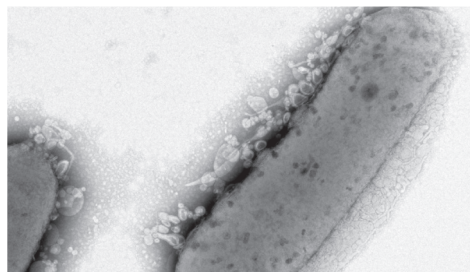
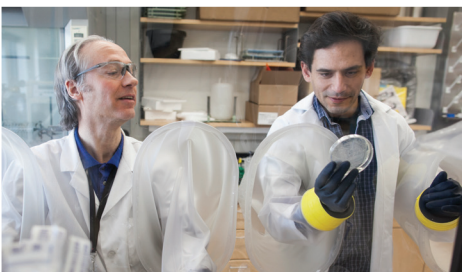
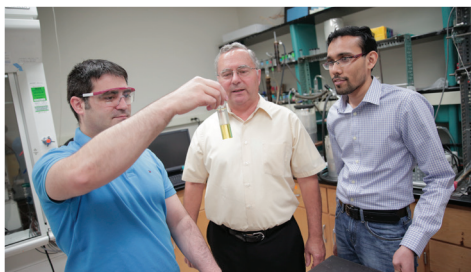
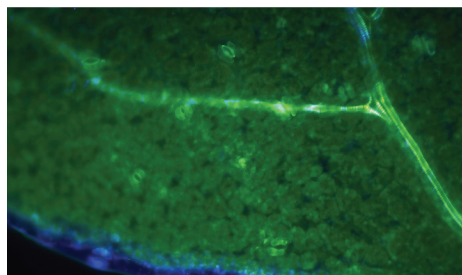
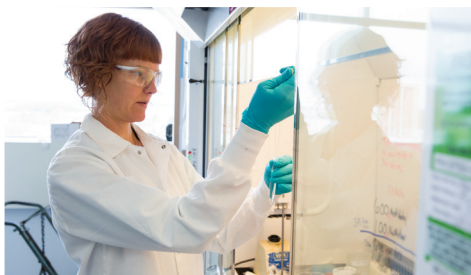
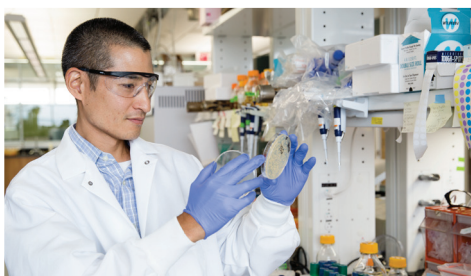


GREAT LAKES
BIOENERGY
RESEARCH CENTER



Department of Energy

Y1-10 FINAL REPORT



Great Lakes Bioenergy Research Center Final Report

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GLBRC RESEARCH HIGHLIGHTS (2007-2017)

Genomic approaches to engineer plant lignin.

The ability to rationally engineer plant metabolism thereby producing cell walls with modified lignin has the potential to make major contributions to producing fuels and chemicals from biomass. Altering the chemical composition of lignin can increase the feasibility of converting the aromatic building blocks of this polymer into fuels or other valuable products, and make it easier to access the sugars in plant cell walls for conversion to fuels. In a widely publicized 2014 paper, GLBRC researchers reported on deployment of the “Zip-Lignin approach” in which plants are engineered to incorporate monomer-conjugates (named coniferyl- and sinapyl-ferulate into lignin), thereby introducing bonds that are easier to break but retain lignin’s structural value to the plant. The Zip-Lignin approach has since been hailed as having “the potential to fundamentally change the economics of lignin degradation”

(<http://www.sciencemag.org/news/2014/04/cheaper-fuel-self-destructing-trees>) and was nominated for Science’s 2014 Breakthrough of the Year. Patents have been filed on this approach and agribusiness leaders are interested in licensing this innovation.

More recently we have demonstrated that hybrid poplar trees engineered to contain Zip-Lignin result in enhanced lignin levels via chemical pulping, indicating the utility of Zip-Lignin for poplar processing. Additionally, we have engineered pine tracheary elements to make a monomeric precursor of Zip-Lignin (sinapyl alcohol), indicating the potential for Zip-Lignin production in this species. Surprisingly, a survey of Zip-Lignin content in spermatophytes (seed plants) indicated that many angiosperm species naturally contain low, but detectable, levels of Zip-Lignins, knowledge that may aid in identification of superior sources of biomass that are more easily and economically deconstructed. Finally, we have identified additional genetic engineering approaches for improved processing of plants; for example, augmentation of available pools of soluble sucrose in poplar yields reduced lignin and altered cell wall structure, while mutation of a gene encoding a lignin biosynthetic enzyme increases Zip-Lignin levels in maize. GLBRC continues to evaluate Zip-Lignin performance in bioenergy-relevant crops such as grasses and identify ways to engineer plant lignins for improved processing.

By the end of Year 10, we have analyzed the impact of Zip-Lignin modification in poplar trees on the release of sugars with the use of different deconstruction methods. We have also built upon the Zip-Lignin strategy by genetically altering lignin biosynthesis in poplar and maize, resulting in increased Zip levels and greater sugar release. Field trials of Zip-Lignin poplar began in June 2016, thus we are two growing seasons into testing how translatable the Zip-Lignin approach is from greenhouse-grown material to field-grown trees.

Next-generation plant deconstruction strategies.

The energy and economic costs of deconstructing plant biomass represent a significant barrier to the production of cost-effective lignocellulosic fuels and chemicals. GLBRC developed an innovative, next-generation deconstruction method that is based on using γ -valerolactone (GVL), a “green” solvent that can be produced from plant material, to dissolve biomass followed by acid

hydrolysis to release free sugars. The GVL technology is a feedstock agnostic, enzyme-free, and renewable strategy to produce sugar and lignin streams for conversion at lower cost than existing methods. The use of GVL for biomass deconstruction has been hailed by others as “a better way to make biofuels” (<http://www.popularmechanics.com/cars/hybrid-electric/a10028/a-better-way-to-make-biofuels-16388312/>), with the potential to “save billions on the cost to bring to market new biofuels” (<http://www.jsonline.com/business/uw-madison-researchers-are-building-better-biofuels-b99190278z1-241974641.html>). In addition to yielding high levels of C5 and C6 sugars from lignocellulosic biomass, the GVL method produces high-quality lignin with a native-like structure. When lignin production is integrated into the sugar platform, the method produces high-value products from each of the major biomass fractions in up to 80% yield. Recently a technoeconomic modeling study indicated that a method utilizing co-solvents to produce sugar and lignin streams and enable GVL recovery appears to be economically feasible, with a predicted modest decrease in minimum selling price per gasoline gallon equivalent as compared to other benchmarked processes.

By the end of Year 10, we have increased GVL hydrolysate production capacity within the Center, providing liter amounts of material to researchers for additional studies. We continue to characterize the chemical composition of hydrolysate to identify major inhibitors and evaluate their effect on microbial fermentation and conversion. A soon-to-be published manuscript describes the effect of GVL on yeast bioconversion and genetic engineering strategies to overcome toxicity, thus furthering the Center’s mission to understand and mitigate microbial stress responses to plant hydrolysate. The Center continues research on depolymerization of GVL lignin into aromatic monomers or small oligomers, which may be biologically converted by specialized microbes into fuels and high-value products.

The GVL approach is one of two licensed GLBRC next-generation deconstruction strategies and has the potential to increase the economic viability of producing fuels and chemicals from a wide variety of lignocellulosic plant material, and both are currently being evaluated by industry.

The potential for marginal lands to meet a significant portion of cellulosic biomass needs.

Growing cellulosic biofuel crops on existing farmland conflicts with the necessity to sustainably meet the food needs of a burgeoning global population. Taking land out of food production can raise food prices and motivate continued conversion of non-cropland to agriculture, thereby creating a significant carbon debt. In a landmark paper that was hailed by others as laying the foundation for “biofuel production at the margins”

(<http://www.npr.org/2013/01/16/169538570/could-some-midwest-land-support-new-biofuel-refineries>), GLBRC researchers showed that there is sufficient marginal land in the U.S. Midwest to grow a significant fraction of expected biofuel needs. Michigan field experiments were used to calibrate a cellulosic crop model that was applied to non-forested, non-farmed private land at high resolution across a 13-state region of the central U.S. Forty-five percent of available land fell within 50 miles of a potential biorefinery, and modeling showed that 25 percent of the legislated cellulosic biomass target could be met by productivity on these lands alone, for a substantial net carbon benefit. Biomass production on marginal lands avoids both food-fuel conflict and indirect land use costs. GLBRC continues to extend these analyses to a wider

geographic region and incorporate techno-economic modeling to show reasonable potential configurations for future feedstock supplies.

Techniques to valorize lignin.

Lignin is a major component of plant cell walls. The ability to convert this polymer into aromatics and other valuable products has the potential to improve the sustainability and economic viability of lignocellulosic biorefineries. In a study that received a Presidential Green Chemistry Challenge Award in 2014, GLBRC researchers described a process that could “capture the full and unrealized value of biofuels” by converting what is currently burned as fuel into valuable products. At the heart of this process is the ability to convert processed lignin fractions into chemically defined products for conversion into high-value products.

By the end of Year 10, we have made several strides in our efforts to enable lignin valorization. GLBRC has taken several approaches, including structural and biochemical analyses of bacterial enzymes that cleave lignin bonds; chemical analysis of dimeric degradation products released by lignin depolymerization to verify and quantify components used in lignification; utilization of formaldehyde to facilitate lignin monomer release during biomass depolymerization; identification of valuable extractable “clip-off” bioproducts like the flavonoid, tricetin, shown to be involved in lignification in monocots; and identification of new and complex lignin compositions in palm species, including polyphenolic compounds, hydroxystilbenes, incorporated into lignin, and an unprecedented range of monolignol conjugates not previously known to be involved in lignification. All of these approaches help to inform our understanding of lignification and enable lignin valorization, both through extraction of naturally-occurring components and of compounds that have been engineered into bioenergy crops.

Genome- and systems biology-enabled identification of valuable co-products from biomass hydrolysates.

All published methods for processing plant biomass release materials that have a negative impact on the ability of biofuel microbes to produce products. Consequently, GLBRC uses a genome-enabled platform to identify toxic compounds within biomass hydrolysates, determine their mode of action, and develop ways to bypass their negative impact on biofuel microbes. A 2015 paper reported on an unexpected product of this activity, the discovery of a compound (poacic acid) within biomass hydrolysates that is a potent antifungal agent. As a natural plant-derived product, poacic acid has been described as a “valuable new weapon in our armamentarium of antifungals” (<http://www.nature.com/nature/journal/v521/n7551/full/521168a.html>). As patenting of poacic acid is in progress, an investment from our technology transfer partner has been used to prepare kilogram quantities of this compound for field trials as a natural fungicide against agricultural pests. In the near future poacic acid could be a prime example of how genome- and systems biology-enabled advances can provide fuels and valuable products that can be produced by future lignocellulosic biorefineries.

Feedstock variability and effects on deconstruction and microbial conversion.

Variation in environmental conditions during the growth of bioenergy crops could have significant detrimental effects on microbes during biofuel production. However, the effects of environmental stress on the “field-to-fuel” pipeline have not been well studied. A 2016 GLBRC paper examined the effects of variable precipitation on two bioenergy feedstocks, corn stover and switchgrass, sampling from a major drought year and two years with average precipitation. The study examined effects on hydrolysate chemical composition and the ability of yeast (*Saccharomyces cerevisiae*) or bacteria (*Zymomonas mobilis*) to ferment them to ethanol. While most hydrolysates were readily fermented to biofuel, the growth of yeast, but not *Z. mobilis*, was completely inhibited in hydrolysate generated from drought-stressed switchgrass. Biomass and compositional analysis revealed that drought-year switchgrass accumulated greater concentrations of soluble sugars, likely a stress response to drought, that were degraded to inhibitory compounds during pretreatment. These findings indicate that environmental variation can have significant effects on biomass hydrolysate properties and subsequent fermentation to biofuels. Knowledge of how these factors impact biofuel production can guide pretreatment and biocatalyst choices to mitigate inhibitory effects. GLBRC continues to study the effect of environmental stress on downstream processes, with planned studies on several other variables such as nitrogen fertilizer treatments, exposure to pathogens or pests, and variation in the plant microbiome. Ultimately, we aim to integrate the effects of environmental conditions and microbial molecular signatures into a predictive model that can guide biocatalyst choice or suggest engineering strategies to overcome inhibitory effects.

GLBRC OUTREACH (2007-2017)

Over the past ten years, GLBRC's broad research activities have had significant impact on the research community, industry, and society at large. Through formal educational programming, targeted activities, and informal interactions, innumerable individuals, groups, and organizations have been exposed to the impactful research being performed by the Center. These efforts have increased awareness of bioenergy research and the role it can play in addressing societal problems related to energy usage, supply, and security.

Stakeholder Outreach

GLBRC considers our stakeholders to not only be the taxpayers funding the program and the research community but also local, federal, and foreign government officials including the president, congressional members and staff, U.S. Department of Energy personnel, and other relevant officials.

GLBRC researchers and leadership have impacted the scientific community through research accomplishments and via membership in a diverse, yet relevant, set of advisory boards as well as society/organization officer roles. These include but are not limited to the Army Research Office, American Society of Microbiology, University of North Texas, University of Michigan, and the other BRCs.

In 2016, GLBRC answered the call from the Government Accountability Office (GAO) to represent the BRC program and aid in providing information that generated a [report](#) regarding the status of advanced biofuels research and development in the federal government. The two research objectives of the report were: (1) How does the federal government support advanced biofuels research and development, and where are current and planned federal efforts focused; and (2) What are expert and stakeholder views on the commercial viability of advanced biofuels in the short-, medium-, and long-term?

Specific examples of interactions: Over the period of the grant, GLBRC has hosted a wide range of distinguished leaders. Noteworthy are the following events: President Barack Obama and Secretary of Agriculture Tom Vilsack [visited](#) GLBRC pretreatment facility at MSU in 2014, prior to the President's signing of the Farm Bill on the MSU campus. In 2012, we proudly [hosted](#) DOE Secretary Steven Chu to discuss the research portfolio of the Center. Lastly, in 2015, shortly after congressional confirmation, DOE Undersecretary for Science Franklin Orr [visited](#) GLBRC at UW–Madison to see the impact of DOE's significant BRC program investment and to hear about other DOE-funded research on the UW–Madison campus.

Education and Outreach (E&O)

The goal of our E&O efforts has been to broaden the understanding of current issues in bioenergy for the general public, students, and educators at all levels. Bioenergy research and development are extremely important contemporary issues. Therefore, our E&O team used a variety of programs, events, university courses, and even publications (see complete publications

list) to present research from GLBRC labs in a way that is accessible and interesting to a broad array of audiences. E&O resources and projects included (1) inquiry-based bioenergy classroom activities, (2) libraries of information on bioenergy, energy, and sustainability, (3) summer undergraduate research programs, (4) public speaking engagements, and (5) collaboration with existing education and outreach programs that address bioenergy and related issues, such as the [First-Year Interest Groups](#) (FIGs) program at the UW–Madison and [iGEM](#) at Michigan State University (MSU).

Over the past ten years we have directly connected more than 100 GLBRC faculty, students and staff with over 1,000 educators, a significant number of whom were curriculum coordinators or master teachers that went on to introduce our materials to other teachers in their area or school district. We roughly calculate that our E&O activities engaged more than 50,000 students per year through the cumulative work of these teachers and related GLBRC education materials.

Specific examples of impact: Craig Kohn and Lisa Sorlie were participants in both our Research Experience for Teachers (RET) and Bioenergy Institute for Educators (BIE) programs. Kohn participated in the BIE program in 2010 and in the RET program in Cameron Currie's lab in 2011, where he created curriculum materials. A national leader in high school agriculture education, Kohn is currently a graduate student in Joyce Parker's lab at MSU. He has an NSF fellowship for his bioenergy-related graduate studies. In 2013, Kohn worked with TED talks to create a [video](#) on biofuels and bioprospecting.

Sorlie is a teacher at Bonduel Middle School in Crawford County, WI. She participated in GLBRC's BIE program as well as the RET program as part of Chris Hittinger's lab. GLBRC wrote a story about her in April 2017 that was distributed broadly by University Communications. This story inspired UW Foundation to choose Sorlie to represent Crawford County for their 72 Counties project, and even put up a billboard in Crawford County with Sorlie on it.

Our summer undergraduate research programs brought over 150 students to GLBRC from an international applicant pool, many of whom were underrepresented minorities in the sciences. These students studied at three different sites (MSU, UW–Madison, and the Kellogg Biological Station) and were involved in bioenergy-related projects ranging from molecular biology to landscape-level ecology. Over 60% of these students went on to pursue post-graduate degrees.

Specific examples of impact: Gina Lewin and Grace Klinger both began with GLBRC as Research Experience for Undergraduates (REU) participants. Lewin was a 2009 participant of the program in Brian Pfleger's lab. Given she attended a small school for her undergraduate degree, the program was Lewin's first opportunity to experience doing research at a world-class research institution. She became a graduate student in Cameron Currie's lab and is now a postdoctoral fellow at Georgia Institute of Technology. The importance of the REU program and E&O activities continued to resonate with Lewin throughout her time with GLBRC that she continued to participate in many E&O activities. A [story we wrote on her in March 2015 here](#).

Klinger started with GLBRC as a UW–Madison REU participant in 2013 while she was an undergraduate student at Wright State University. She is now a PhD student with Eric Hegg at

MSU. Klinger is studying a biomimetic approach to lignin deconstruction, and is a coauthor on a patent application for technology she developed as part of her graduate studies.

Community Outreach

Outreach to the general public was diverse and included participation in science events and festivals, attendance at events where bioenergy was a topic of interest, and working with individuals and groups that visited GLBRC. We also connected with the media via regularly occurring segments on the [Larry Meiller Show](#) on Wisconsin Public Radio and active Facebook and Twitter accounts. Such wide reach has allowed GLBRC to not only inform the general public but also attract talented individuals to the Center.

Specific examples of events: GLBRC representatives have been active, invited participants in the [French American Science Festival](#) in Chicago, an annual event sponsored by the French Consulate exposing hundreds of individuals to cutting-edge science. GLBRC ambassadors have hosted multiple yearly activities at the [Wisconsin Science Festival](#), a four-day statewide science event connecting people to science, technology, and engineering topics at UW–Madison. Various investigators within GLBRC have been featured presenters for [Wednesday Night at the Lab](#) events and other broad outreach activities through the Wisconsin Alumni Association. Many MSU investigators were key speakers and advocates for hosting [Fascination of Plants Day](#) in 2017, which has been proposed to be an annual event forthcoming due to its inaugural success.

