Environment and Natural Resources Trust Fund
2010 Request for Proposals (RFP)

LCCMR ID: 052-B1

Project Title:

Robust Bioconversion Approaches to make Crop Polycultures Viable

LCCMR 2010 Funding Priority:

B. Renewable Energy Related to Climate Change

Total Project Budget: $ $298,098

Proposed Project Time Period for the Funding Requested: 2 years, 2010 - 2012

Other Non-State Funds: $ $0

Summary:

Growing biomass crops in mixtures (polycultures) is far superior to monocultures, environmentally, including being potentially carbon negative. Our goal is to improve polyculture bioconversion to stimulate their marketability in Minnesota.

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Sponsoring Organization: U of MN

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Location:

Region: Statewide

County Name: Statewide

City / Township:

_____ Knowledge Base _____ Broad App. _____ Innovation

_____ Leverage _____ Outcomes

_____ Partnerships _____ Urgency _______ TOTAL

MAIN PROPOSAL (Schilling and Tschirner)

PROJECT TITLE: Robust bioconversion approaches to make crop polycultures viable

I. PROJECT STATEMENT

In Minnesota, farming crops like poplar and switchgrass to yield non-edible 'biomass' is poised for unprecedented growth, and the environmental impacts of this business hinge on which agricultural practices we adopt. In the near-term, biomass crops will likely be grown in single-species monocultures, as corn is presently grown, with yields improved by hybridization or genetic manipulation. Despite knowing that diverse plant mixtures (polycultures) reliably yield more biomass than single-species monocultures[1], with less environmental impact, <u>farmers will not plant polycultures unless there are market-ready approaches to process mixtures of crops.</u>

Compared to edible plant tissues, non-edible lignocellulosic biomass can yield more energy per hectare while reducing net greenhouse gas emissions, limiting maintenance, minimizing water and fertilizer inputs, and providing flexibility in crop selection. These assets would be enhanced if we utilized polycultures and if we could bioconvert crop mixtures. Key environmental benefits gained through more robust bioconversion are as follows: 1) Local processing facilities whose crop inputs are seasonal and local farmers who select crops based in part on their market value would have flexibility to make sustainable choices. 2) Polyculturing high diversity crops that fully utilize ecological niches, including marginal soils, and produce more biomass per hectare could increase land-use efficiency without relying on unproven technologies. 3) Polycultures better resist weed and pathogen invasions, and give predictable yields with far less maintenance than monocultures. 4) Site-specific native plants would be more attractive as non-edible crops, giving extraordinary potential for a market force to encourage ecosystem restoration by farmers.

Minnesota leads the U.S. in polyculture research, but we lack the data to evaluate their market value. We risk losing an unprecedented chance to give incentives for biomass industries to plant more natural cropping systems. Our team aims to define and lower a 'downstream' barrier in processing mixed crop residues, and is thus well-aligned with the LCCMR Trust Fund mission.

OUR GOALS: 1) to identify crop characteristics that affect bioconversion of crop mixtures, 2) to increase predictive capacity for novel crop mixtures, and 3) to share our data across Minnesota.

OUR APPROACH: characterize individual crops, and correlate these characters with yields from mixtures, using the pretreatment→saccharification→fermentation process (known as the 'sugar platform') as the bioconversion route most relevant near-term. Statistics gained from our past work will guide our design, and by standardizing key steps, our design will facilitate comparisons with other work. We will present our work locally and nationally and will update progress online.

II. DESCRIPTION OF PROJECT RESULTS

Result 1: <u>Characterization of Mixed Crops</u> **Budget:** $ 88,683

A six-species native prairie grass consortium, supplied by Dave Tilman (Univ. of Minnesota), will be characterized. Along with this *'native' group*, tissues from Minnesota's chief commodity crops, corn, grains (wheat straw), soybeans (*'crop' group*), and forest biomass spruce, pine, aspen (*'wood' group*) will be characterized. Data will include wet chemistry (eg. lignin), inductively-coupled plasma spectroscopy (ICP) for cations, EPA organics, and x-ray diffraction (XRD) for cellulose crystallinity. This data, alone, is valuable, and defining key characters that affect final yields should allow us to better predict yields in novel mixtures of Minnesota crops.

