

ACADEMIC APPOINTMENTS

California University of Pennsylvania – California, PA
   August 2007-present
   Department of Biological and Environmental Sciences
   Assistant Professor

Denison University – Granville, OH
   August 2004
   Department of Biology through
   Visiting Assistant Professor  May 2007

Ohio University Lancaster – Lancaster, OH
   August 2005
   Department of Biology through
   Adjunct Professor  December 2005

Ohio University (main campus) – Athens, OH
   January 2004
   Department of Plant Biology through
   Adjunct Professor  June 2004

RESEARCH INTERESTS
   Microbiology and Environmental Microbiology; Human and Plant pathogens, mycology, symbiotic relationships
   including human, plant and fungal. Medicinal Plants, tropical biology and sustainable agriculture.
RESEARCH EXPERIENCE

Effects of various chemicals on microbial growth - Last semester I advised an honors addendum of Jackie Davis. She grew up bacteria and examined the affect a variety of chemicals had on the microbial growth. Fall 2008

Creating a cosmid bacterial genomic library of Rhizobium sp. - Currently, I am Avritha Singh’s Senior Research Advisor at Denison University. Together, we are creating a genomic library of the bacteria Rhizobium leguminosarum. We are isolating genomic DNA, using restriction digests and pLAFR cosmid to create the library. September 2005 through May 2006

Biochemical characterization of exudates produced by Rhizobia sp. – Currently, I am Harpreet Grewal’s Research Advisor at Denison University. Together, we are Isolating Rhizobial bacterial exudates and chemically analyzing them using HPLC, Liquid chromatography, mass spectrometry and NMR. January 2006 through May 2006

Ames testing of biological pollutants: - Currently working with senior Adam White to examine pollutants for their possible mutagenic affects. We are using the Ames test to see if compounds such as EDTA, plant pesticides, and household cleaners could mutate a bacteria. January 2006 through May 2006

Characterization of three Rhizobial strains with the ability to nodulate a mutant Phaseolus vulgaris- Doctoral research advised by Dr. Art Trese at Ohio University. I investigated the legume rhizobium symbiosis. I isolated a mutant P. vulgaris that selectively restricts nodulation. I examined what characteristics of the soil bacteria cause them to be restricted by the plant. I focused on the bacterial symbiont, due to the ease of working with prokaryotes. August 2000 through August 2005

FIELD EXPERIENCE

Integrated Tropical Botany Course in Belize and Guatemala Ohio University, Athens OH December 1999

Neotropical Biology Course in Peru Bloomsburg University, Bloomsburg, PA January 1997

PROFESSIONAL AFFILIATIONS

American Society of Microbiology 1998- present
American Society of Plant Biology 2001-present
Tri-Beta Biological Honor Society 1998-present
Botanical Society of America 1998-present
Ohio Academy of Science 1998-present
International Society for Molecular Plant Microbe Interactions 1998-present
The Nature Conservancy / The Ocean Conservancy 1998-present

PUBLICATIONS


Wyatt, Sarah and Sarah Bashore, PBIO 114 Laboratory Manual, Cellular Foundations in Plant Biology