Specific examples of our wide reach: Jeff Vinokur, a former GLBRC student, has been featured through multiple media ([print](#), [television](#), [touring](#), etc.) for his scientific passion and unique way of reaching many people. Vinokur's entertaining approach has made him quite memorable to all those who have seen or interacted with him.

Jan Hellinger is a high school student from Germany who has come to GLBRC each summer since 2015 and plans to return in 2018. Hellinger's participation in GLBRC was directly a result of our outreach activities and corresponding reputation.

Industry Outreach

Industry Outreach takes many forms at GLBRC, from Scientific Advisory Board (SAB) participation and conference attendance to intellectual property options, licenses and material transfer agreements. The relationship GLBRC has with its SAB members is a strength of the Center. Over the period of the grant there have been numerous, influential members of the bioeconomy involved with the board including venture capitalists, academic researchers, leadership from the other BRCs, as well as executives from the automotive, energy, chemical, and biobased industries. This balanced perspective has had a great influence on the GLBRC portfolio and has pushed knowledge of GLBRC technologies into industry.

Outside of the SAB, industry relations have taken a multipronged approach. Direct industry interactions include representatives from roughly sixty companies relevant to Center research either visiting or hosting GLBRC key personnel. The diversity of industry sectors that have had

touch points with GLBRC is representative of the full scope of the research portfolio of the Center. Many of these interactions have resulted in successful technology transfer negotiations or internships for students in the Center with these companies. Additionally, GLBRC has participated in many rounds of the SBIR-STTR program resulting in further relationships being built with corporate partners. Start-up companies resulting from GLBRC intellectual property have strengthened the Center's reputation outside of strictly research-based institutions. Finally, GLBRC strategically utilizes attendance at meetings and conferences to deepen and/or broaden relationships with industry partners.

Importantly, GLBRC partners with the Wisconsin Alumni Research Foundation (WARF) and MSU-Technologies (MSU-T) to identify potential opportunities in all of the interactions described above. Relationships with these technology transfer entities have been critical to the success of our industry outreach.

Specific examples of interactions: Novozymes has been a critical partner with GLBRC. Access to their standardized enzyme cocktails at no cost has allowed Center researchers to develop industry-relevant standard operating procedures that have resulted in strong validation and applicability of Center studies.

GLBRC PERSONNEL DEVELOPMENT (2007-2017)

GLBRC is made of a diverse group of faculty, scientists, technicians, staff, undergraduate and graduate students, and postdocs. These include those funded by the DOE award as well as students or postdocs who have fellowships, other funding, or perform unfunded research in support of the Center.

Personnel Development

GLBRC has employed a wide-range of strategies to develop its personnel for greater impact either within the Center or beyond. Examples of activities that are used to provide either diversification or strengthening of skill-sets beyond that of scientific training include, but are not limited to, planning of the annual science meeting program as well as presenting at the event, sponsoring personnel to attend and present at meetings, workshops and conferences, exposing individuals to impactful outreach opportunities, and providing the opportunity for students to intern at relevant bioindustry companies and organizations.

Featured personnel (in alphabetical order):

[Lauren Meyers](#)

Lauren Meyers is the Wisconsin Energy Institute (WEI) and GLBRC Director of Administrative Services at the University of Wisconsin–Madison. Lauren has been with GLBRC since 2008, serving in a variety of administrative roles from being a business operations office employee to pre- and post-award research administrator positions and, finally, director of administrative operations, which affords her a seat on the GLBRC Management Team. She is a shining example of developing talent from within the organization.

[Rebecca Garlock Ong](#)

Dr. Ong is an Assistant Professor at Michigan Technological University. Rebecca was with GLBRC from the beginning as a graduate research assistant in Dr. Bruce Dale's Biomass Conversion Research Laboratory at Michigan State University until 2011. After obtaining her Ph.D. in 2012, Dr. Ong continued on as a research technician within the GLBRC at MSU and was then promoted to research assistant professor in 2013. In 2016 she began a faculty position in the Department of Chemical Engineering at Michigan Technological University. Dr. Ong has supervised, advised, and taught many students and staff through her career as well as chaired and presented at meeting and conference sessions, become a member of many scientific societies, and is a respected reviewer of publications in her field. Rebecca contributed to GLBRC's recently awarded grant and is listed as key personnel. Dr. Ong went from a graduate assistant to a faculty project lead in her time with GLBRC.

[Jennifer \(Jennie\) Reed](#)

Dr. Reed is the Harvey D. Spangler Faculty Scholar and Associate Professor in the College of Engineering at the University of Wisconsin–Madison. At the time of the initial GLBRC proposal,

Jennie was a faculty fellow at the University of California, San Diego. Upon arriving at the University of Wisconsin–Madison, she began to make an immediate impact resulting in [numerous awards](#), recognitions and increased demand to serve on committees and boards. These include but are not limited to: the [Presidential Early Career Award for Scientists and Engineers](#) (2013), a DOE Early Career Award (2012), an NSF Career Award (2011), the [KBase Scientific Advisory Committee](#), and the GLBRC Conversion Area Leadership Council. Additionally, Dr. Reed was a major contributor to the recently awarded GLBRC grant and will serve on the new GLBRC Management Team.

[Matt Wisniewski](#)

Matt Wisniewski is the WEI and GLBRC Director of Marketing and Communications. Matt has been with GLBRC since 2010, serving in a variety of communication roles from photographer to web-, graphic-, and document designer and, finally director of the entire communications team for the Center. Matt’s work has been featured on multiple forms of media from print to web, including DOE materials. From having received his initial job offer from GLBRC on the day he graduated from UW–Madison to becoming the director, Matt is an incredible example of promoting talented individuals.

Center Alumni

Among the alumni of the Center that have been tracked, roughly 60% are in academia (35% of these are in faculty positions), 25% are in industry (including one Vice President, three Founders of business, 3 Directors and 3 CTOs) and 15% are working in non-profit research organizations. These individuals have spread across 28 states and 17 nations, making GLBRC’s impact to the scientific community truly global.

Featured alumni (in alphabetical order):

[Jeremy Luterbacher](#)

As a Swiss National Science Foundation Postdoctoral Scholar, Dr. Luterbacher joined [Great Lakes Bioenergy Research Center](#) via Dr. Jim Dumesic’s lab at the University of Wisconsin–Madison. Jeremy’s contributions to the Center were numerous and significant during his short, two-year tenure working on solvent-aided chemical biomass depolymerization. He was a pivotal component of many high-profile publications such as the recent Shuai, L. *et al.* (2016) *Science* publication (Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science* **354**, 329-333 (2016) [DOI: 10.1126/science.aaf7810]). Dr. Luterbacher is now an Assistant Professor and head of the Laboratory for Sustainable and Catalytic Processing at École Polytechnique Fédérale de Lausanne (EPFL) in Lausanne, Switzerland, where he continues to collaborate with GLBRC researchers.

[Maria Sardi](#)

As an NSF Graduate Research Fellow in Microbiology, Maria Sardi joined the Center via Dr.

Audrey Gasch's lab. During her doctoral work, Maria not only published prolifically, but also generated intellectual property on *Saccharomyces cerevisiae* strains relevant to biofuels. Additionally, in 2016 Maria completed a successful summer internship in Cargill's Research and Development Biotechnology team. After completion of a Ph.D. at the University of Wisconsin–Madison, Dr. Sardi returned to [Cargill](#) as a Research Biotechnologist. Maria is an outstanding example of a Center alumna that took advantage of the development opportunities afforded to her by being a member of GLBRC.

[Karen Stahlheber](#)

Dr. Stahlheber was a post-doctoral researcher in the Sustainability area of GLBRC, studying the stability of perennial grassland communities during the establishment phase. Though Karen was officially assigned to Dr. Kay Gross' lab at the Michigan State University's W. K. Kellogg Biological Station, she worked in close collaboration with Dr. Randy Jackson's lab at the University of Wisconsin–Madison. Dr. Stahlheber was a poster presenter at the 2015 Genomic Sciences Program PI Meeting before moving on to her current position of Assistant Professor at the [University of Wisconsin – Green Bay](#) beginning in 2016. Karen shows how leveraging multiple GLBRC partners and field sites can be advantageous for all.

[Garret Suen](#)

Dr. Suen was a post-doctoral researcher in Dr. Cameron Currie's lab during the early years of GLBRC. In 2011, Garret became an Assistant Professor at the University of Wisconsin–Madison as part of the Wisconsin Bioenergy Initiative cluster hire. He has since been promoted to Associate Professor studying how symbiotic microbes convert biomass into usable nutrients for their herbivore hosts and how this is applicable to the production of biofuels and bioproducts.

GLBRC RESEARCH ACCOMPLISHMENTS (2007-2017)

Year 1 (Dec. 1, 2007 - Nov. 30, 2008)

- ***Arabidopsis* XXT1, XXT2, and XXT5 encode xylosyltransferases.** In this first year of funding, we report on two studies of genes involved in xyloglucan biosynthesis in *Arabidopsis*. The first paper demonstrates that *XXT1* and *XXT2* encode xylosyltransferases that are required for xyloglucan biosynthesis; the second paper reports on the identification of a third xylosyltransferase, *XXT5*. These studies further our understanding of the mechanisms of xyloglucan biosynthesis, and offer opportunities for the direct modification of this polysaccharide in order to modify plant cell walls of economically important crop species.

Cavalier DM, Lerouxel O, Neumetzler L, Yamauchi K, Reinecke A, Freshour G, Zabolina OA, Hahn MG, Burgert I, Pauly M, Raikhel NV, Keegstra K (2008) Disrupting two *Arabidopsis thaliana* xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. *Plant Cell* **20**: 1519-1537.

<http://www.plantcell.org/content/20/6/1519>

Zabolina OA, van de Ven WT, Freshour G, Drakakaki G, Cavalier DM, Mouille G, Hahn MG, Keegstra K, Raikhel NV (2008) *Arabidopsis* *XXT5* gene encodes a putative α -1,6-xylosyltransferase that is involved in xyloglucan biosynthesis. *The Plant Journal* **56**: 101-115.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2008.03580.x/abstract>

- **The role of native plants in maximizing arthropod-mediated ecosystem services in agricultural landscapes.** We provide a template for the evaluation of local plant genotypes in various areas, and show ways in which arthropod-mediated ecosystem services (AMES), which include crop pollination and pest control, can help maintain agricultural productivity and reduce the need for pesticide inputs. Agricultural producers can benefit from understanding how to best utilize native plants to enhance AMES.

Isaacs R, Tuell JK, Fiedler A, Gardiner MA, Landis DA (2009) Maximizing arthropod-mediated ecosystem services in agricultural landscapes: The role of native plants. *Frontiers in Ecology and the Environment* **7**: 196-203.

<http://www.esajournals.org/doi/pdf/10.1890/080035>

Year 2 (Dec. 1, 2008 - Nov. 30, 2009)

- **Maize traits relevant to the production of cellulosic ethanol.** In two studies we show that traits used for silage production and also for forage quality are predictors of cellulosic ethanol yield. Our results suggest that utilizing standard genetic variation of maize in breeding programs could substantially increase the amount of biofuels produced from stover per unit area of land.

Lorenz AJ, Coors JG, de Leon N, Wolfrum EJ, Hames BR, Sluiter AD, Weimer PJ (2009) Characterization, genetic variation, and combining ability of maize traits relevant to the production of cellulosic ethanol. *Crop Science* **49**: 85-98.

<https://dl.sciencesocieties.org/publications/cs/abstracts/49/1/85>

Lorenz AJ, Anex RP, Isci A, Coors JG, de Leon N, Weimer PJ (2009) Forage quality and composition measurements as predictors of ethanol yield from maize (*Zea mays* L.) stover. *Biotechnology for Biofuels* **2**: 5.

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-2-5>

- **Radical coupling reactions produce a series of cross-coupled ferulate-coniferyl alcohol dimers.** Radical coupling reactions between ethyl ferulate, a simple model for feruloyl polysaccharides in plants, and coniferyl alcohol, a monolignol, were studied in

order to better understand the polymer cross-coupling interactions among polysaccharides and monolignols or lignin, mediated by ferulate (FA), in plant cell walls. Results from this study showed that ferulate readily cross-couples with coniferyl alcohol through free radical coupling mechanisms producing a series of cross-coupled FA/CA dimers. Thus, these results show that ferulates in plants indeed react with monolignols through free radical mechanisms producing a diverse array of cross-coupled dimers than previously reported.

Zhang A, Lu F, Sun R, Ralph J (2009) Ferulate-coniferyl alcohol cross-coupled products formed by radical coupling reactions. *Planta* **229**: 1099-1108.

<https://link.springer.com/article/10.1007%2Fs00425-009-0894-6>

- **High syringyl (S) poplar.** Poplar with exceedingly high syringyl monomer levels was produced by overexpression of the ferulate-5-hydroxylase (F5H) gene driven by a cinnamate-4-hydroxylase (C4H) promoter. The resultant transgenic lignins were more linear and displayed a lower degree of polymerization. The altered structural features of the transgenic lignin polymer, as revealed here, support the contention that there are significant opportunities to improve biomass utilization by exploiting the malleability of plant lignification processes.

Stewart JJ, Akiyama T, Chapple C, Ralph J, Mansfield SD (2009) The effects on lignin structure of overexpression of ferulate 5-hydroxylase in hybrid poplar. *Plant Physiology* **150**: 621-635.

<http://www.plantphysiol.org/content/150/2/621>

- **A new NMR tool for cell wall research.** We demonstrate how multivariate analysis of 2D NMR spectra can be used to visualize cell wall changes between sample types, where changes in composition and structure for both lignin and polysaccharides can subsequently be interpreted on a molecular level. Different *Populus* wood samples were used and we were able to show that differences in both lignin and polysaccharide composition that are difficult to detect with traditional spectral analysis were revealed by the multivariate approach. 2D NMR of dissolved cell wall samples combined with multivariate analysis constitutes a novel approach in cell wall analysis and provides a new tool that will benefit cell wall research.

Hedenstrom M, Wiklund-Lindstrom S, Oman T, Lu F, Gerber L, Schatz P, Sundberg B, Ralph J (2009) Identification of lignin and polysaccharide modifications in *Populus* wood by chemometric analysis of 2D NMR spectra from dissolved cell walls. *Molecular Plant* **2**: 933-942.

<http://www.sciencedirect.com/science/article/pii/S1674205214607097?via%3Dihub>

- **Insect symbioses: fungus growing ant research.** Approaches for studying insect-microbe symbioses using the attine ant-microbial symbiosis as a framework draw attention to particular challenges in the field of symbiosis including the establishment of symbiotic associations and symbiont function.

Caldera EJ, Poulsen M, Suen G, Currie CR (2009) Insect symbioses: a case study of past, present, and future fungus-growing ant research. *Environmental Entomology* **38**: 78-92.

<https://academic.oup.com/ee/article-lookup/doi/10.1603/022.038.0110>

Pinto-Tomas AA, Anderson MA, Suen G, Stevenson DM, Chu FST, Cleland WW, Weimer PJ, Currie CR (2009) Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. *Science* **326**: 1120-1123.

<http://www.sciencemag.org/cgi/content/abstract/326/5956/1120>

- **Ethanol production from AFEX-CS using *Saccharomyces cerevisiae*.** Enzymatic hydrolysis at high solids loading was identified as the primary bottleneck affecting overall ethanol yield and titer. Nutrients inherently present in corn stover and those resulting in the processed biomass can support microbial growth during fermentation. This process can improve economics of cellulosic ethanol production.

Lau MW, Dale BE (2009) Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proceedings of the National Academy of Sciences of the United States of America **106**: 1368-1373.

<http://www.pnas.org/content/106/5/1368.abstract>

- **Chemical transformation of biomass into chemicals without pretreatment or enzymes.** Lignocellulosic biomass is a complex, heterogenous mixture of material that is highly recalcitrant to deconstruction, often requiring harsh chemical treatments and cellulolytic enzymes. This study describes the use of a privileged solvent, *N,N*-dimethylacetamide containing lithium chloride, that enables the synthesis of the renewable platform chemical 5-hydroxymethylfurfural in a single step from lignocellulosic biomass. The simplicity of the chemical transformation provides a new paradigm for the use of biomass as a raw material for renewable energy and chemical industries.
Binder JB, Raines RT (2009) Simple chemical transformation of lignocellulosic biomass into furans for fuels and chemicals. Journal of the American Chemical Society **131**: 1979-1985.
<http://pubs.acs.org/doi/abs/10.1021/ja808537j>
- **Economic and environmental impacts of alternative biofuel crops.** This study describes the initial steps in estimating the cost associated with substantial conversion of land from production of traditional crops to switchgrass and its potential environmental consequences. Initial modeling results indicate that farmers will convert to switchgrass production only with significant subsidies.
Jha M, Babcock BA, Gassman PW, Kling CL (2009) Economic and environmental impacts of alternative energy crops. International Agricultural Engineering Journal **18**: 15-23.
<http://www.iaej.cn/EN/abstract/abstract15.shtml>
- **Unexplored variables in land change, biofuels, and greenhouse emissions.** Appropriate cropping management strategies significantly reduce the payback period and enhance greenhouse gas benefits associated with the E85 fuel system compared to gasoline.
Kim H, Kim S, Dale BE (2009) Biofuels, land use change, and greenhouse gas emissions: some unexplored variables. Environmental Science & Technology **43**: 961-967.
<http://pubs.acs.org/doi/abs/10.1021/es802681k>
- **Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes.** This study provides estimates of the value of natural biological control of the soybean aphid, a major pest in agricultural landscapes, and the economic impacts of reduced biocontrol caused by increased corn production in the Midwest. Findings suggest that the value of biocontrol services in the US economy may be underestimated, and furthermore, the development of cellulosic ethanol production processes that use various feedstocks could foster increased diversity in agricultural landscapes.
Landis DA, Gardiner MM, van der Werf W, Swinton SM (2008) Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. Proceedings of the National Academy of Sciences of the United States of America **105**: 20552-20557.
<http://www.pnas.org/content/105/51/20552.long>
- **Projected scenarios for conversion of cellulosic biomass to ethanol and environmental impacts of biomass refining.** Seven processes for ethanol and co-products production from switchgrass, as well as 14 mature biomass-refining technologies were evaluated. Results suggest that fossil fuel displacement is decidedly positive and production costs are competitive with gasoline. Furthermore, they offer reduced GHG emissions.
Laser M, Jin H, Jayawardhana K, Dale BE, Lynd LR (2009) Projected mature technology scenarios for conversion of cellulosic biomass to ethanol with coproduction thermo-chemical fuels, power, and/or animal feed protein. Biofuels, Bioproducts and Biorefining **3**: 231-246.
<http://onlinelibrary.wiley.com/doi/10.1002/bbb.131/full>

Laser M, Larson E, Dale BE, Wang M, Greene N, Lynd LR (2009) Comparative analysis of efficiency, environmental impact, and process economics for mature biomass refining scenarios. *Biofuels, Bioproducts and Biorefining* **3**: 247-270.
<http://onlinelibrary.wiley.com/doi/10.1002/bbb.136/full>

Year 3 (Dec. 1, 2009 - Nov. 30, 2010)

- Acyltransferases in triacylglycerol biosynthesis.** We report two significant advances in our understanding of triacylglycerol (TAG) biosynthesis: 1) the first paper establishes *in vivo* involvement of a second confirmed acyltransferase in TAG biosynthesis, and 2) the second paper demonstrates the utility of deep transcriptional profiling with multiple tissues as a gene discovery strategy for low-abundance proteins; we identified the acetyltransferase necessary and sufficient for the production of AcTAGs in *Euonymus* seeds and demonstrate that this activity can be introduced into the seeds of other plants. These advances allow for the evaluation of TAGs for biofuels and other applications.