Deliverable	Completion Date
1. All crop residues acquired and stored dry at 4^oC	9/30/10
2. Each crop residue fully characterized and milled to relevant size	3/31/11

Result 2: Biodegradation and Bioconversion of Mixtures **Budget:** $ 72,524

At this milestone, we will have hydrolyzed residue polysaccharides (saccharification) following National Renewable Energy Laboratory (NREL) protocols and will have tested in-group tissue mixtures to compare with single-feedstock runs. Stock enzymes (Celluclast 1.5L, Novozyme 188, Multifect xylanase) will be loaded at a set activity per gram of cellulose. Pretreatments will be dilute acid (1% H_2SO_4, 170^oC, 1 hr) or ammonia fiber explosion (AFEX), commercially viable pretreatments with distinct modes of action and that are replicable in the lab. Sugar yields will be determined by refractive index and HPLC. In tandem, these mono- and mixed-substrates will be degraded by the fungus *Gloeophyllum trabeum* over 15 weeks. Preliminary data from the 'wood' group is provided (*see* Figure suppl.), and it yields important basic biodegradability data.

Deliverable	Completion Date
1. Mixtures + monosubstrates bioconverted with enzymes or biodegraded	6/30/11
2. Sugar yields and substrate weight loss quantified in all samples	9/30/11

Result 3: Fermentation of Hydrolysate to Gauge Process Inhibition **Budget:** $ 84,960

In hydrolysate from the bioconversion runs, inhibitors including furfural, acetyl, and lignin fragments will be measured by wet-chemistry and HPLC. Inhibition will be gauged in situ using shake flask fermentation through a time series to the ethanol maximum using the model yeast *Saccharomyces cereviseae*. We will gauge process inhibition, not with the goal of optimizing ethanol production, but to identify and avoid general barriers to downstream conversion.

Deliverable	Completion Date
1. Hydrolysate inhibitors quantified and fermentation barriers identified	3/31/12

Result 4: Final Recommendations and Data Sharing **Budget:** $ 51,931

Concurrent with final analyses, a Drupal online content management system (CMS) will be used to share project specifics and metadata, and our work will be presented locally and nationally.

Deliverable	Completion Date
1. Data analyses, conference proceedings and website metadata available	ongoing

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III. PROJECT STRATEGY

A. Project Team/Partners

Dr. Schilling (www.bbe.umn.edu/faculty/schilling) has published often on native biodegradation and bioconversion mechanisms, and works on several projects with Dr. Tschirner (www.tc.umn.edu/~xuxxx158/group/visit.html), whose expertise is plant chemistry and effects of tissues on industrial bioprocessing. Post-doctoral Associates Duncan and Wafa AlDajani work in the current group and supervise students. We acknowledge Dr. David Tilman as a Partner for supplying prairie grasses from the most likely long-term native polycultures in Minnesota.

B. Timeline Requirements

Through March, 2011, we will acquire and characterize crop residues (12 total). Through September, 2011, will pretreat/saccharify residues in mixtures and monocultures (21 in-group mixes, run in quadruplicate = 84 runs + controls). Through March 2012, we will test inhibitors and ferment all hydrolysates. In the last quarter, through June 2012, we will analyze and communicate findings. Our experience guides our sample sizes and our budget is appropriate.

C. Long-Term Strategy

The future of this project will involve creative ventures into economic modeling and process integration. Making improvements in process engineering is not a conventional environmental science project, but this is a case where engineering can remove a sustainability bottleneck. It needs targeted funding. Our long-term strategy is to identify high-feasibility polyculturing opportunities in Minnesota and create the partnerships necessary to achieve a viable industry.

Project Budget (Schilling and Tschirner)

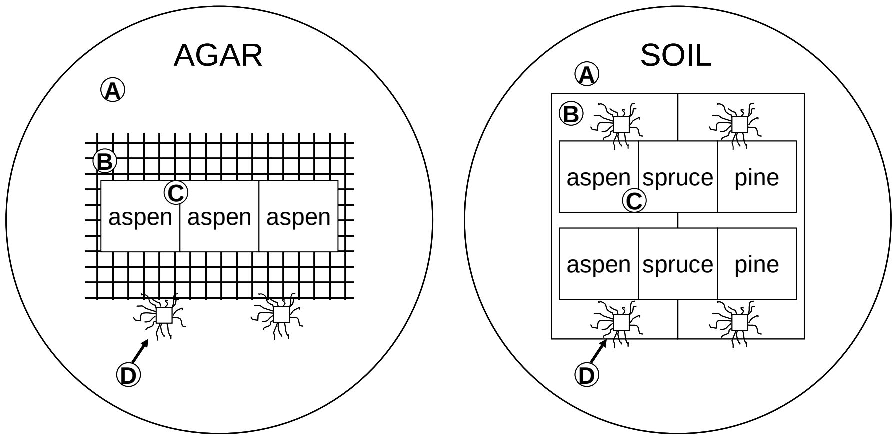
IV. TOTAL PROJECT REQUEST BUDGET (2 years)