Zhang M, Fan J, Taylor DC, Ohlrogge JB (2009) *DGAT1* and *PDAT1* acyltransferases have overlapping functions in *Arabidopsis* triacylglycerol biosynthesis and are essential for normal pollen and seed development. *Plant Cell* **21**: 3885-3901.
<http://www.plantcell.org/content/21/12/3885>

Durrett TP, McClosky DD, Tumaney AW, Elzinga DA, Ohlrogge J, Pollard M (2010) A distinct DGAT with *sn*-3 acetyltransferase activity that synthesizes unusual, reduced-viscosity oils in *Euonymus* and transgenic seeds. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 9464-9469.
<http://www.pnas.org/content/107/20/9464.abstract>
- A revolutionary method improvement for NMR of plant cell walls.** Here, the NMR fingerprint method to obtain solution-state NMR spectra of the entire array of plant cell wall polymers without the need for component fractionation has been improved by use of a new gel mixture, resulting in considerably elevated intensities and improved resolution of the NMR spectra. This modification therefore provides a more rapid method for comparative structural evaluation of plant cell walls than is currently available. With the new potential for chemometric analysis using the 2D NMR fingerprint, this gel-state method may provide the basis for an attractive approach to providing a secondary screen for selecting biomass lines and for optimizing processing and conversion efficiencies.

Kim H, Ralph J (2010) Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-*d*₆/pyridine-*d*₅. *Organic & Biomolecular Chemistry* **8**: 576-591.
<http://pubs.rsc.org/en/Content/ArticleLanding/2010/OB/B916070A#!divAbstract>
- Natural variation of flowering time and vernalization responsiveness in *Brachypodium*.** Using *Brachypodium* as a model to better understand grass physiology and ecology, we report on the flowering time variability of natural *Brachypodium* accessions in response to temperature and light cues. Changes in both environmental parameters greatly influence when a given accession will flower, and natural *Brachypodium* accessions broadly group into winter and spring annuals. We show that some of the phenotypic variation is associated with changes in expression of orthologs of VRN genes, and thus VRN genes are a possible target for modifying flowering time in grass family bioenergy crops.

Schwartz CJ, Doyle MR, Manzaneda AJ, Rey PJ, Mitchell-Olds T, Amasino RM (2010) Natural variation of flowering time and vernalization responsiveness in *Brachypodium distachyon*. *BioEnergy Research* **3**: 38-46.
<https://link.springer.com/article/10.1007/s12155-009-9069-3>
- Cell wall traits relevant to cellulosic ethanol production.** Maize hybrids with improved stover yield and quality are needed to support the emerging cellulosic biofuels industry, and

knowledge of the genetic variation, covariation, and genetic architecture of agronomic and cell wall traits will help maize breeders improve relevant traits through selective breeding and genetic engineering. We utilized a maize recombinant inbred line (RIL) population to map quantitative trait loci (QTL) and to evaluate the genetic relationships among traits relevant for cellulosic biofuel production, including grain yield, stover yield, neutral detergent fiber digestibility (NDFD), and glucan, xylan, and lignin concentration. This study provides information that will support the development of dual-purpose maize hybrids that are high in grain yield and also high in yield of digestible cellulosic feedstock.

Lorenz AJ, Coors JG, Hansey CN, Kaeppler SM, de Leon N (2010) Genetic analysis of cell wall traits relevant to cellulosic ethanol production in maize (*Zea mays* L.). *Crop Science* **50**: 842-852.

<https://dl.sciencesocieties.org/publications/cs/articles/50/3/842?highlight=&search-result=1>

- **Mass spectroscopy sequencing strategy for lignin oligomers.** Current methods to analyze lignin structure focus only on the frequency of the major monomeric units and inter-unit linkage types but do not provide information on the presence of less abundant unknown units and linkage types, nor on how linkages affect the formation of neighboring linkages. Such information can only be obtained using a sequencing approach. Here we describe, to our knowledge for the first time, a sequencing strategy for lignin oligomers using mass spectrometry. This paper launched a large area of study in “lignomics” and became the *de facto* standard for elucidating gene misregulation impacts on plant cell wall development.

Morreel K, Dima O, Kim H, Lu F, Niculaes C, Vanholme R, Dauwe R, Goeminne G, Inzé D, Messens E, Ralph J, Boerjan W (2010) Mass spectrometry-based sequencing of lignin oligomers. *Plant Physiology* **153**: 1464-1478.

<http://www.plantphysiol.org/content/153/4/1464>

- **Plant cell wall precursors separated and quantified by a liquid chromatography tandem mass spectroscopy method.** The biosynthesis of cell wall polymers involves enormous fluxes through central metabolism that are not fully delineated and whose regulation is poorly understood. We have established and validated a liquid chromatography tandem mass spectrometry method using multiple reaction monitoring mode to separate and quantify the levels of plant cell wall precursors.

Alonso AP, Piasecki RJ, Wang Y, LaClair RW, Shachar-Hill Y (2010) Quantifying the labeling and the levels of plant cell wall precursors using ion chromatography tandem mass spectrometry. *Plant Physiology* **153**: 915-924.

<http://www.plantphysiol.org/content/153/3/915.long>

- **Evaluation of AFEX pretreatment of switchgrass harvested in different seasons and locations in the production of ethanol.** This paper studied the effect of agronomic factors such as harvest time and location, as well as pretreatment- specific parameters, such as ammonia and water loading, and reaction time and temperature, on downstream production of biofuels. The findings indicate that all of these parameters have significant effects on production, suggesting that an integrative approach is best.

Bals B, Rogers C, Jin M, Balan V, Dale BE (2010) Evaluation of ammonia fiber expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations. *Biotechnology for Biofuels* **3**: 11.

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-3-1>

Bals B, Wedding C, Balan V, Sendich E, Dale B (2011) Evaluating the impact of ammonia fiber expansion (AFEX) pretreatment conditions on the cost of ethanol production. *Bioresource Technology* **102**: 1277-1283.

<http://www.sciencedirect.com/science/article/pii/S0960852410014185>

- Enzyme mixtures for various pretreatments of different feedstocks.** Here we studied several combinations of enzymes during AHP and AFEX pretreatment of corn stover using GENPLAT, a high-throughput biomass digestion platform that can be used to rapidly produce enzyme cocktails for specific pretreatment-biomass combinations. The technique assists in identifying optimal combinations for biomass deconstruction.

Banerjee G, Car S, Scott-Craig J, Borrusch M, Walton J (2010) Rapid optimization of enzyme mixtures for deconstruction of diverse pretreatment/biomass feedstock combinations. *Biotechnology for Biofuels* **3**: 22.
<http://www.biotechnologyforbiofuels.com/content/3/1/22>

Banerjee G, Car S, Scott-Craig JS, Borrusch MS, Aslam N, Walton JD (2010) Synthetic enzyme mixtures for biomass deconstruction: production and optimization of a core set. *Biotechnology and Bioengineering* **106**: 707-720.
<http://onlinelibrary.wiley.com/doi/10.1002/bit.22741/full>

Banerjee G, Car S, Scott-Craig JS, Borrusch MS, Bongers M, Walton JD (2010) Synthetic multi-component enzyme mixtures for deconstruction of lignocellulosic biomass. *Bioresource Technology* **101**: 9097-9105.
<http://www.sciencedirect.com/science/article/pii/S0960852410012162>

Banerjee G, Scott-Craig JS, Walton JD (2010) Improving enzymes for biomass conversion: a basic research perspective. *BioEnergy Research* **3**: 82-92.
<https://link.springer.com/article/10.1007%2Fs12155-009-9067-5>

Walton J, Banerjee G, Car S (2011) GENPLAT: an automated platform for biomass enzyme discovery and cocktail optimization. *Journal of Visualized Experiments*: e3314.
<https://www.jove.com/video/3314/genplat-an-automated-platform-for-biomass-enzyme-discovery-cocktail>
- Characterization of cell wall deconstruction during pretreatment.** Multi-scale visualization and characterization of corn stover cell walls was carried out by various microscopy, magnetic resonance, and spectroscopy techniques to elucidate the mechanism of AFEX pretreatment. It was found that AFEX results in ultrastructural and physicochemical modifications within the cell wall that enhance enzymatic hydrolysis yield by 4- to 5-fold over that of untreated cell walls.

Chundawat SPS, Donohoe BS, da Costa Sousa L, Elder T, Agarwai UP, Lu F, Ralph J, Himmel M, Balan V, Dale BE (2011) Multi-scale visualization and characterization of plant cell wall deconstruction during thermochemical pretreatment. *Energy and Environmental Science* **4**: 973-984.
<http://pubs.rsc.org/en/Content/ArticleLanding/2011/EE/c0ee00574f>
- Impacts of pretreatment on the fermentability of AFEX- and dilute acid-pretreated lignocellulosic biomass.** A detailed mass balance for major cell wall decomposition products and extractables was provided for AFEX- and dilute acid- treated corn stover, finding that the development of inexpensive, low thermochemical severity pretreatments that minimize formation of biological inhibitors while maximizing cell wall deconstruction is an important objective of the cellulosic ethanol industry.

Chundawat SPS, Vismeh R, Sharma LN, Humpala JF, daCosta Sousa L, Chambliss CK, Jones AD, Balan V, Dale BE (2010) Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute-acid based pretreatments. *Bioresource Technology* **101**: 8429-8438.
<http://www.sciencedirect.com/science/article/pii/S0960852410010084?via=ihub>

Lau M, Gunawan C, Dale B (2009) The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment. *Biotechnology for Biofuels* **2**: 30.
<http://www.biotechnologyforbiofuels.com/content/2/1/30>

- **Two-step simultaneous saccharification and co-fermentation (SSCF) to convert AFEX switchgrass to ethanol using *Saccharomyces cerevisiae*.** To overcome the reduced yield of ethanol production from AFEX-treated switchgrass, a 2-step saccharification and co-fermentation (SSCF) process was developed in which xylan is first hydrolyzed and fermented followed by the hydrolysis and fermentation of glucan. Compared to the traditional SSCF, this process results in higher xylose consumption, higher ethanol yield, and increased sugar conversion.

Jin M, Lau M, Balan V, Dale BE (2010) Two-step SSCF to convert AFEX-treated switchgrass to ethanol using commercial enzymes and *Saccharomyces cerevisiae* 424A (LNH-ST). *Bioresource Technology* **101**: 8171-8178.
<http://www.sciencedirect.com/science/article/pii/S0960852410010072>
- **An insect herbivore microbiome with high plant biomass-degrading capacity.** Using a combination of sugar composition analyses, metagenomics and sequencing, it was revealed that the fungus garden microbiome of leaf-cutter ants is composed of a diverse community of bacteria with high plant biomass-degrading capacity. Understanding how large-scale and rapid plant biomass degradation occurs in this highly evolved insect can help understand deconstruction processes.

Suen G, Scott JJ, Aylward FO, Adams SM, Tringe SG, Pinto-Tomás AA, Foster CE, Pauly M, Weimer PJ, Barry KW, Goodwin LA, Bouffard P, Li L, Osterberger J, Harkins TT, Slater SC, Donohue TJ, Currie CR (2010) An insect herbivore microbiome with high plant biomass-degrading capacity. *PLOS Genetics* **6**: e1001129.
<http://dx.doi.org/10.1371/journal.pgen.1001129>
- **New algorithm aids in refinement of metabolic models.** One of the challenges in constructing integrated metabolic and regulatory models is limited information on transcription factor-gene target interactions and computational methods to quickly refine models based on additional datasets. Researchers developed *GeneForce*, an algorithm to identify incorrect regulatory rules and gene-protein-reaction associations in such models and showed that it improves accuracy in the ability to predict growth phenotypes. New tools for the refinement of metabolic modeling will greatly aid in systems biology approaches to biofuel production.

Barua D, Kim J, Reed JL (2010) An automated phenotype-driven approach (*GeneForce*) for refining metabolic and regulatory models. *PLOS Computational Biology* **6**: e1000970.
<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1000970>
- **Ionic liquid deconstruction of biomass produces sugars in high yield.** Although the use of ionic liquids to obtain fermentable sugars from biomass has been reported, the yields are typically low. Here we report a method for high-yielding sugars from untreated corn stover involving the gradual addition of water to the ionic liquid-catalytic acid mixture. Ionic liquids represent a promising approach to biomass deconstruction, provided that there be increased efficiency of sugar production and solvent recovery.

Binder JB, Raines RT (2010) Fermentable sugars by chemical hydrolysis of biomass. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 4516-4521.
<http://www.pnas.org/content/107/10/4516>
- **Biomass-derived gamma valerolactone (GVL) converted to liquid alkenes.** GVL, a solvent that can be obtained from biomass-derived carbohydrates, was integrated into a process for production of condensable alkenes with molecular weights that can be targeted for gasoline or jet fuel. GVL is a potentially game-changing method for biomass deconstruction that avoids the use of expensive enzymes. Moreover, the method is green and renewable as GVL can be obtained from the biomass itself.

Bond JQ, Alonso DM, Wang D, West RM, Dumesic JA (2010) Integrated catalytic conversion of γ -valerolactone to liquid alkenes for transportation fuels. *Science* **327**: 1110-1114.

<http://science.sciencemag.org/content/327/5969/1110>

- **Computational method integrates transcriptional regulatory and metabolic networks to identify gene deletion or overexpression strategies to improve biofuel production.**

Limitations of currently available computational methods for metabolic engineering are that they are often based on reaction deletions rather than gene deletions and do not consider the regulatory networks that control metabolism. The new algorithm, OptORF, takes into account known regulatory information to predict optimized production of biofuel from metabolic models.

Kim J, Reed J (2010) OptORF: optimal metabolic and regulatory perturbations for metabolic engineering of microbial strains. *BMC Systems Biology* **4**: 53.

<https://bmcsystbiol.biomedcentral.com/articles/10.1186/1752-0509-4-53>

- **Examination of natural genetic variation in wild yeast strains identifies traits for improved ethanol tolerance.** Stress due to biofuels themselves is a major factor that limits efficient microbial fermentation. The authors of this study show that a common lab strain cannot acquire higher ethanol tolerance after a mild ethanol pretreatment whereas many wild strains are adaptable. The genetic basis for this was examined and a number of genes were observed to be differentially expressed in wild vs. lab strains, including some that were previously not known to play a role in ethanol tolerance. These results demonstrate the power of comparative genomics and natural genetic variability in designing efficient biofuel microbes.

Lewis JA, Elkon IM, McGee MA, Higbee AJ, Gasch AP (2010) Exploiting natural variation in *Saccharomyces cerevisiae* to identify genes for increased ethanol resistance. *Genetics* **186**: 1197-1205.

<http://www.genetics.org/content/186/4/1197>

- **Water and energy footprints of bioenergy crops on marginal lands.** Water and energy demands associated with bioenergy crop production on marginal lands are inextricably linked with land quality and land use history. To illustrate the effect of land marginality on bioenergy crop yield and associated water and energy footprints, we analyzed several large-scale sites converted from either Conservation Reserve Program (CRP) or conventional agricultural land use to no-till soybean for biofuel production. The analysis of biophysical characteristics of the sites in relation to water and energy use suggests that crops and management systems similar to CRP grasslands may provide a potential strategy to grow biofuels that would minimize environmental degradation while improving the productivity of marginal lands.

Bhardwaj AK, Zenone T, Jasrotia P, Robertson GP, Chen J, Hamilton SK (2011) Water and energy footprints of bioenergy crop production on marginal lands. *Global Change Biology – Bioenergy* **3**: 208-222.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1757-1707.2010.01074.x/abstract>

- **Evaluation of bacterial communities in the rhizosphere of biofuel crops grown on marginal lands.** We studied bacterial communities in the rhizosphere of five biofuel crops cultivated in four locations in Michigan to determine which factors were correlated to changes in the structure of those communities. Bacterial communities grouped by location rather than by crop and their structures were correlated to soil attributes, principally pH, organic matter, and nutrients. The effect of plant species was low but significant, and interactions between locations, plant species, and soil attributes account for most of the explained variation in the structure of bacterial communities, showing a complex relationship between bacterial populations and their environment.

da C. Jesus E, Susilawati E, Smith SL, Wang Q, Chai B, Farris R, Rodrigues JLM, Thelen KD, Tiedje JM (2010) Bacterial communities in the rhizosphere of biofuel crops grown on marginal lands as evaluated by 16S rRNA gene pyrosequences. *BioEnergy Research* **3**: 20-27.

<https://link.springer.com/article/10.1007/s12155-009-9073-7>

- **Modeling of biofuel production based on efficient land use without affecting animal feed production.** Our analysis explores potential changes in agriculture that utilize new technology and approaches to meet and reconcile what appear to be competing demands for food, biofuels, and environmental services. We conclude that the United State can produce 400 billion liters of ethanol annually on farmland and reduce greenhouse gas emissions by 10% without harming food production.

Dale BE, Bals BD, Kim S, Eranki P (2010) Biofuels done right: land efficient animal feeds enable large environmental and energy benefits. *Environmental Science & Technology* **44**: 8385-8389.

<http://pubs.acs.org/doi/abs/10.1021/es101864b>

- **Understanding the microbial role in soil carbon stabilization.** Here, we describe the use of an Absorbing Markov Chain (AMC) to model the dynamics of soil C transformations among three microbial states: living microbial biomass, microbial necromass, and C removed from living and dead microbial sources. We find that AMC provides a powerful quantitative approach that allows prediction of how C will be distributed among these three states, and how long it will take for the entire amount of initial C to pass through the biomass and necromass pools and be moved into atmosphere. Our work represents a first step in attempting to quantify the flow of C through microbial pathways, and has the potential to increase our understanding of the microbial role in soil C dynamics

Liang C, Cheng G, Wixon D, Balser T (2010) An Absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization. *Biogeochemistry* **106**: 303-309.

<https://link.springer.com/article/10.1007/s10533-010-9525-3>

- **Bird communities in future bioenergy landscapes of the Upper Midwest.** Here, we use data from the North American Breeding Bird Survey to forecast the impact of potential bioenergy crops on avian species richness and the number of bird species of conservation concern in Midwestern landscapes. Our analysis suggests that expanded production of annual bioenergy crops (e.g., corn and soybeans) on marginal land will lead to declines in avian richness between 7% and 65% across 20% of the region, and will make managing at-risk species more challenging. In contrast, replacement of annual with diverse perennial bioenergy crops (e.g., mixed grasses and forbs) is expected to bring increases in avian richness between 12% and 207% across 20% of the region, and possibly aid the recovery of several species of conservation concern.

Meehan TD, Hurlbert AH, Gratton C (2010) Bird communities in future bioenergy landscapes of the Upper Midwest. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 18533-18538.

<http://www.pnas.org/content/107/43/18533.abstract>

Year 4 (Dec. 1, 2010 - Nov. 30, 2011)

- **Genome-wide maize transcription atlas.** Maize is an important model species and has also emerged as a potential feedstock and model system for bioenergy research due to recent worldwide interest in developing plant biomass-based, carbon-neutral liquid fuels. To understand how the underlying genome sequence results in specific plant phenotypes, information on the temporal and spatial transcription patterns of genes is crucial. Here we

present a comprehensive atlas of global transcription profiles across developmental stages and plant organs, creating a valuable resource for gene discovery and functional characterization in maize.

Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, Kaeppler SM (2011) Genome-wide atlas of transcription during maize development. *The Plant Journal* **66**: 553-563.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2011.04527.x/abstract>

- **Photoprobes for studying *in vitro* lignification.** Here, we report synthetic protocols for preparing fluorescent tagged analogues of coniferyl alcohol (CA) and demonstrate their compatibility with normal monolignols for forming synthetic lignins. We anticipate that fluorescence-tagged monolignols will be useful for *in vitro* and *in vivo* studies of cell wall lignification.

Tobimatsu Y, Davidson CL, Grabber JH, Ralph J (2011) Fluorescence-tagged monolignols: synthesis, and application to studying *in vitro* lignification. *Biomacromolecules* **12**: 1752-1761.

<http://pubs.acs.org/doi/abs/10.1021/bm200136x>

- **Xylan synthesis in *Arabidopsis*.** Xylan is the principal hemicellulose in the secondary cell walls of eudicots and in the primary and secondary cell walls of grasses and cereals. The biosynthesis of this important cell wall component has yet to be fully determined although a number of proteins have been shown to be required for xylan synthesis. To discover new genes involved in xylan biosynthesis we explored the psyllium (*Plantago ovata Forsk*) seed mucilaginous layer through EST profiling; we conclude that two genes, *IRREGULAR XYLEM (IRX) 15* and *IRX15-LIKE (IRX15-L)*, function in a redundant manner and are involved in xylan biosynthesis.

Jensen JK, Kim H, Cocuron J-C, Orlor R, Ralph J, Wilkerson CG (2011) The DUF579 domain containing proteins IRX15 and IRX15-L affect xylan synthesis in *Arabidopsis*. *The Plant Journal* **66**: 387-400.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2010.04475.x/abstract;jsessionid=1C30408815FAC56321024C8F5F5DE0A2.f02t02>

- **CGR3, a protein involved in pectin methylesterification.** Pectins are a large group of complex polysaccharides of the plant cell wall that are made in the Golgi and secreted to the wall. The methylesterification of pectins is believed to be an important factor for the dynamic properties of the cell wall. Here, we report on a protein of unknown function discovered using an extensive proteomics analysis of cotton Golgi; our results suggest that CGR3 plays a role in the methylesterification of homogalacturonan in *Arabidopsis*.

Held MA, Be E, Zemelis S, Withers S, Wilkerson C, Brandizzi F (2011) CGR3: a golgi-localized protein influencing homogalacturonan methylesterification. *Molecular Plant* **4**: 832-844.

<http://www.sciencedirect.com/science/article/pii/S1674205214607693?via%3Dihub>

- **Cellulose-degrading bacteria associated with invasive woodwasp *Sirex noctilio*: from ecosystem to enzyme structure.** We developed a detailed understanding of how the microbes in the *Sirex noctilio* ecosystem act together to degrade biomass by characterizing the *Sirex*-associated microbiome, and focusing on the *Streptomyces* sp. *SirexAA-E*, which has a particularly strong biomass degrading capacity.

Adams AS, Jordan MS, Adams SM, Suen G, Goodwin LA, Davenport KW, Currie CR, Raffa KF (2011) Cellulose-degrading bacteria associated with the invasive woodwasp *Sirex noctilio*. *The International Society for Microbial Ecology Journal* **5**: 1323-1331.

<http://www.nature.com/ismej/journal/v5/n8/full/ismej201114a.html>

- **Alkaline peroxide pretreatment of corn stover.** Alkaline hydrogen peroxide (AHP) has several attractive features as a pretreatment for lignocellulosic biomass, especially since it

works well on woody materials. We studied the feasibility of scaling-up the AHP process, examining impacts on enzyme loading, enzyme composition, and ethanol yields.

Banerjee G, Car S, Liu T, Williams DL, López Meza S, Walton JD, Hodge DB (2012) Scale-up and integration of alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis, and ethanolic fermentation. *Biotechnology and Bioengineering* **109**: 922-931.

<http://onlinelibrary.wiley.com/doi/10.1002/bit.24385/abstract>

Banerjee G, Car S, Scott-Craig J, Hodge DB, Walton JD (2011) Alkaline peroxide pretreatment of corn stover: effects of biomass, peroxide, and enzyme loading and composition on yields of glucose and xylose. *Biotechnology for Biofuels* **4**: 16.

<http://www.biotechnologyforbiofuels.com/content/4/1/16>

- **Corn harvest strategies for combined starch and cellulosic bioprocessing to ethanol.** We compared the ethanol yield of several sources of biomass to better understand the effects of whole-plant harvesting vs. traditional harvesting methods. Results suggest that whole-plant harvesting coupled with whole-plant bioconversion to ethanol is a viable alternative to the convention of separate grain and stover harvesting and bioprocessing.

Gao J, Qian L, Thelen KD, Hao X, da Costa Sousa L, Balan V, Dale BE (2011) Corn harvest strategies for combined starch and cellulosic bioprocessing to ethanol. *Agronomy Journal* **103**: 844-850.

<https://dl.sciencesocieties.org/publications/aj/articles/103/3/844>
- **CBP performance of *Clostridium phytofermentans* on AFEX-treated corn stover for ethanol production.** Consolidated bioprocessing (CBP) is believed to be a potentially cost-efficient and commercially viable way to produce cellulosic biofuels. We evaluated the performance of *Clostridium phytofermentans* on AFEX-treated corn stover, and found that decomposition products could help increase the ethanol yield during CBP and that particle size plays a crucial role in the enhancement of sugar conversion by CBP.

Jin M, Balan V, Gunawan C, Dale BE (2011) Consolidated bioprocessing (CBP) performance of *Clostridium phytofermentans* on AFEX-treated corn stover for ethanol production. *Biotechnology and Bioengineering* **108**: 1290-1297.

<http://dx.doi.org/10.1002/bit.23059>
- **Mining for new glycoside hydrolases.** From the metagenome sequences of an anaerobic microbial community actively decaying poplar biomass, we identified approximately 4,000 glycoside hydrolase (GHase) homologues. Based on homology to GHase families/activities of interest and the quality of the sequences, candidates were selected for full-length cloning and subsequent expression in *E. coli*.

Li L-L, Taghavi S, McCorkle S, Zhang Y-B, Blewitt M, Brunecky R, Adney W, Himmel M, Brumm P, Drinkwater C, Mead D, Tringe S, van der Lelie D (2011) Bioprospecting metagenomics of decaying wood: mining for new glycoside hydrolases. *Biotechnology for Biofuels* **4**: 23.

<http://www.biotechnologyforbiofuels.com/content/4/1/23>
- **Genome sequence of the leaf cutter ant revealed insights into its symbiotic lifestyle. GLBRC Researchers Successfully Leverage DOE Funding for Ant Genome Research.** With a Roche Diagnostics 10 Gigabase Sequencing and Transcriptomics Analysis Grant to sequence the leaf-cutter *Atta cephalotes* ant genome we found that leaf-cutters are unique among ants because they farm a specialized, mutualistic fungus that serves as their primary food source. The ants maintain fungal gardens and the waste dumps harbor other microbial symbionts including nitrogen-fixing bacteria and others that appear to help the fungus degrade plant biomass.

Suen G, Teiling C, Li L, Holt C, Abouheif E, Bornberg-Bauer E, Bouffard P, Caldera EJ, Cash E, Cavanaugh A, Denas O, Elhaik E, Favé M-J, Gadau J, Gibson JD, Graur D, Grubbs KJ, Hagen DE, Helmkamp M, Hu H, Johnson BR, Kim J, Marsh SE, Moeller JA, Muñoz-

Torres MC, Murphy MC, Naughton MC, Nigam S, Overson R, Rajakumar R, Reese JT, Scott JJ, Smith CR, Tao S, Tsutsui ND, Viljakainen L, Wissler L, Yandell MD, Zimmer F, Harkins TT, Taylor J, Slater SC, Clifton SW, Warren WC, Elisk CG, Smith CD, Weinstock GM, Gerardo NM, Currie CR (2011) The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. PLOS Genetics 7: e1002007. <http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1002007>

- **Technoeconomic analysis of liquid hydrocarbon fuel production from lignocellulosic biomass suggests an economically competitive process.** This study developed and evaluated a novel catalytic strategy for the transformation of lignocellulosic biomass to levulinic acid (LA), LA to gamma valerolactone (GVL), and GVL to butene and other heavy alkenes. Economic evaluation indicated that the process could be economically competitive; further improvements to the process were identified that are predicted to enable additional cost savings.

Sen MS, Henao CA, Braden DJ, Dumesic JA, Maravelias CT (2012) Catalytic conversion of lignocellulosic biomass to fuels: process development and technoeconomic evaluation. Chemical Engineering Science 67: 57-67.

<http://www.sciencedirect.com/science/article/pii/S0009250911004830>

- **New method to identify lignocellulose-degrading bacteria.** Environmental microbes represent an abundant and diverse source of lignocellulose-degrading enzymes however identification of such microorganisms is slow due to a lack of rapid screening methodologies. This study describes a new method that employs acrylamide rather than agar as the solidifying agent. Both Gram-positive and Gram-negative organisms possessing cellulose and hemicellulose degrading activities were identified from environmental samples.

Gardner JG, Zeitler LA, Wigstrom WJS, Engel KC, Keating DH (2011) A high throughput solid phase screening method for identification of lignocellulose-degrading bacteria from environmental isolates. Biotechnology Letters 34: 81-89.

<https://link.springer.com/article/10.1007%2Fs10529-011-0742-1>

- **Fluorous ionic liquid that dissolves cellulose and is recoverable.** Ionic liquids are an attractive class of solvents for biomass deconstruction however their chemical properties hinder recovery. Here we report that of seven fluorous ionic liquids examined, 1-ethyl-3-methylimidazolium [EMIM]Cl dissolves cellulose and can be recovered from glucose product. The ability to recover and recycle the ionic liquids is necessary due to the high cost of these promising solvents.

Caes BR, Binder JB, Blank JJ, Raines RT (2011) Separable fluorous ionic liquids for the dissolution and saccharification of cellulose. Green Chemistry 13: 2719-2722.

<http://pubs.rsc.org/en/content/articlelanding/2011/gc/c1gc15776k> - !divAbstract

- **Cellular response to endogenous overproduction of free fatty acids (FFA) has a large membrane stress component.** A strain of *E. coli* that overproduced medium-chain FAs was shown to have greatly reduced inner membrane integrity and viability by the early stationary phase of growth. Membrane stresses were further implicated by gene and protein expression analyses, which indicated an increase in phage shock proteins and the marA/rob/soxS stress regulon. These studies show that endogenous overproduction of FAs is negatively correlated with viability and efforts to obtain higher titers of FAs will require mitigation of the toxic effects of FA overproduction.

Lennen RM, Kruziki MA, Kumar K, Zinkel RA, Burnum KE, Lipton MS, Hoover SW, Ranatunga DR, Wittkopp TM, Marner WD, 2nd, Pflieger BF (2011) Membrane stresses induced by overproduction of free fatty acids in *Escherichia coli*. Applied and Environmental Microbiology 77: 8114-8128.

<http://aem.asm.org/content/77/22/8114.long>

- Comparative genomics approach identifies yeast genes important for xylose utilization.** Many microbes are unable to metabolize pentose sugars such as xylose yet efficient conversion of lignocellulosic biomass requires that microbial catalysts be able to utilize both C5 and C6 sugars. Thus study compared the genomes of two xylose utilizing species of yeast (*Spathaspora passalidarum* and *Candida tenuis*) to 14 other Ascomycete genomes and identified many genes involved in xylose utilization. Several of these genes significantly improved xylose utilization when engineered into *S. cerevisiae*, indicating that comparative genomics is a useful approach to identify genes important for biofuel production.

Wohlbach DJ, Kuo A, Sato TK, Potts KM, Salamov AA, LaButti KM, Sun H, Clum A, Pangilinan JL, Lindquist EA, Lucas S, Lapidus A, Jin M, Gunawan C, Balan V, Dale BE, Jeffries TW, Zinkel R, Barry KW, Grigoriev IV, Gasch AP (2011) Comparative genomics of xylose-fermenting fungi for enhanced biofuel production. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 13212-13217.
<http://www.pnas.org/content/108/32/13212.abstract>
- A spatially explicit bioeconomic modeling approach for biomass supply from alternative cellulosic and crop residues.** A new bioeconomic model predicts biomass supply and its environmental impacts; the model captures the opportunity cost of switching to new cellulosic crops. A key finding is that biomass from cellulosic crops has superior climate and water quality outcomes.

Egbendewe-Mondzozo A, Swinton SM, Izaurrealde RC, Manowitz DH, Zhang X (2011) Biomass supply from alternative cellulosic crops and crop residues: a spatially explicit bioeconomic modeling approach. *Biomass and Bioenergy* **35**: 4636-4647.
<http://www.sciencedirect.com/science/article/pii/S0961953411004648>
- Assessment of regional biomass processing depots.** In the first of two reports this year, we conducted comparative life cycle assessment (LCA) of distributed and centralized biomass processing systems combined with farm-scale landscapes of varying acreages allocated to a “corn-system” consisting of corn grain, stover, and rye (grown as a winter double crop) and two perennial grasses, switchgrass and miscanthus. In the second publication, to address supply chain challenges, we explored the concept of Regional Biomass Processing Depots (RBDs), strategically distributed facilities that procure, pre-process/pre-treat and densify biomass into stable intermediate products that are compatible with existing bulk commodity logistical systems. These reports advance our knowledge of features important for a successful biofuel industry.

Eranki PL, Dale BE (2011) Comparative life cycle assessment of centralized and distributed biomass processing systems combined with mixed feedstock landscapes. *Global Change Biology – Bioenergy* **3**: 427-438.
<http://onlinelibrary.wiley.com/doi/10.1111/j.1757-1707.2011.01096.x/abstract>

Eranki P, Bals BD, Dale BE (2011) Advanced regional biomass processing depots: a key to the logistical challenges of the cellulosic biofuel industry. *Biofuels, Bioproducts and Biorefining* **5**: 621-630.
<http://onlinelibrary.wiley.com/doi/10.1002/bbb.318/abstract>
- Relationship between agricultural landscape simplification and insecticide use in the Midwestern US.** A relationship between landscape simplification, pest pressure, and insecticide use has long been assumed, but direct evidence has been lacking. Results from this study provide unique correlative support for this relationship over an unprecedented range of cropping systems and environmental conditions, spanning a globally important farming region.

Meehan TD, Werling BP, Landis DA, Gratton C (2011) Agricultural landscape simplification and insecticide use in the Midwestern United States. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 11500-11505.

<http://www.pnas.org/content/108/28/11500.abstract>

- **Virus susceptibility of native grasses modified for biofuel production.** In this study, we examined relationships between growth rates and biomass recalcitrance in five switchgrass populations, ranging from near-wildtype to highly selected cultivars, in a common garden trial. In experiments, susceptibility varied notably among switchgrass populations and was more strongly predicted by potential biomass accumulation rates than by foliar digestibility; highly selected, productive cultivars were most virus-susceptible and most preferred by aphids. Evaluation and mitigation of virus susceptibility of new biofuel crops is recommended to avert possible unintended consequences of biofuel production on regional pathogen dynamics.

Schrotenboer AC, Allen MS, Malmstrom CM (2011) Modification of native grasses for biofuel production may increase virus susceptibility. *Global Change Biology – Bioenergy* **3**: 360-374.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1757-1707.2011.01093.x/abstract>

- **Carbon debt of Conservation Reserve Program (CRP) grasslands converted to bioenergy production.** Here we provide measurements of the greenhouse gas consequences of converting US Conservation Reserve Program (CRP) land to continuous corn, corn-soybean, or perennial grass for biofuel production. We consider five contrasting scenarios for subsequent management: continuous corn and corn-soybean rotations, each either tilled or in permanent no-till; a fifth scenario is CRP grassland harvested for cellulosic ethanol production. We present the permanent no-till scenario as the best-practice option, agronomically realistic with the proper incentives.

Gelfand I, Zenone T, Jasrotia P, Chen J, Hamilton SK, Robertson GP (2011) Carbon debt of Conservation Reserve Program (CRP) grasslands converted to bioenergy production. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 13864-13869.

<http://www.pnas.org/content/108/33/13864.abstract>

Year 5 (Dec. 1, 2011 - Nov. 30, 2012)

- **Acylation of grass monolignols with *p*-coumarate.** In grasses, monolignols can be enzymatically preacylated and incorporated into lignin with unknown effects on lignin function. Here we show that the grass-specific acyltransferase enzyme OsPMT, expressed in *Escherichia coli*, acylated monolignols with *p*-coumarate; thus, OsPMT likely encodes an enzyme responsible for acylation of monolignols in grasses. With a greater understanding of monolignol *p*-coumarate conjugates, grass lignins could be engineered to contain fewer pendent *p*-coumarate groups and more monolignol conjugates that improve lignin cleavage.

Withers S, Lu F, Kim H, Zhu Y, Ralph J, Wilkerson CG (2012) Identification of grass-specific enzyme that acylates monolignols with *p*-coumarate. *The Journal of Biological Chemistry* **287**: 8347-8355.

<http://www.jbc.org/content/287/11/8347.long>

- **C-lignin produced naturally in the Vanilla orchid.** Here we report a lignin in the monocotyledonous angiosperm Vanilla orchid (*Vanilla planifolia*) that is naturally biosynthesized from the unusual C monolignol, caffeyl alcohol. Similar polymers are found in the seeds of other vanilla species and several species of cacti (which are dicots). The *V. planifolia* polymer was structurally characterized by various chemical methods, 2D NMR

spectroscopic techniques, and gel-permeation chromatography (GPC); all evidence indicates that the C-lignin is formed by combinatorial oxidative radical coupling under simple chemical control, a mechanism analogous to that occurring in classic lignification.