BUDGET ITEM	AMOUNT
Personnel:	
2 yrs. Undergraduate employee (8% fringe) (laboratory assistant, website)	$ 6,480
3 total yrs. Post-doctoral Assoc. w/ full-time salary (20% benefits) for Dr. Shona Duncan (2 yrs, Biodegradation) and Dr. Waleed Wafa AlDajani (1 yr, Inhibitors/fermentation)	$ 149,400
2 yrs. Graduate Student w/ full-time salary (70.5% tuition, 16.8% benefits)	$ 79,144
Equipment/Tools/Supplies:	
Variable wavelength microplate reader (BioTek uQuant) - to be used for high-throughput analyses of multiple enzyme activities post saccharification, inlcuding low UV application. We currently use a dial-in single-channel spec-20.	$ 12,440
I-2500KC programmable incubator/shaker with refridgeration to handle our large run sizes and automatically halt saccharification at a repeatable set time	$ 15,000
Chemicals and Biologicals: acids, bases, buffers, solvents, growth media, HPLC eluent, hydrolytic enzymes, microbial organisms	$ 15,000
Supplies: HPLC columns, glassware, consumables	$ 13,000
Travel:	
In-state meetings (E3 in Saint Paul and the MN chapter of the Society for Conservation Biology)	$ 1,500
Out-of-state meetings (American Chemical Society-Biofuels and Chemicals, Society for Conservation Biology)	$ 3,000
Additional Budget Items:	
Web-hosting for database on U. Minnesota-housed URL using Java and Web Services (JAWS) at UMN ($60/mo for 2 yrs, with $144 set up fee)	$ 1,584
ICP-OES analytical fees for cation analysis at the Soils Analytical Laboratory at the University of Minnesota ($10/sample with 105 samples)	$ 1,050
Publication fees (page charges, etc.)	$ 500
TOTAL PROJECT BUDGET REQUEST TO LCCMR	$ 298,098

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
In-kind Services During Project Period: High-performance liquid chromatograph (HPLC) - This Agilent model is new and will be used for all HPLC in this project.	$ 51,500	
Funding History: We have several grants in which we have acquired and optimized the procedures needed for this proposed work. These include PI Schilling's $578,000 DOE Biomass Initiative grant and a $350,000 UofM IonE Discovery Grant, as well as some smaller grants from the University of Minnesota (Initiative for Renewable Energy and the Environment - IREE $42,732 and a Technology-Enhanced Learning Grant $9974, which will form the basis for our Drupal-based metadata online resources). Also, the preliminary data generated for mixed subtrates (attached as a figure) were funded by a University of Minnesota start-up fund for the PI Schilling and they funded Adam Norcutt, an undergraduate student, plus supplies ($2500).	$ 983,206	The DOE, Discovery, and TEL grants are all active ($937,974)

Preliminary Data– **Mixed Substrate Biodegradation** – Below are depictions of the microcosms used to study mixed substrate biodegradation by the fungus *Gloeophyllum trabeum* (D). Nutrient-limited agar or soil mixes (A) were used, with mesh or birch supports (B) for aspen, pine, and/or spruce (C). A monosubstrate shown on AGAR at left – a polysubstrate shown in SOIL at right.



Pine monosubstrates were degraded faster by *G. trabeum* than polysubstrates (see **bold**), demonstrating an effect of mixtures on bioconversion. We believe the compensation for weight loss in 'combined' wood groups ('combined' rates are equal) indicates substrate preference and an influence of pine inhibitors, a bioprocessing dynamic that would need to be addressed commercially.

Wood Substrate	Microcosm Set-up	Replicates (n)	Week 8 Weight Loss (%)	Week 16 Weight Loss (%)
Aspen	Monosubstrate	12	35.4 (2.5)[a]	63.9 (1.8)[a]
	Polysubstrate	12	46.7 (1.8)[a]	66.5 (1.8)[a]
	Control	12	0.01 (0.03)	0.89 (0.71)
Spruce	Monosubstrate	12	36.7 (3.6)[a]	56.4 (4.6)[a]
	Polysubstrate	12	38.8 (3.6)[a]	64.8 (0.9)[a]
	Control	12	0.00 (0.03)	0.04 (0.03)
E. White Pine	Monosubstrate	12	**33.9 (3.0)[a]**	**44.7 (3.9)[a]**
	Polysubstrate	12	**14.6 (4.0)[b]**	**26.1 (4.8)[b]**
	Control	12	0.15 (0.09)	0.56 (0.16)
Combined	Monosubstrate	36	35.3 (1.7)[a]	55.0 (2.4)[a]
	Polysubstrate	36	33.4 (3.0)[a]	52.5 (3.6)[a]
	Control	36	0.05 (0.05)	0.50 (0.30)

Project Manager Qualifications and Organization Description

Dr. Jonathan S. Schilling is an Assistant Professor in Bioproducts & Biosystems Engineering at the University of Minnesota. Jonathan is Adjunct Faculty in the Department of Plant Pathology and is a current Resident Fellow in the Institute on the Environment (IonE). Among his awards, Jonathan received a prestigious Junior Faculty grant in the Conservation and the Environment program of the Andrew W. Mellon Foundation and was winner of the competitive Maine Dow Griffee Research Award. Jonathan received a Ph.D. from the University of Maine in biology, with his focus on wood microbiology. Jonathan received his M.S. in Environmental Studies from Longwood University and did his undergraduate work at Rhodes College in Biology. He is author of over 30 publications and proceedings related to lignocellulose biodegradation mechanisms. He teaches 5 annual credit hours in biodegradation topics, and he advises 15 undergraduate students in the lab or through their curricula. His lab group is currently 6 members, including Dr. Shona Duncan. Jonathan also manages a Tech-enhanced Learning grant, using Drupal as a CMS platform to share biodeterioration information with other scientists and the general public. He is involved in significant service and outreach, including society technical committees, conference chairperson duties, and journal refereeing.

<u>Jonathan's responsibilities</u> related to this project, in addition to being the primary manager, are to guide Dr. Duncan in the characterization of plant materials and to lead his group's efforts in pretreatment, saccharification, and biodegradation. He will also advise the graduate student involved in the project, making a concise and useful graduate-level project, and will oversee the development of online tools with the aid of an undergraduate student working on the project.

Dr. Ulrike Tschirner is an Associate Professor in Bioproducts & Biosystems Engineering at the University of Minnesota. Ulrike received her Ph.D. at the University of Karlsruhe, Germany, in polymer chemistry, specifically lignin chemistry. She had 9 years of industry experience in industrial processing (pulping chemistry and papermaking) before returning to academia. Ulrike did a post-doc at State University of New York (SUNY-ESF) before her position as Research Chemist at Scott Paper Company in Philadelphia. Around her industry research, Ulrike has published many papers on polymer processing chemistry, including work on mixed-fiber processing, and she has a synergistic project on fermentation inhibitor production during hemicellulose extraction from agricultural residues. Ulrike has a strong commitment to teach others in the State of Minnesota about the potential in renewable resources and about the importance of engineering in process efficiency as well as environmental sustainability.

<u>Ulrike's responsibilities</u> related to this project are to participate directly at the outset in co-advising the characterization effort, particularly x-ray diffraction and wet-chemistry of these novel plant mixtures. We have budgeted post-doctoral money for this stage, led by Dr. Waleed Wafa AlDajani. Her other primary responsibilities are managing the characterization of inhibitors and testing inhibition using a model fermentation system. These efforts will be in the 5^{th}, 6^{th}, and 7^{th} quarters, primarily. Ulrike will also spearhead our efforts to disseminate findings to industry.

The University of Minnesota, Twin Cities (UMTC), is among the largest public research universities in the country, offering undergraduate, graduate, and professional students a multitude of opportunities for study and research. Spanning the Mississippi River, the flagship campus includes the West Bank, East Bank, and Saint Paul campuses. As the State of Minnesota's main land-grant institution, the University of Minnesota is committed to affordable higher education and it is tied directly and responsibly to our State's agriculture.