Chen F, Tobimatsu Y, Havkin-Frenkel D, Dixon RA, Ralph J (2012) A polymer of caffeyl alcohol in plant seeds. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 1772-1777.

<http://www.pnas.org/content/109/5/1772.full>

- **MYB46, master regulator of secondary cell wall synthesis.** While many aspects of primary cell wall have been extensively elucidated, our current understanding of secondary wall biosynthesis is limited. Recently, transcription factor MYB46 has been identified as a master regulator of secondary wall biosynthesis in *Arabidopsis thaliana*. To gain better understanding of this MYB46-mediated transcriptional regulation, we conducted several lines of research related to MYB46, resulting in the three publications listed below for this year.

Kim W-C, Ko J-H, Han K-H (2012) Identification of a *cis*-acting regulatory motif recognized by MYB46, a master transcriptional regulator of secondary wall biosynthesis. *Plant Molecular Biology* **78**: 489-501.

<https://link.springer.com/article/10.1007%2Fs11103-012-9880-7>

Ko J-H, Kim W-C, Kim J-Y, Ahn S-J, Han K-H (2012) MYB46-mediated transcriptional regulation of secondary wall biosynthesis. *Molecular Plant* **5**: 961-963

<http://www.sciencedirect.com/science/article/pii/S1674205214600617>

Kim W-C, Ko J-H, Kim J-Y, Kim J-M, Bae H-J, Han K-H (2013) MYB46 directly regulates the gene expression of secondary wall-associated cellulose synthases in *Arabidopsis*. *The Plant Journal* **73**: 26-36.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313x.2012.05124.x/abstract>
- **Galactomannan biosynthesis.** Galactomannans, hemicellulosic polysaccharides, are entirely composed of yeast-fermentable hexoses and are easily digestible, making them an ideal polymer to provide sugars for biofuel production from plant biomass. In our first report this year, to better understand the galactomannan biosynthetic pathway and its regulatory mechanism, we conducted deep EST sequencing from cDNA libraries using various stages of developing fenugreek seeds. In a second report, we identified a likely glycosyltransferase important for mannan biosynthesis.

Wang Y, Alonso A, Wilkerson C, Keegstra K (2012) Deep EST profiling of developing fenugreek endosperm to investigate galactomannan biosynthesis and its regulation. *Plant Molecular Biology* **79**: 243-258.

<https://link.springer.com/article/10.1007%2Fs11103-012-9909-y>

Wang Y, Mortimer JC, Davis J, Dupree P, Keegstra K (2012) Identification of an additional protein involved in mannan biosynthesis. *The Plant Journal* **73**: 105-117.

<http://onlinelibrary.wiley.com/doi/10.1111/tpj.12019/full>
- **Influence of variable species composition on the saccharification of AFEX pretreated biomass.** We evaluated the impact of AFEX pretreatment conditions on hydrolysis sugar yields for various old-field treatments comprised of different mixtures of annual forbs and grass species to corn stover. We found that the grass-dominated feedstocks were as digestible as corn stover, but corn stover had higher total glucose and xylose yields.

Garlock RJ, Bals B, Jasrotia P, Balan V, Dale BE (2012) Influence of variable species composition on the saccharification of AFEX™ pretreated biomass from unmanaged fields in comparison to corn stover. *Biomass and Bioenergy* **37**: 49-59.

<http://www.sciencedirect.com.ezproxy.library.wisc.edu/science/article/pii/S0961953411006556>

- **An integrated biological process for ethanol production featuring enzyme recycling.** We developed a process that saves time by quickly fermenting xylose, processing the easily digestible biomass first to fully utilize the high enzymatic hydrolysis rate period, and hydrolyzing the more recalcitrant part of biomass with less inhibition by degradation products and sugar, enhancing the process ethanol productivity. This process also allows for better use of biocatalysts resources by recycling yeast cells and enzymes and reducing overall processing cost.
Jin M, Gunawan C, Uppugundla N, Balan V, Dale BE (2012) A novel integrated biological process for cellulosic ethanol production featuring high ethanol productivity, enzyme recycling and yeast cells reuse. *Energy & Environmental Science* **5**: 7168-7175.
<http://dx.doi.org/10.1039/C2EE03058F>
- **Integrated model for cellulosic biorefineries.** We demonstrated a cellulosic biorefinery model, which produces ethanol and food precursors using lignocellulosic biomass as the exclusive source of carbohydrates and minerals. This biorefinery model includes in-house saccharolytic enzyme production using AFEX-pretreated corn stover as carbohydrate sources, which reduces the dependence on commercial enzymes.
Lau MW, Bals BD, Chundawat SPS, Jin M, Gunawan C, Balan V, Jones AD, Dale BE (2012) An integrated paradigm for cellulosic biorefineries: utilization of lignocellulosic biomass as self-sufficient feedstocks for fuel, food precursors and saccharolytic enzyme production. *Energy & Environmental Science* **5**: 7100-7110.
<http://dx.doi.org/10.1039/C2EE03596K>
- **Structural characterization of AHP-treated grasses with diverse lignin phenotypes.** We determined that the digestibility of four grasses with relatively diverse lignin phenotypes could be linked to quantifiable changes in the cell wall composition and properties of the lignin of the grasses, while S/G ratios did not appear to contribute to the enzymatic digestibility of delignification.
Li M, Foster CE, Pu Y, Holmes D, Saffron C, Ragauskas A, Hodge DB (2012) Structural characterization of alkaline hydrogen peroxide pretreated grasses exhibiting diverse lignin phenotypes. *Biotechnology for Biofuels* **5**: 38.
<http://www.biotechnologyforbiofuels.com/content/5/1/38>
- **Techno-Economic Analysis (TEA) of butene oligomer production from lignocellulosic biomass.** We developed and evaluated an integrated process for the conversion of lignocellulose to liquid fuels through the esterification of levulinic acid (LA). We performed TEA and our model shows that the biomass feedstock price is the main cost driver for the lignocellulose conversion.
Sen M, Gurbuz EI, Wettstein SG, Alonso DM, Dumesic JA, Maravelias CT (2012) Production of butene oligomers as transportation fuels using butene for esterification of levulinic acid from lignocellulosic biomass: process synthesis and technoeconomic evaluation. *Green Chemistry* **14**: 3289-3294.
<http://pubs.rsc.org/en/content/articlelanding/2012/gc/c2gc35881f>
- **Solvent derived from lignin used in catalytic conversion of cellulose and hemicellulose biomass fractions.** Catalytic depolymerization of lignin was used to produce a lignin-derived solvent, shown to be effective for selective biomass conversion processes. Like biomass-derived gamma valerolactone, the ability to derive solvent from lignin represents a green, renewable process for production of fuels and chemicals.
Azadi P, Carrasquillo-Flores R, Pagan-Torres YJ, Gurbuz EI, Farnood R, Dumesic JA (2012) Catalytic conversion of biomass using solvents derived from lignin. *Green Chemistry* **14**: 1573-1576.
<http://pubs.rsc.org/en/content/articlelanding/2012/gc/c2gc35203f> - !divAbstract

- An improved computational method for predicting the effects of metabolic and regulatory perturbations in systems biology.** Predicting cellular responses to perturbations is an important goal in systems biology. The authors of this study describe a new approach, RELATCH, which uses flux and gene expression data from a reference state to predict metabolic responses in a genetically or environmentally perturbed state. The generation of accurate genome-scale models of microbial metabolism will greatly aid host strain engineering and biofuel production.

Kim J, Reed JL (2012) RELATCH: relative optimality in metabolic networks explains robust metabolic and regulatory responses to perturbations. *Genome Biology* **13**: R78.
<https://genomebiology.biomedcentral.com/articles/10.1186/gb-2012-13-9-r78>
- Unique ability of *Spathaspora* yeast to co-ferment sugars provides insight into regulatory mechanisms that limit conversion of lignocellulosic biomass.** For many microorganisms the ability to co-utilize sugars is limited by distinct regulatory mechanisms, with glucose generally preferred over other carbon sources. However *Spathaspora passalidarum*, a native xylose-fermenting yeast, is unique in its ability to co-ferment glucose, xylose, and cellobiose and analyses indicated there was increased glycolytic flux in cultures grown on xylose compared to glucose. Study of this yeast's unique metabolism could provide insight into ways in which cellular metabolism may be rewired for increased conversion of sugars to biofuels.

Long TM, Su Y-KK, Headman J, Higbee A, Willis LB, Jeffries TW (2012) Cofermentation of glucose, xylose, and cellobiose by the beetle-associated yeast, *Spathaspora passalidarum*. *Applied and Environmental Microbiology* **78**: 5492-54500.
<http://aem.asm.org/content/78/16/5492.abstract?sid=8fe0097c-37ac-4f8d-bfe3-887728712884>
- Fermentative performance and ethanol production in AFEX corn stover hydrolysate (ACSH) is limited by multiple stress responses in *E. coli*.** Fermentation of ACSH was performed and the effects on *E. coli* growth, sugar utilization, gene expression, and biofuel production were monitored. The results suggest that the high energetic cost of mitigating numerous stressors limits growth and biofuel production in lignocellulosic hydrolysates and identifies genetic targets for strain improvement.

Schwalbach MS, Keating DH, Tremaine M, Marner WD, 2nd, Zhang YH, Bothfeld WH, Higbee AJ, Grass JA, Cotten C, Reed JL, da Costa Sousa L, Jin M, Balan V, Ellinger JJ, Dale B, Kiley PJ, Landick R (2012) Complex physiology and compound stress responses during fermentation of alkali-pretreated corn stover hydrolysate by an *Escherichia coli* ethanologen. *Applied and Environmental Microbiology* **78**: 3442-3457.
<http://aem.asm.org/content/78/9/3442.abstract>
- Yields of free fatty acid (FA) are higher in continuous culture compared to batch culture.** A plasmid-free strain of *E. coli* expressing three heterologous copies of a thioesterase gene was grown in continuous culture under carbon-limiting conditions and compared to batch culture. The yields of free FAs were 15% higher in continuous culture at low dilution rate as compared to batch culture, and could be further increased to ~40% when carbon was no longer limiting. Modeling suggests that maintenance requirements for the FA-producing strain are much higher than wild-type *E. coli*.

Youngquist JT, Lennen RM, Ranatunga DR, Bothfeld WH, Marner II WD, Pfleger BF (2011) Kinetic modeling of free fatty acid production in *Escherichia coli* based on continuous cultivation of a plasmid free strain. *Biotechnology and Bioengineering* **109**: 1518-1527.
<http://onlinelibrary.wiley.com/doi/10.1002/bit.24420/abstract>
- Hierarchical marginal land assessment framework for land use planning and management.** The objective of this study was to develop a hierarchical marginal land

assessment framework for land use planning and management. The hierarchical assessment framework has advantages of quantitatively reflecting land functions and multiple concerns. This provides a foundation upon which focused studies can be identified in order to improve the assessment framework by quantifying high-resolution land functions associated with environment and ecosystem services as well as their criteria needed to improve the assessment framework.

Kang S, Post WM, Wang D, Nichols J, Bandaru VP (2013) Hierarchical marginal land assessment for land use planning. *Land Use Policy* **30**: 106-113.

<http://www.sciencedirect.com/science/article/pii/S0264837712000312>

- **Soil microbial communities under biofuel cropping systems in southern Wisconsin.** In the first of two papers, in which we analyzed microbial lipids and various soil physicochemical factors under model biofuel cropping systems of corn, switchgrass, and mixed prairie in southern Wisconsin, we conclude that the cropping system shifted the microbial community composition at this regional scale, which may also affect the microbial processes associated with these differing communities. In the second report, we evaluated the impact of soil attributes, microbial functional group biomass, and cropping system type on soil amino sugar profiles in three model biofuel cropping systems in southern Wisconsin; our results highlight some of the complications present in using and interpreting time-integrative measures of ecosystem properties such as amino sugar profiles (microbial residues), in contrast to more immediately responsive metrics such as microbial lipid profiles (microbial biomass).

Liang C, Jesus EdC, Duncan DS, Jackson RD, Tiedje JM, Balser TC (2012) Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: impact of crop species and soil properties. *Applied Soil Ecology* **54**: 24-31.

<http://www.sciencedirect.com/science/article/pii/S0929139311003209>

Liang C, Duncan DS, Balser TC, Tiedje JM, Jackson RD (2012) Soil microbial residue storage linked to soil legacy under biofuel cropping systems in southern Wisconsin, USA. *Soil Biology and Biochemistry* **57**: 939-942.

<http://www.sciencedirect.com/science/article/pii/S0038071712003549>

- **Pest suppression potential of Midwestern landscapes under contrasting bioenergy scenarios.** In this study we use predation rates of three sentinel crop pests to develop a biocontrol index (BCI) summarizing pest-suppression potential in corn and perennial grass-based bioenergy crops in southern Wisconsin, lower Michigan, and northern Illinois. We show that BCI is higher in perennial grasslands than in corn, and increases with the amount of perennial grassland in the surrounding landscape. Through comparisons with other independent studies, we find that our biocontrol index is negatively related to insecticide use across the Midwest, suggesting that strategically positioned, perennial bioenergy crops could reduce insect damage and insecticide use on neighboring food and forage crops.

Meehan TD, Werling BP, Landis DA, Gratton C (2012) Pest-suppression potential of Midwestern landscapes under contrasting bioenergy scenarios. *PLOS ONE* **7**: e41728.

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0041728>

- **Pastureland conversion effects on soil greenhouse gas fluxes.** We assessed the short-term effects of converting pastureland to hybrid poplar and willow bioenergy plantation on soil greenhouse gas (GHG) fluxes and nitrogen (N) leaching in northern Michigan. Our results demonstrate the need to include soil disturbance impacts on the N cycle in future life cycle assessment of these bioenergy crops.

Nikiema P, Rothstein DE, Miller RO (2012) Initial greenhouse gas emissions and nitrogen leaching losses associated with converting pastureland to short-rotation woody bioenergy crops in northern Michigan, USA. *Biomass and Bioenergy* **39**: 413-426.

<http://www.sciencedirect.com/science/article/pii/S0961953412000475>

- **Devising a new data structure for metagenome assembly.** In this work, we describe a simple probabilistic representation for storing short-read sequencing de Bruijn graphs in memory; we apply this graph representation to reduce the memory needed to assemble a soil metagenome sample through the use of read partitioning. By applying the probabilistic de Bruijn graph representation to the problem of partitioning, we achieve a dramatic decrease of nearly 40-fold in the memory required for assembly of a soil metagenome. This probabilistic graph representation is a significant theoretical advance in storing assembly graphs and also yields immediate leverage on metagenomic assembly.
Pell J, Hintze A, Canino-Koning R, Howe A, Tiedje JM, Brown CT (2012) Scaling metagenome sequence assembly with probabilistic de Bruijn graphs. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 13272-13277.
<http://www.pnas.org/content/109/33/13272>

Year 6 (Dec. 1, 2012 - Nov. 30, 2013)

- **Ectopic expression of a diacylglycerol acyltransferase results in lipids with increased energy density.** Enhancement of acyl-CoA-dependent triacylglycerol (TAG) synthesis in vegetative tissues is widely discussed as a potential avenue to increase the energy density of crops. Here, we report the identification and characterization of *Chlamydomonas reinhardtii* diacylglycerol acyltransferase type two (DGTT) enzymes and use DGTT2 to alter acyl carbon partitioning in plant vegetative tissues. The nutritional value and/or energy density of the transgenic lines was increased by ectopic expression of *DGTT2* and acyl groups were diverted from different pools into TAGs, demonstrating the interconnectivity of acyl metabolism in leaves.
Sanjaya, Miller R, Durrett TP, Kosma DK, Lydic TA, Muthan B, Koo AJK, Bukhman YV, Reid GE, Howe GA, Ohlrogge J, Benning C (2013) Altered lipid composition and enhanced nutritional value of *Arabidopsis* leaves following introduction of an algal diacylglycerol acyltransferase 2. *The Plant Cell* **25**: 677-693.
<http://www.plantcell.org/content/25/2/677.long>
- **New maize sequencing information complements existing databases.** Here we provide two reports: 1) optimizing read depth to obtain desired marker coverage when using genotyping-by-sequencing (GBS), and 2) analysis of RNA-Seq data to enhance the coverage and resolution of the maize gene atlas.
Beissinger TM, Hirsch CN, Sekhon RS, Foerster JM, Johnson JM, Muttoni G, Vaillancourt B, Buell CR, Kaeppler SM, de Leon N (2013) Marker density and read-depth for genotyping populations using genotyping-by-sequencing. *Genetics* **193**: 1073-1081.
<http://www.genetics.org/content/193/4/1073>
Sekhon RS, Briskine R, Hirsch CN, Myers CL, Springer NM, Buell CR, de Leon N, Kaeppler SM (2013) Maize gene atlas developed by RNA sequencing and comparative evaluation of transcriptomes based on RNA sequencing and microarrays. *PLOS ONE* **8**: e61005.
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061005>
- **Dynamic metabolic flux analysis of plant cell wall synthesis.** A dynamic metabolic flux model was developed based on pulse labeling experiments in *Arabidopsis*. Candidates for engineering cell wall synthesis rates and composition were identified.
Chen X, Alonso AP, Shachar-Hill Y (2013) Dynamic metabolic flux analysis of plant cell wall synthesis. *Metabolic Engineering* **18**: 78-85.
<http://www.sciencedirect.com/science/article/pii/S1096717613000487>
- **Unconventional lignin identified in a *Medicago* mutant.** Here, we describe the effect of cinnamyl alcohol dehydrogenase (CAD) gene knockout in the model legume *Medicago*

truncatula. Retrotransposon insertions in the *M. truncatula* *CAD1* (*MtCAD1*) gene result in stable mutant plants that grow normally under standard growth conditions. However, 2D NMR analysis revealed that lignins in *MtCAD1* mutants are, surprisingly, composed almost exclusively (~95%) of hydroxycinnamaldehyde-derived units. The almost exclusive derivation of lignin polymers in the *cad1* mutants from nontraditional lignin monomers extends the scope of modifications that we may yet consider for lignin modification toward improved biomass processing.

Zhao Q, Tobimatsu Y, Zhou R, Pattathil S, Gallego-Giraldo L, Fu C, Jackson LA, Hahn MG, Kim H, Ralph J, Chen F, Dixon RA (2013) Loss of function of cinnamyl alcohol dehydrogenase 1 leads to unconventional lignin and a temperature-sensitive growth defect in *Medicago truncatula*. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 13660-13665.

<http://www.pnas.org/content/110/33/13660.full>

- **Identification of a new enzyme in lignin biosynthesis.** Lignin is a major component of plant secondary cell walls. Here we have identified a new step in the biosynthetic pathway of lignin in *Arabidopsis* in which caffeoyl shikimate esterase (CSE) is a central enzyme. Our data necessitate the revision of currently accepted models of the lignin biosynthetic pathway.

Vanholme R, Cesarino I, Rataj K, Xiao Y, Sundin L, Goeminne G, Kim H, Cross J, Morreel K, Araujo P, Welsh L, Hastraete J, McCellan C, Vanholme B, Ralph J, Simpson GG, Halpin C, Boerjan W (2013) Caffeoyl shikimate esterase (CSE) is an enzyme in the lignin biosynthetic pathway in *Arabidopsis*. *Science* **341**: 1103-1106.

<http://science.sciencemag.org/content/341/6150/1103.long>

- **Demonstrating biomass deconstruction of *Streptomyces* sp. SirexAA-E.** When grown on plant biomass, ActE secretes a suite of enzymes including endo- and exo-cellulases and hemicellulases. We performed extensive analyses of this enzyme to provide insight into its function. Transcriptomic and proteomic analysis have revealed the key enzymes used in the biomass deconstruction process, and we found it is comparable to a cellulolytic enzyme cocktail from *Trichoderma reesei*.

Bianchetti CM, Hermann CH, Takasuka TE, Hura GL, Dyer K, Fox BG (2013) Fusion of dioxygenase and lignin-binding domains in a novel secreted enzyme from cellulolytic *Streptomyces* sp. SirexAA-E. *Journal of Biological Chemistry* **288**: 18574-18587.

<http://www.jbc.org/content/288/25/18574.abstract>

Takasuka TE, Book AJ, Lewin GR, Currie CR, Fox BG (2013) Aerobic deconstruction of cellulosic biomass by an insect-associated *Streptomyces*. *Scientific Reports* **3**: 1030.

<http://www.nature.com/srep/2013/130107/srep01030/full/srep01030.html>

- **Increased enzyme binding to substrate is not necessary for more efficient cellulose hydrolysis.** We found that altering the crystalline structure of plant-derived cellulose I to III can lower fungal cellulose loading by 5- fold to achieve comparable saccharification yields, despite a reduced effective enzyme affinity for the III substrate. We developed a mechanistic kinetic model coupling enzyme binding, cellulose surface chain extraction, and glycosidic bond hydrolysis to explain our findings. This can help engineer more efficient cellulases to work in native and non-native (i.e. chemically pretreated) celluloses.

Gao D, Chundawat SPS, Sethi A, Balan V, Gnanakaran S, Dale BE (2013) Increased enzyme binding to substrate is not necessary for more efficient cellulose hydrolysis. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 10922-10927.

<http://www.pnas.org/content/110/27/10922.full>

- **Hydrolysis of AFEX-pretreated corn stover. Effects of supply and storage conditions.** We investigated the effects of storage conditions on the composition and fermentability of

hydrolysate prepared from AFEX-pretreated corn stover, including pelletized biomass as a source using high solid loadings. We found that there is no significant difference in the fermentability between fresh hydrolysate and stored hydrolysate, and that the slurries resulting from pelletized stover remained well mixed. Furthermore, pelletization slightly increased the initial rate of hydrolysis compared to unpelletized biomass, suggesting that pelletization after AFEX pretreatment could have additional advantages beyond the logistical handling of biomass.

Bals BD, Gunawan C, Moore J, Teymouri F, Dale BE (2013) Enzymatic hydrolysis of pelletized AFEX™-treated corn stover at high solid loadings. *Biotechnology and Bioengineering* **111**: 264-271.

<http://onlinelibrary.wiley.com/doi/10.1002/bit.25022/abstract>

Jin M, Bothfeld W, Austin S, Sato TK, LaReau A, Li H, Foston M, Gunawan C, LeDuc RD, Quensen JF, McGee M, Nuppugundia N, Higbee A, Ranatunga R, Donald CW, Bone G, Raguskas AJ, Tiedje JM, Noguera DR, Dale BE, Zhang Y, Balan V (2013) Effect of storage conditions on the stability and fermentability of enzymatic lignocellulosic hydrolysate. *Bioresource Technology* **147**: 212-220.

<http://dx.doi.org/10.1016/j.biortech.2013.08.018>

- **Framework for biomass-to-fuel conversion strategies.** We developed a framework for the identification and evaluation of biomass-to-fuel production strategies; a technology superstructure consisting of a wide range of conversion technologies along with the corresponding feedstocks, intermediates, and final products. The proposed framework can be used to study a range of questions including “What is the best strategy for the production of a specific fuel? What is the best use strategy for a specific feedstock? We illustrated our methodology using the production of ethanol from hard woody biomass as a case study.

Kim J, Sen SM, Maravelias CT (2013) An optimization-based assessment framework for biomass-to-fuel conversion strategies. *Energy & Environmental Science* **6**: 1093-1104.

<http://pubs.rsc.org/en/Content/ArticleLanding/2013/EE/C3EE24243A>
- **Pretreatment of woody biomass via Alkaline Hydrogen Peroxide catalyzed by a metal complex.** We discovered that AHP Cu^{II}(bpy) significantly improves the sugar release from poplar relative to uncatalyzed AHP pretreatment, and we characterized the relationship between pretreatment conditions and enzymatic sugar release.

Li Z, Chen CH, Hegg EL, Hodge DB (2013) Rapid and effective oxidative pretreatment of woody biomass at mild reaction conditions and low oxidant loadings. *Biotechnology for Biofuels* **6**: 119.

<http://www.biotechnologyforbiofuels.com/content/6/1/119>

Li Z, Chen CH, Liu T, Mathrubootham V, Hegg EL, Hodge DB (2013) Catalysis with Cu^{II}(bpy) improves alkaline hydrogen peroxide pretreatment. *Biotechnology and Bioengineering* **110**: 1078-1086.

<http://dx.doi.org/10.1002/bit.24793>
- **Algorithm for high-resolution identification of transcription factor binding sites in ChIP-seq data.** Chromatin-immunoprecipitation followed by high-throughput sequencing (ChIP-seq) is a powerful technique to identify transcription factor binding sites throughout the genome. Microbial genomes often contain closely spaced or overlapping promoters and it can be difficult to identify binding sites with a high degree of resolution. The authors of this study developed the algorithm dPeak, which can estimate the locations of binding events with higher resolution than standard methods. High resolution mapping of promoter binding sites is an important tool for synthetic biology and biofuel host strain development.

Chung D, Park D, Myers K, Grass J, Kiley P, Landick R, Keleş S (2013) dPeak: High resolution identification of transcription factor binding sites from PET and SET ChIP-Seq data. *PLOS Computational Biology* **9**: e1003246.

<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1003246>

- Identification of pathway utilized for microbial metabolism of meta-hydroxy aromatic compounds.** Researchers used a genetic approach to show that metabolism of meta-hydroxy-aromatic acids by *R. palustris* occurs via induction of the native benzyol-CoA pathway. The results open the possibility of *R. palustris* metabolizing aromatic compounds from lignin and the ability to derive greater value from all of the major components of biomass.

Gall DL, Ralph J, Donohue TJ, Noguera DR (2013) Benzoyl coenzyme A pathway-mediated metabolism of meta-hydroxy-aromatic acids in *Rhodopseudomonas palustris*. *Journal of Bacteriology* **195**: 4112-4120.
<http://jlb.asm.org/content/195/18/4112.long>
- Genome-scale mapping of transcription factor binding sites provide insights into the global response to oxygen.** Two studies utilized ChIP-seq to map promoter occupancy of the *Escherichia coli* transcription factors FNR and ArcA in response to oxygen availability. Interestingly, FNR promoter occupancy was strongly influenced by nucleoid associated proteins, which in several cases appear to restrict promoter access by FNR. In the case of ArcA, an abundance of non-cannonical binding sites within promoters dictates the length and concentration-sensitive occupancy of DNA. Knowledge of promoter architecture and the mechanisms that govern global gene regulation informs synthetic biology approaches for improved biofuel production.

Myers KS, Yan H, Ong IM, Chung D, Liang K, Tran F, Keles S, Landick R, Kiley PJ (2013) Genome-scale analysis of *Escherichia coli* FNR reveals complex features of transcription factor binding. *PLOS Genetics* **9**: e1003565.
<http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1003565>

Park DM, Akhtar MS, Ansari AZ, Landick R, Kiley PJ (2013) The bacterial response regulator ArcA uses a diverse binding site architecture to regulate carbon oxidation globally. *PLOS Genetics* **9**: e1003839.
<http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1003839>
- New method of alcohol oxidation in lignin.** An efficient organocatalytic method for chemoselective aerobic oxidation of secondary benzylic alcohols within lignin model compounds has been identified. Extension to selective oxidation of natural lignins has also been demonstrated. Preliminary studies highlight the prospect of combining this method with a subsequent oxidation step to achieve C–C bond cleavage.

Rahimi A, Azarpira A, Kim H, Ralph J, Stahl SS (2013) Chemoselective metal-free aerobic alcohol oxidation in lignin. *Journal of the American Chemical Society* **135**: 6415-6418
<http://pubs.acs.org/doi/abs/10.1021/ja401793n>
- Multimiomics data sets provide estimation of *in vivo* kinetic parameters for constraint-based metabolic models.** Constraint-based models typically omit kinetic considerations due to limitations in determining kinetic information for an entire metabolic network. To overcome these limitations, researchers produced a method for *in vivo* kinetic parameter estimation, using multi-omic data sets for *Escherichia coli*. The result was a simplified kinetic model, with rate laws for a subset of metabolic enzymes. These results demonstrate the utility of multiomic data sets for identifying metabolic bottlenecks for biofuel production.

Cotten C, Reed JL (2013) Mechanistic analysis of multi-omics datasets to generate kinetic parameters for constraint-based metabolic models. *BMC Bioinformatics* **14**: 32.
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-14-32>
- Net primary production in three bioenergy crop systems following land conversion.** This is the first study of this kind to investigate and compare production on two common types of land available for bioenergy production in the Midwest. The results lead to the conclusion that there was no significantly higher production in lands that were converted

from the conservation reserve program (CRP) as originally hypothesized. This study should be followed up with a more comprehensive investigation evaluating these differences in a long-term field trial, with increased replication, simultaneous microclimate study and analysis of CO₂, H₂O and energy flux coupled with NPP.

Deal MW, Xu J, John R, Zenone T, Chen J, Jasrotia P, Kahmark K, Bossenbroek J, Mayer C (2014) Net primary production in three bioenergy crop systems following land conversion. *Journal of Plant Ecology* **7**: 451-460.

<https://academic.oup.com/jpe/article/7/5/451/939272/Net-primary-production-in-three-bioenergy-crop?related-urls=yes&legid=jpe;rtt057v1>

- **Sustainable bioenergy production from marginal lands in the US Midwest.** Here we evaluate the potential for marginal lands in ten Midwestern US states to produce sizeable amounts of biomass and concurrently mitigate greenhouse gas (GHG) emissions. In a comparative assessment of six alternative cropping systems over 20 years, we found that successional herbaceous vegetation, once well established, has a direct GHG emissions mitigation capacity that rivals that of purpose-grown. Our results suggest that such vegetation could produce about 21 giga litres of ethanol per year from around 11 million hectares, or approximately 25 per cent of the 2022 target for cellulosic biofuel mandated by the US Energy Independence and Security Act of 2007, with no initial carbon debt nor the indirect land-use costs associated with food-based biofuels.
Gelfand I, Sahajpal R, Zhang X, Izaurrealde RC, Gross KL, Robertson GP (2013) Sustainable bioenergy production from marginal lands in the US Midwest. *Nature* **493**: 514-517.

<https://www.nature.com/articles/nature11811>

- **Miscanthus establishment and overwintering in the Midwest.** This study described a dataset for annual extreme minimum temperatures across the Midwest US for both soils that are managed without leaving residue on the soil surface after harvest of crops as well as for soils that have a full cover (5 cm thick prostrate residue layer for maize in place, and for varying thicknesses of miscanthus straw). Our results indicated that strategic residue management in the region has potential to help increase extreme minimum soil temperatures that can threaten overwintering of miscanthus rhizomes in the first year of establishment and potentially in later years; also, the thickness of that residue layer has significant bearing on the insulating effect.
Kucharik CJ, VanLoocke A, Lenters JD, Motew MM (2013) Miscanthus establishment and overwintering in the Midwest USA: a regional modeling study of crop residue management on critical minimum soil temperatures. *PLOS ONE* **8**: e68847.
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0068847>
- **Initial N₂O, CO₂, and methane costs of converting CRP grassland to row crops under no-till vs. conventional tillage.** In our earlier work by Gelfand *et al.* (in Year 4), we reported that the conversion of Conservation Reserve Program (CRP) land to no-till soybean production released significant amounts of CO₂ and N₂O and had little effect on CH₄ oxidation rates. Here, we extend the results to examine the impact of conventional tillage practices on greenhouse gas (GHG) fluxes during conversion. Of particular note, we conclude that no-till management can reduce GHG costs by ~60% compared to conventional tillage during initial CRP conversion. **Ruan L, Robertson GP** (2013) Initial nitrous oxide, carbon dioxide, and methane costs of converting conservation reserve program grassland to row crops under no-till vs. conventional tillage. *Global Change Biology* **19**: 2478-2489.
<http://onlinelibrary.wiley.com/doi/10.1111/gcb.12216/abstract>
- **Effects of land use change on carbon balance.** We studied the effect of agricultural land-use change on two historical land use types for three different cropping systems using eddy

covariance and ecosystem carbon balance approaches. We evaluated the impact of the crop choices and yields together with biomass end-use to show the relative effects of these factors on the carbon balance of the converted ecosystems. These results can be readily incorporated into management recommendations for future establishment of biofuels feedstock and row agriculture systems with different management intensities.

Zenone T, Gelfand I, Chen J, Hamilton SK, Robertson GP (2013) From set-aside grassland to annual and perennial cellulosic biofuel crops: effects of land use change on carbon balance. *Agricultural and Forest Meteorology* **182-183**: 1-12.

<http://www.sciencedirect.com/science/article/pii/S0168192313001950>

Year 7 (Dec. 1, 2013 - Nov. 30, 2014)

- **PMT acts specifically in monolignol acylation.** In this study we tested the authenticity of *p*-coumaroyl-CoA:monolignol transferase (PMT) as a lignin biosynthetic pathway enzyme, by examining *Brachypodium distachyon* plants with altered *BdPMT* gene function. Using newly developed cell wall analytical methods, we determined that the transferase was involved specifically in monolignol acylation. Our data are consistent with a defined role for grass PMT genes in encoding BAHD acyltransferases that specifically acylate monolignols with *p*-coumarate (*p*CA) and produce monolignol *p*-coumarate conjugates that are used for lignification *in planta*.

Petrik DL, Karlen SD, Cass CL, Padmakshan D, Lu F, Liu S, Le Bris P, Antelme S, Santoro N, Wilkerson CG, Sibout R, Lapierre C, Ralph J, Sedbrook JC (2014) *p*-Coumaroyl-CoA:monolignol transferase (PMT) acts specifically in the lignin biosynthetic pathway in *Brachypodium distachyon*. *The Plant Journal* **77**: 713-726.

<http://onlinelibrary.wiley.com/doi/10.1111/tpj.12420/abstract>

- **Photoperiodic flowering time in *Brachypodium*.** In this year we published two papers on flowering time in *Brachypodium*. In the first report, our results indicate there is rich natural diversity in photoperiod and vernalization requirements to exploit for understanding gene networks controlling flowering; further, we provide evidence for conserved roles of *VRN1* and *FT* in promoting flowering, whereas the role of *VRN2* is less clear. In the second report, we show that *PHYC* is required for the transcriptional activation of several photoperiod-pathway genes, including *PPD1*, *CO*, and *FT*, and that loss of *PHYC* alters the expression of certain circadian input and output genes, revealing a difference in how light is perceived for the photoinduction of flowering between the well-studied plants *Arabidopsis* and rice compared to the temperate grass *Brachypodium*.

Ream T, Woods D, Schwartz C, Sanabria C, Mahoy J, Walters E, Kaeppler H, Amasino R (2013) Interaction of photoperiod and vernalization determine flowering time of *Brachypodium distachyon*. *Plant Physiology* **164**: 694-709.

<http://www.plantphysiol.org/content/164/2/694.long>

Woods DP, Ream TS, Minevich G, Hobert O, Amasino RM (2014) PHYTOCHROME C is an essential light receptor for photoperiodic flowering in the temperate grass, *Brachypodium distachyon*. *Genetics* **198**: 397-408.

<http://www.genetics.org/content/198/1/397>

- **Maize pan-genome and pan-transcriptome.** Genomes at the species level are dynamic, with genes present in every individual (core) and genes in a subset of individuals (dispensable) that collectively constitute the pan-genome. Using transcriptome sequencing of seedling RNA from maize (*Zea mays*) inbred lines to characterize the maize pan-genome, we identified 8681 representative transcript assemblies (RTAs) with 16.4% expressed in all lines and 82.7% expressed in subsets of the lines; furthermore, genome-wide association studies based on single nucleotide polymorphisms (SNPs) and transcript abundance in the

pan-genome revealed loci associated with the timing of the juvenile-to-adult vegetative and vegetative-to-reproductive developmental transitions, two traits important for fitness and adaptation. This study revealed the dynamic nature of the maize pan-genome and demonstrated that a substantial portion of variation may lie outside the single reference genome for a species.

Hirsch CN, Foerster JM, Johnson JM, Sekhon RS, Muttoni G, Vaillancourt B, Peñagaricano F, Lindquist E, Pedraza MA, Barry K, de Leon N, Kaeppler SM, Buell CR (2014) Insights into the maize pan-genome and pan-transcriptome. *The Plant Cell* **26**: 121-135.

<http://www.plantcell.org/content/26/1/121>

- **Zip-lignin poplar.** Redesigning lignin, the aromatic polymer fortifying plant cell walls, to be more amenable to chemical depolymerization can lower the energy required for industrial processing. We have engineered poplar trees to introduce ester linkages into the lignin backbone by augmenting the monomer pool with monolignol ferulate conjugates, and demonstrate that the resulting “zip-lignin” poplar trees showed no difference in growth habit under greenhouse conditions, but that their lignin showed improved digestibility. Tailoring plants to use such conjugates during cell wall biosynthesis is a promising way to produce plants that are designed for deconstruction.

Wilkerson CG, Mansfield SD, Lu F, Withers S, Park J-Y, Karlen SD, Gonzales-Vigil E, Padmakshan D, Unda F, Rencoret J, Ralph J (2014) Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone. *Science* **344**: 90-93.

<http://science.sciencemag.org/content/344/6179/90>

- **Sorghum modeling of genetics maps.** Here, we build off an existing model of differential zygotic viability to incorporate a heterozygosity maintenance term for plant recombinant inbred populations and find a new solution for the genotype probabilities used to calculate recombination frequencies. Using sorghum, this modeling allows more accurate generation of genetic maps and retention of more genetic information by accounting for the biological phenomenon of differential fitness of heterozygous loci. More accurate estimations of recombination fractions, and thus linkage, will improve the accuracy of methods that use linkage information to detect and use causal genetic variants.

Truong SK, McCormick RF, Morishige DT, Mullet JE (2014) Resolution of genetic map expansion caused by excess heterozygosity in plant recombinant inbred populations. *G3: Genes | Genomes | Genetics* **4**: 1963-1969.

<http://www.g3journal.org/content/4/10/1963>

- **Evolution of substrate specificity in bacterial lytic polysaccharide monooxygenases (LPMOs).** We analyzed the sequences, structures, and evolution of LPMOs to understand the factors that may influence substrate specificity within and between these enzyme families. We found that these families share an ancient ancestral protein, but the different clades have specialized their selection towards different substrate bindings. Our work provided a phylogenetic basis for identifying and classifying these LPMOs.

Book AJ, Yennamalli RM, Takasuka TE, Currie CR, Phillips GN, Fox BG (2014) Evolution of substrate specificity in bacterial AA10 lytic polysaccharide monooxygenases. *Biotechnology for Biofuels* **7**: 109.

<http://www.biotechnologyforbiofuels.com/content/7/1/109>

- **Comparison of enzymatic reactivity from corn stover pretreated via three different pretreatments.** In a biorefinery, biomass pretreatment will significantly influence the efficacy of enzymatic hydrolysis and fermentation. In this BRC collaboration, we compared the effects of three pretreatments (dilute acid, AFEX, and ionic liquid) on downstream operations. We found that AFEX treated corn stover showed no significant change in composition, whereas 85% of the hemicellulose was solubilized after dilute acid

pretreatment, and about 90% of the lignin was removed after ionic liquid pretreatment. We also found that no single factor absolutely dominated the early and long-term sugar yields from enzymatic hydrolysis.

Gao X, Kumar R, Singh S, Simmons B, Balan V, Dale B, Wyman CE (2014) Comparison of enzymatic reactivity of corn stover solids prepared by dilute acid, AFEX™, and ionic liquid pretreatments. *Biotechnology for Biofuels* **7**: 71.

<http://www.biotechnologyforbiofuels.com/content/7/1/71>

Uppugundla N, Sousa L, Chundawat SPS, Yu X, Simmons B, Singh S, Gao X, Kumar R, Wyman CE, Dale BE, Balan VA (2014) A comparative study of ethanol production using dilute acid, ionic liquid and AFEX™ pretreated corn stover. *Biotechnology for Biofuels* **7**: 72.

<http://www.biotechnologyforbiofuels.com/content/7/1/72/abstract>

- **Pyrolysis of grass species in North America.** We analyzed various North American native grass species to understand the effects of feedstock compositional variability on fast pyrolysis and associated products. The grasses studied were: big bluestem, coastal panicgrass, deertongue, indiagrass, Miscanthus, prairie sandreed, sideoats grama, and switchgrass. We identified significant variability in the pyrolysis products but could find no connection to the taxonomic tribe. The results help identify which grasses might be better suited for different biofuels technologies.

Kelkar S, Li Z, Bovee J, Thelen KD, Kriegel RM, Saffron CM (2014) Pyrolysis of North-American grass species: effect of feedstock composition and taxonomy on pyrolysis products. *Biomass and Bioenergy* **64**: 152-161.

<http://www.sciencedirect.com/science/article/pii/S0961953414001500?via=ihub>

- **Bacterial enzyme sheds light on cellulose deconstruction.** Using a CBM first isolated from a highly cellulolytic actinobacterium discovered by our own researchers, we described a simple strategy to produce fully functional bacterial CBMs, showing that, unlike previous studies of bacterial CBMs, these bacterial CBMs bind reversibly to cellulose.

Lim S, Chundawat SPS, Fox BG (2014) Expression, purification and characterization of a functional carbohydrate-binding module from *Streptomyces* sp. SirexAA-E. *Protein Expression and Purification* **98**: 1-9.

<http://www.sciencedirect.com/science/article/pii/S1046592814000473>

- **The RaBIT process: Rapid bioconversion of lignocellulosic sugars into ethanol using high cell density fermentations with enzyme recycling.** We developed a process called RaBIT (Rapid Bioconversion with Integrated recycle Technology) that reduces capital costs, processing times, and biocatalyst cost for biochemical conversion of cellulosic biomass to biofuels. The process reduces total bioprocessing time to 48 h, increases biofuel productivity 2-fold, and recycles biocatalysts using 24-h high cell density fermentations along with cell recycling to solve the slow/incomplete xylose fermentation issue. We tested various microbial strains in the fermentation process, and while not all of them are capable of effectively performing in the RaBIT process, we found that their performance is largely correlated to the specific xylose consumption rate.

Sarks C, Jin M, Sato TK, Balen V, Dale BE (2014) Studying the rapid bioconversion of lignocellulosic sugars into ethanol using high cell density fermentations with cell recycle. *Biotechnology for Biofuels* **7**: 73.

<http://www.biotechnologyforbiofuels.com/content/7/1/73>

- **Ethanol shown to target transcriptional and translational machinery in *E. coli*.** Various omics methods, including ribosome profiling, were used to characterize ethanol-tolerant mutants to show that biofuel directly targets central dogma processes. These findings provide conceptual frameworks for the study of ethanol toxicity in microbes and for the engineering of ethanol tolerance that may be extensible to other microbes and to other short-chain alcohols.

Haft, R. J. F., Keating, D. H., Schwaegler, T., S., S. M., Vinokur, J., Tremaine, M., Peters, J. M., Kotlajich, M. V., Pohlmann, E. L., Ong, I. M., Grass, J. A., Kiley, P. J., and Landick, R. (2014) Correcting direct effects of ethanol on translation and transcription machinery confers ethanol tolerance in bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E2576-E2585.

<http://www.pnas.org/content/111/25/E2576.long>

- Aromatic inhibitors derived from lignocellulosic biomass hinder bacterial ethanologenesis by activating energy-depleting stress-response and detoxification pathways.** This study utilized transcriptomic, proteomic, and metabolomic approaches to investigate inhibitor toxicity and microbial response mechanisms in the model bacterium *E. coli*. The findings indicate that the major regulatory responses to lignocellulose-derived inhibitors are mediated by transcriptional rather than translational regulators and that energy consumed for inhibitor efflux and detoxification may limit biofuel production.

Keating, D. H., Zhang, Y., Ong, I. M., McIlwain, S., Morales, E. H., Grass, J. A., Tremaine, M., Bothfeld, W., Higbee, A., Ulbrich, A., Balloon, A., Westphall, M. S., Aldrich, J., Lipton, M., Kim, J., Moskvina, O. V., Bukhman, Y. V., Coon, J., Kiley, P. J., Bates, D. M., and Landick, R. (2014) Aromatic inhibitors derived from ammonia-pretreated lignocellulose hinder bacterial ethanologenesis by activating regulatory circuits controlling inhibitor efflux and detoxification. *Frontiers in Microbiology* **5**, 402.

<http://journal.frontiersin.org/article/10.3389/fmicb.2014.00402/full>
- Examination of bacterial lipid production reveals a novel furan-containing fatty acid that could potentially serve as a biofuel precursor or additive.** A previously undescribed pathway for furan-containing fatty acid synthesis in *Rhodobacter sphaeroides* was discovered. The oxygen atom in the furan ring is reactive and could have potentially useful applications either as a fuel or fuel additive.

Lemke, R. A. S., Peterson, A. C., Ziegelhoffer, E. C., Westphall, M. S., Tjellström, H., Coon, J. J., and Donohue, T. J. (2014) Synthesis and scavenging role of furan fatty acids. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E3450-E3457.

<http://www.pnas.org/content/early/2014/07/31/1405520111.full.pdf+html?sid=dc6a6a79-baa9-4d4d-8510-16bc677a028b>
- GVL-based deconstruction method for high-yielding sugars.** This novel deconstruction method produces both C5 and C6 sugars from biomass in high yield without the use of enzymes. It is also a green, renewable technology since GVL may be derived from the biomass itself and recycled. The ability to bypass the use of enzymes to deconstruct biomass could provide a substantial cost-savings and thus is a promising new technology.

Luterbacher, J. S., Rand, J. M., Martin Alonso, D., Han, J., Youngquist, J. T., Maravelias, C. T., Pfleger, B. F., and Dumesic, J. A. (2014) Non-enzymatic sugar production from biomass using biomass-derived γ -valerolactone. *Science* **343**, 277-280.

<http://www.sciencemag.org/content/343/6168/277.abstract>
- Lignin depolymerization method increases monomeric yield.** Oxidation of a C-O bond adjacent to the β -aryl ether bond in lignin enhances depolymerization and aromatic monomer yield. This study demonstrates a more efficient process that may potentially enhance the lignin value chain and improve the commercial and economic viability of lignocellulosic biofuels.

Rahimi, A., Ulbrich, A., Coon, J. J., and Stahl, S. S. (2014) Formic-acid-induced depolymerization of oxidized lignin to aromatics. *Nature* **515**, 249-252.

<http://www.nature.com/nature/journal/v515/n7526/full/nature13867.html>
- Tackling soil diversity with the assembly of large, complex metagenomes.** Investigations of complex environments rely on large volumes of sequence data to

adequately sample the genetic diversity of a microbial community. We present approaches that make *de novo* assembly of complex metagenomes more accessible; these approaches scale data size with community richness and subdivide the data into tractable subsets representing individual species. We applied these methods toward the assembly of two large soil metagenomes to identify important metagenomic references and show that considerably more data are needed to study the terrestrial microbiome comprehensively.

Howe AC, Jansson JK, Malfatti SA, Tringe SG, Tiedje JM, Brown CT (2014) Tackling soil diversity with the assembly of large, complex metagenomes. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 4904-4909.

<http://www.pnas.org/content/111/13/4904>

- **Modeling a biorefinery for production of furfural and furfuryl alcohol in a green, high-yield process.** The biodegradation pathway for the reduction of the fermentation inhibitor furfural was utilized to produce furfuryl alcohol using both a commercial Bakers' yeast and six other native strains, selected for their high tolerance towards the inhibitory effects of furfural. This study explores the potential of the microbial method as an environmentally-benign alternative to the conventional catalytic hydrogenation process for producing furfuryl alcohol used extensively in industry. Results showed that the yields of furfuryl alcohol using the laboratory yeast strains exceeded 90% of the theoretical yield at a furfural concentration of 25 g l⁻¹, which are comparable to yields obtained using the catalytic process; thus, piecing together novel high-yield conversion processes for furfural and furfuryl alcohol, an integrated biorefinery model based on the production of furans has been envisioned.

Mandalika A, Qin L, Sato TK, Runge T (2014) Integrated biorefinery model based on production of furans using open-ended high yield processes. *Green Chemistry* **16**: 2480-2489.

<http://pubs.rsc.org/en/content/articlelanding/2014/gc/c3gc42424c#!divAbstract>
- **Nitrous oxide (N₂O) in soil.** We have published two notable soil N₂O reports in Year 7; the first provides results showing that it is possible to estimate N₂O diffusivity with sparse measurements, and that accuracy can likely be further improved with knowledge of soil moisture and texture in the immediate vicinity of the injection and sampling ports, as uncertainty in water modeling is reduced. In our second report, we clarify the response of the greenhouse gas nitrous oxide (N₂O) to nitrogen (N) fertilizer additions, a topic of considerable debate; our results suggest a general trend of exponentially increasing N₂O emissions as N inputs increase to exceed crop needs. Use of this knowledge in greenhouse gas inventories should improve assessments of fertilizer-derived N₂O emissions, help address disparities in the global N₂O budget, and refine the accuracy of N₂O mitigation protocols.

Shcherbak I, Philip Robertson G (2014) Determining the diffusivity of nitrous oxide in soil using in situ tracers. *Soil Science Society of America Journal* **78**: 79-88.

<https://dl.sciencesocieties.org/publications/sssaj/abstracts/78/1/79>

Shcherbak I, Millar N, Robertson GP (2014) Global metaanalysis of the nonlinear response of soil nitrous oxide (N₂O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 9199-9204.

<http://www.pnas.org/content/111/25/9199.abstract>
- **Mechanism of soil carbon accrual and storage in bioenergy cropping systems.** Annual row cropping systems converted to perennial bioenergy crops tend to accrue soil carbon (C), likely a function of increased root production and decreased frequency of tillage. To address this uncertainty, we assessed the formation and stability of aggregates and soil organic C (SOC) pools under switchgrass, giant miscanthus, and native perennial grass mix, and continuous corn treatments in Michigan and Wisconsin soils differing in both texture and mineralogy. Our results help explain cross-site variability in soil C accrual under

perennial bioenergy crops by demonstrating how interactions between belowground productivity, soil type, aggregation processes and microbial communities influence the rates and extent of SOC stabilization.

Tiemann LK, Stuart Grandy A (2015) Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *Global Change Biology – Bioenergy* **7**: 161-174.

<http://onlinelibrary.wiley.com/doi/10.1111/gcbb.12126/abstract>

- **The impact of perennial grasslands in bioenergy landscapes.** Science-based policies are needed to inform sustainable bioenergy landscape design. Our key finding is that the linkage between biodiversity and ecosystem services is dependent not only on the choice of bioenergy crop but also on its location relative to other habitats; the implication is that careful design of bioenergy landscapes has the potential to enhance multiple services in food and energy crops, leading to important synergies that have not yet informed the ongoing bioenergy debate. This study is especially timely as high commodity prices are driving conversion of marginal lands to annual crop production, reducing future flexibility. **Werling BP, Dickson TL, Isaacs R, Gaines H, Gratton C, Gross KL, Liere H, Malmstrom CM, Meehan TD, Ruan L, Robertson BA, Robertson GP, Schmidt TM, Schrotenboer AC, Teal TK, Wilson JK, Landis DA** (2014) Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 1652-1657.
<http://www.pnas.org/content/111/4/1652.abstract>

Year 8 (Dec. 1, 2014 - Nov. 30, 2015)

- **Ac-TAGs from transgenic *Camelina*.** In these two studies, we report the engineering and subsequent field production of acetyl-triacylglycerol (acetyl-TAG) in *Camelina*. The first study produced multi-liter quantities of conventional oils suitable for testing in a range of applications and with different fatty acid compositions, whereas the second study resulted in the highest accumulation of unusual oil achieved so far in transgenic plants. **Liu J, Tjellström H, McGlew K, Shaw V, Rice A, Simpson J, Kosma D, Ma W, Yang W, Strawsine M, Cahoon E, Durrett TP, Ohlrogge J** (2015) Field production, purification and analysis of high-oleic acetyl-triacylglycerols from transgenic *Camelina sativa*. *Industrial Crops and Products* **65**: 259-268.
<http://www.sciencedirect.com/science/article/pii/S0926669014007067>
Liu J, Rice A, McGlew K, Shaw V, Park H, Clemente T, Pollard M, Ohlrogge J, Durrett TP (2015) Metabolic engineering of oilseed crops to produce high levels of novel acetyl glyceride oils with reduced viscosity, freezing point and calorific value. *Plant Biotechnology Journal* **13**: 858-865.
<http://onlinelibrary.wiley.com/doi/10.1111/pbi.12325/full>
- **Topology of the MLG synthase BdCLSF6.** In our studies to better understand the biosynthesis of mixed-linkage glucan (MLG), a major carbon source for grass-based biofuel production, we sought to establish the topology of the BdCLSF6 enzyme. Our results demonstrate BdCLSF6 localization in the Golgi and supports a model where: BdCLSF6 alone can synthesize MLG using UDP-glucose in the cytoplasm, and MLG is channeled through the biosynthetic membranes of the Golgi and secreted into the cell wall through the default pathway. While the evidence supports our proposed model, the results do not preclude the possibility of other isoforms expressed in other cellular locations. **Kim S-J, Zemelis S, Keegstra K, Brandizzi F** (2015) The cytoplasmic localization of the catalytic site of CLSF6 supports a channeling model for the biosynthesis of mixed-linkage glucan. *The Plant Journal* **81**: 537-547.
<http://onlinelibrary.wiley.com/doi/10.1111/tpj.12748/abstract>

- Maize cell-wall properties contributing to improved deconstruction and hydrolysis.** To gain a better understanding of how maize cell-wall properties impact initial recalcitrance as well as alkaline pre-treatment, we investigated correlations between cell-wall properties and hydrolysis yields. We found that several properties did not correlate with yield, indicating that while enzymatic hydrolysis yields may be set by the cell-wall lignin content, the cell wall's response to delignifying pre-treatment is not necessarily set by the initial lignin content. This knowledge may inform strategies for plant breeding or genetic engineering that may improve cell-wall deconstruction, thereby improving bioenergy production.

Li M, Heckwolf M, Crowe JD, Williams DL, Magee TD, Kaeppler SM, de Leon N, Hodge DB (2015) Cell-wall properties contributing to improved deconstruction by alkaline pre-treatment and enzymatic hydrolysis in diverse maize (*Zea mays* L.) lines. *Journal of Experimental Botany* **66**: 4305-4315.
<https://academic.oup.com/jxb/article-lookup/doi/10.1093/jxb/erv016>
- CGR2 and CGR3 are involved in pectin methylesterification.** To gain a better understanding of pectin biosynthesis and methylesterification and their effects on cell wall features critical for plant growth and development, we conducted various genetic and biochemical analyses of *Arabidopsis* CGR2 and CGR3. Our results indicate that CGR2 and CGR3 share high sequence similarity, are localized to the Golgi, and are type II membrane proteins with the bulk of the protein, including the methyltransferase domain, exposed to the Golgi lumen where pectin methylesterification occurs; further, *cgr2-1 cgr3-1* double mutants exhibit dwarfism and apparent reduced pectin methylesterification, while overexpression lines demonstrate the opposite phenotypes, suggesting overlapping roles in plant growth and development for the CGR2 and CGR3 proteins. This knowledge may inform the design of new bioenergy crops with altered cell wall properties.

Kim S-J, Held MA, Zemelis S, Wilkerson C, Brandizzi F (2015) CGR2 and CGR3 have critical overlapping roles in pectin methylesterification and plant growth in *Arabidopsis thaliana*. *The Plant Journal* **82**: 208-220.
<http://onlinelibrary.wiley.com/doi/10.1111/tpj.12802/abstract>
- Tricin: the first phenolic from outside the lignin biosynthetic pathway to be discovered in the lignin polymer.** After triclin had been found in lignin preparations from wheat and all monocots examined, we sought to provide proof that triclin is involved in lignification and to establish the mechanism by which it incorporates into the lignin polymer. We demonstrated that triclin is able to couple with monolignols and participate in lignification, and regularly does so in monocots where triclin is found covalently bound within the lignin polymer itself; further, triclin acts as an initiator, as it is only found at the beginning of the polymer chain. Incorporation of triclin into monocot lignins provides a new, extractable source of this valuable flavonoid, recognized for its antioxidant, antiaging, anticancer, and cardioprotective properties.

Lan W, Lu F, Regner M, Zhu Y, Rencoret J, Ralph SA, Zakai UI, Morreel K, Boerjan W, Ralph J (2015) Tricin, a flavonoid monomer in monocot lignification. *Plant Physiology* **167**: 1284-1295.
<http://www.plantphysiol.org/content/167/4/1284>
- Exome capture sequencing of switchgrass.** Switchgrass is present primarily in two ecotypes: a northern upland ecotype, and a southern lowland ecotype. We employed high-coverage exome capture sequencing to genotype 537 individuals from 45 upland and 21 lowland ecotypes. In total, 45,719 genes were affected by nucleotide variants across the panel, providing a firm foundation to identify functional variation associated with phenotypic traits of interest for biofuel feedstock production.

Evans J, Crisovan E, Barry K, Daum C, Jenkins J, Kunde-Ramamoorthy G, Nandety A, Ngan CY, Vaillancourt B, Wei C-L, Schmutz J, Kaeppler SM, Casler MD, Buell CR (2015) Diversity and population structure of northern switchgrass as revealed through exome capture sequencing. *The Plant Journal* **84**: 800-815.

<http://onlinelibrary.wiley.com/doi/10.1111/tpj.13041/full>

- **Improving alkaline hydrogen peroxide pretreatment of woody biomass via transition metals.** Having previously demonstrated that the Cu-catalyzed AHP pretreatment of hybrid poplar resulted in substantial improvement of sugar yields after enzymatic hydrolysis, we correlated biomass properties to pretreatment efficacy by comparing enzymatic hydrolysis yields after AHP pretreatment of four different hardwoods and correlated these results with various cell wall properties, finding that lignin removal was correlated with an increase in enzymatic hydrolysis yields. We also demonstrated the role of cell wall-associated redox-active transition metals in impacting the effectiveness of AHP pretreatment, finding that transition metals can play a positive role in oxidative cell wall deconstruction.

Bansal N, Bhalla A, Pattathil S, Adelman SL, Hahn MG, Hodge DB, Hegg EL (2015) Cell wall-associated transition metals improve alkaline-oxidative pretreatment in diverse hardwoods. *Green Chemistry* **18**: 1405-1415.

<http://pubs.rsc.org/en/content/articlelanding/2016/gc/c5gc01748c#!divAbstract>

- **Active site and laminarin hydrolase in glycosidase hydrolase family 55 (GH55).** In collaboration with JGI, we used a combination of gene synthesis, cell-free protein translation, catalytic assays, and X-ray crystallography to provide a correlated biochemical and structural characterization of the GH55 family. The high resolution crystal structures with substrates bound and ensemble refinement suggest a simple new mechanism for promoting processive reactivity, which is a new way to annotate bioenergy phylogenetic space.

Bianchetti CM, Takasuka TE, Deutsch S, Udell HS, Yik EJ, Bergeman LF, Fox BG (2015) Active site and laminarin binding in glycoside hydrolase family 55. *Journal of Biological Chemistry* **290**: 11819-11832.

<http://www.jbc.org/content/290/19/11819.long>

- **Use of Nanostructure-initiator Mass Spectrometry (NIMS) to deduce selectivity of reaction in glycoside hydrolases.** In collaboration with JBEI, we used chemically synthesized NIMS to study the reactivity of several enzymes representative of the glycoside hydrolase (GH) function. We modeled time-dependent reactions of these enzymes in order to provide a quantitative basis to make functional distinctions among reactive properties, thus offering a new approach to enhance the annotation of GH phylogenetic trees with functional measurements.

Deng K, Takasuka TE, Heins R, Cheng X, Bergeman LF, Shi J, Aschenbrener R, Deutsch S, Singh S, Sale KL, Simmons BA, Adams PD, Singh AK, Fox BG, Northen TR (2014) Rapid kinetic characterization of glycosyl hydrolases based on oxime derivatization and nanostructure-initiator mass spectrometry (NIMS). *ACS Chemical Biology* **9**: 1470-1479.

<http://pubs.acs.org/doi/abs/10.1021/cb5000289>

Deng K, Guenther JM, Gao J, Bowen BP, Tran H, Reyes-Ortiz V, Cheng X, Sathitsuksanoh N, Heins R, Takasuka TE, Bergeman LF, Geertz-Hansen H, Deutsch S, Loque D, Sale K, Simmons B, Adam PD, Singh AK, Fox BG, Northen TR (2015) Development of a high throughput platform for screening glycoside hydrolases based on oxime-NIMS. *Frontiers in Bioengineering and Biotechnology* **3**: 153.

<http://journal.frontiersin.org/article/10.3389/fbioe.2015.00153/full>

Deng K, Takasuka TE, Bianchetti CM, Bergeman LF, Adam PD, Northen TR, Fox BG (2015) Use of nanostructure initiator mass spectrometry to deduce selectivity of reaction in glycoside hydrolases. *Frontiers in Bioengineering and Biotechnology* **3**: 165

- <http://journal.frontiersin.org/article/10.3389/fbioe.2015.00165/abstract>
- **Effects of lignin on cellulase during lignocellulosic biomass saccharification.** We used a fast protein liquid chromatography (FPLC)-based methodology to quantify free *Trichoderma reesei* cellulases (namely CBH I, CBH II, and EG I) concentration within a complex hydrolyzate mixture during the varying time course of biomass saccharification resulting from corn stover samples pretreated via AFEX, dilute acid, and ionic liquids. We showed that family 1 CBMs are highly implicated in the binding of full-length *T. reesei* cellulases to lignin, which aids in further understanding the complex mechanisms of non-productive binding of cellulases to pretreated lignocellulosic biomass.
Gao D, Haarmeyer C, Balan V, Whitehead TA, Dale BE, Chundawat SPS (2014) Lignin triggers irreversible cellulase loss during pretreated lignocellulosic biomass saccharification. *Biotechnology for Biofuels* **7**: 175.
<http://www.biotechnologyforbiofuels.com/content/7/1/175>
 - **Using a designer synthetic media to study inhibitors effect in biomass conversion.** We characterized the plant-derived decomposition products present in AFEX-pretreated corn stover hydrolysate (ACH), and a synthetic hydrolysate (SH) was formulated based on that ACH composition and further used to evaluate the inhibitory effects of various families of decomposition products during fermentation using a strain of *S. cerevisiae*. The SH did not entirely match the ACH performance, but the major groups of inhibitory compounds were identified and used for further evaluation, finding that amides are significantly less inhibitory to both glucose and xylose fermentation, suggesting ACH would be easily fermentable by yeast without any further detoxification.
Tang X, da Costa Sousa L, Jin M, Chundawat SPS, Chambliss CK, Lau MW, Xiao Z, Dale BE, Balan V (2014) Designer synthetic media for studying microbial-catalyzed biofuel production. *Biotechnology for Biofuels* **8**: 1.
<http://www.biotechnologyforbiofuels.com/content/8/1/1/abstract>
 - **Design of biofuel supply chains with regional depots.** We developed optimization models for the design and operational planning of biofuel supply chains including regional depots. These models can be modified to yield solutions where biomass from a harvesting site is shipped to different depots depending on the amount of feedstock available and the needs of the biorefinery, which could lead to a better “steady-state” configuration more aligned with the reality of a biofuel supply chain operation. The proposed models can aid decision-makers studying the trade-offs in biofuel supply chains.
Ng RTL, Maravelias CT (2016) Design of cellulosic ethanol supply chains with regional depots. *Industrial and Engineering Chemistry Research* **55**: 3420-3432.
<http://pubs.acs.org.ezproxy.library.wisc.edu/doi/abs/10.1021%2Facs.iecr.5b03677>
Ng RTL, Maravelias CT (2017) Design of biofuel supply chains with variable regional depot and biorefinery locations. *Renewable Energy* **100**: 90-102.
<http://www.sciencedirect.com/science/article/pii/S0960148116304190>
Kim S, Dale BE (2016) A distributed cellulosic biorefinery system in the US Midwest based on corn stover. *Biofuels, Bioproducts and Biorefining* **10**: 819-832.
<http://onlinelibrary.wiley.com/doi/10.1002/bbb.1712/abstract>
 - **Microbes metabolize aromatic compounds in lignocellulosic hydrolysate, producing easily fermentable sugars.** Many aromatic compounds are toxic to biofuel microbes whereas *R. palustris* is able to metabolically remove these compounds from hydrolysate via a specific pathway. Moreover, the use of mutant strains with blocks in specific steps of the pathway can allow for enrichment of aromatic intermediates that may be recoverable as bioproducts.
Austin, S., Kontur, W. S., Ulbrich, A., Oshlag, J. Z., Zhang, W., Higbee, A., Zhang, Y., Coon, J. J., Hodge, D. B., Donohue, T. J., and Noguera, D. R. (2015) Metabolism of multiple

aromatic compounds in corn stover hydrolysate by *Rhodopseudomonas palustris*. *Environmental Science & Technology* **49**, 8914-8922.

<http://pubs.acs.org/doi/abs/10.1021/acs.est.5b02062>

- **Integrative approach to identify genome-scale transcriptional regulatory networks.** This study combines comparative genomics, motif analysis, and gene expression-based methods to improve the predictive power and information content of transcriptional regulatory networks, informing synthetic biology approaches to design of biofuel microbes. **Imam, S., Noguera, D. R., and Donohue, T. J.** (2015) An integrated approach to reconstructing genome-scale transcriptional regulatory networks. *PLOS Computational Biology* **11**, e1004103.
<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004103>
- **Production of GVL-derived lignin expands deconstruction platform to three biomass fractions.** Lignin was isolated using the GVL method and shown to retain a native-like structure. After hydrogenolysis over a catalyst a significant portion of lignin carbon was converted to monomers. These findings show that the GVL platform could allow easy and efficient upgrading of all three biomass fractions: C5/C6 sugars and lignin. **Luterbacher, J. S., Azarpira, A., Motagamwala, A. H., Lu, F., Ralph, J., and Dumesic, J. A.** (2015) Lignin monomer production integrated into the γ -valerolactone sugar platform. *Energy & Environmental Science* **8**, 2657-2663.
<http://pubs.rsc.org/en/content/articlelanding/2015/ee/c5ee01322d> - !divAbstract
- **Biofuels research uncovers a natural bioproduct with fungicidal properties.** Plant-derived fermentation inhibitors are a challenge to efficient biofuel production yet they may also have added value as bioproducts. In this study a combination of yeast chemical genomics and microscopic profiling was used to identify the cellular target of poacic acid, a compound present in lignocellulosic hydrolysates of grasses. Poacic acid was shown to target the glucan layer in fungal cell walls and protects soybean leaves from the broad-range fungal pathogen *S. sclerotiorum*. This study demonstrates that biofuel research can sometimes reveal potentially useful bioproducts of value, enhancing the economics of biorefineries. **Piotrowski, J. S., Okada, H., Lu, F., Li, S. C., Hinchman, L., Ranjan, A., Smith, D. L., Higbee, A. J., Ulbrich, A., Coon, J. J., Deshpande, R., Bukhman, Y. V., McIlwain, S., Ong, I. M., Myers, C. L., Boone, C., Landick, R., Ralph, J., Kabbage, M., and Ohya, Y.** (2015) Plant-derived antifungal agent poacic acid targets β -1,3-glucan. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E1490-E1497.
<http://www.pnas.org/content/112/12/E1490.abstract?sid=45276789-834f-4a5c-947a-62eea82daf15>
- **Phenolic amides inhibit *de novo* nucleotide biosynthesis.** This study utilized metabolomics, isotopic tracer methods, and biochemical assays to identify the cellular targets of amide inhibition in the model bacterium *E. coli*. Results indicate that phenolic amides directly inhibit a key enzyme involved in nucleotide biosynthesis. Knowledge of the mechanistic effects of hydrolysate inhibitors will inform efforts to engineer new biocatalysts with improved bioconversion efficiencies. **Pisithkul, T., Jacobson, T. B., O'Brien, T. J., Stevenson, D. M., and Amador-Noguez, D.** (2015) Phenolic amides are potent inhibitors of *de novo* nucleotide biosynthesis. *Applied and Environmental Microbiology* **81**, 5761-5772.
<http://aem.asm.org/content/early/2015/06/08/AEM.01324-15.abstract>
- **Influence of cropping systems on soil microbial communities.** We characterized and compared soil microbial communities under restored prairie and three potential cellulosic biomass crops (corn, switchgrass, and mixed prairie grasses) in two spatial experimental designs – side-by-side plots where plant communities were in their second year since

establishment and regionally distributed fields where plant communities had been in place for at least 10 years. We conclude that location, a proxy primarily for soil type but also including site history, landscape, and climate, was the major factor determining microbial communities in our 2-year-old intensive sites and that these study sites were not under cultivation long enough for the crop to impose a strong signature on the microbial communities; in contrast, when the same crop had been grown on a site for 10 years or longer, a crop effect was observed, with communities under corn clearly differentiated from those under perennial grasses. Both presence of perennial plants and higher plant diversity likely favored the accumulation of microbial biomass and fungi, especially arbuscular mycorrhizal fungi (AMF), under switchgrass, mixed grasses, and prairie, leading to a more stable environment and highlighting that these alternatives to corn for biofuels may improve soil functional stability and sustainability.

da C. Jesus E, Liang C, Quensen JF, Susilawati E, Jackson RD, Balser TC, Tiedje JM (2016) Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. *Global Change Biology – Bioenergy* **8**: 481-494.

<http://onlinelibrary.wiley.com/doi/10.1111/gcbb.12289/abstract>

- **Comparative water use by maize, perennial crops, restored prairie, and poplar trees in the US Midwest.** We measured growing-season evapotranspiration (ET) based on daily changes in soil profile water contents in five perennial systems—switchgrass, miscanthus, native grasses, restored prairie, and hybrid poplar—and in annual maize (corn) in a temperate humid climate (Michigan, USA). Our result show that measured water use by perennial systems was similar to maize across normal and drought years, contrasting with earlier modeling studies and suggesting that rain-fed perennial biomass crops in this climate have little impact on landscape water balances, whether replacing rain-fed maize on arable lands or successional vegetation on marginal lands. Results also suggest that crop ET rates, and thus groundwater recharge, streamflow, and lake levels, may be less sensitive to climate change than has been assumed.

Hamilton SK, Hussain MZ, Bhardwaj AK, Basso B, Robertson GP (2015) Comparative water use by maize, perennial crops, restored prairie, and poplar trees in the US Midwest. *Environmental Research Letters* **10**: 064015.

<http://iopscience.iop.org/article/10.1088/1748-9326/10/6/064015/meta;jsessionid=CB6BCD3D5CE3285C1AB068B6E9B18EB5.c1>

- **Potential of electrified vehicles to contribute to petroleum and climate goals and implications for biofuels.** We are interested in the extent to which electrified vehicles could reduce petroleum consumption and greenhouse gas (GHG) emissions and the sensitivity of these impacts across a range of travel demand and technology scenarios. We find that petroleum consumption and GHG emissions are highly sensitive to these inputs, with resulting petroleum consumption and GHG emissions varying widely. The implication for biofuels is important, to the extent they are aimed at meeting climate and petroleum use reduction goals; and to put these implications into perspective, we further estimate the volume of Renewable Fuel Standard (RFS)-compliant advanced biofuel (e.g., cellulosic biofuel) needed to meet petroleum and climate goals.

Meier PJ, Cronin KR, Frost EA, Runge TM, Dale BE, Reinemann DJ, Detlor J (2015) Potential for electrified vehicles to contribute to U.S. petroleum and climate goals and implications for advanced biofuels. *Environmental Science & Technology* **49**: 8277-8286.

<http://pubs.acs.org/doi/abs/10.1021/acs.est.5b01691>

- **Nitrous oxide emissions during establishment phase of various bioenergy cropping systems.** We measured soil N₂O emissions and potential environmental drivers of these fluxes from a three-year establishment-phase bioenergy cropping systems experiment

replicated in southcentral Wisconsin and southwestern Michigan. Our results indicate that perennial bioenergy crops in their establishment phase emit less N₂O than annual crops, especially when not fertilized. These findings should be considered further alongside yield and other metrics contributing to important ecosystem services.

Oates LG, Duncan DS, Gelfand I, Millar N, Robertson GP, Jackson RD (2016) Nitrous oxide emissions during establishment of eight alternative cellulosic bioenergy cropping systems in the North Central United States. *Global Change Biology – Bioenergy* **8**: 539-549. <http://onlinelibrary.wiley.com/doi/10.1111/gcbb.12268/abstract>

- **Comparing productivity of alternative cellulosic bioenergy cropping systems.** Here, we report on the six-year production potential (above ground net primary production, ANPP), post-frost harvested biomass (yield), and gross harvest efficiency (GHE = yield/ANPP) of seven model bioenergy cropping systems in both southcentral Wisconsin (ARL) and southwest Michigan (KBS). Overall the most productive cropping systems were corn > giant miscanthus > and switchgrass, which were significantly more productive than native grasses ≈ restored prairie ≈ early successional ≈ and hybrid poplar, although some systems (e.g. hybrid poplar) differed significantly by location. Results show that well-established, dedicated bioenergy crops are capable of producing as much biomass as corn stover, but with fewer inputs.

Sanford GR, Oates LG, Jasrotia P, Thelen KD, Robertson GP, Jackson RD (2016) Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. *Agriculture, Ecosystems & Environment* **216**: 344-355. <http://www.sciencedirect.com/science/article/pii/S0167880915301250>

Year 9 (Dec. 1, 2015 - Nov. 30, 2016)

- **Accounting for linkage disequilibrium improves the accuracy of genomic prediction in switchgrass.** In this study we empirically evaluate accuracies of different genomic prediction methods to assess the usefulness of genomic selection in switchgrass. Results suggest that marker-data transformations and, more generally, the account of linkage disequilibrium among markers, offer valuable opportunities for improving prediction procedures in genomic selection. High prediction accuracies, particularly with given traits at given locations, should motivate the implementation of genomic selection breeding programs in switchgrass that will help to accelerate the progress needed to reach biomass yield goals.
Ramstein GP, Evans J, Kaeppler SM, Mitchell RB, Vogel KP, Buell CR, Casler MD (2016) Accuracy of genomic prediction in switchgrass (*Panicum virgatum* L.) improved by accounting for linkage disequilibrium. *G3: Genes | Genomes | Genetics* **6**: 1049-1062. <http://www.g3journal.org/content/6/4/1049.long>
- **Genome-wide associations with flowering time in switchgrass using exome-capture sequencing data.** This study used exome capture sequencing to perform genome-wide association studies using flowering time data from a switchgrass association panel. Principal component analysis showed that the greater than 500 switchgrass genotypes in the study represent five genetically distinct regional gene pools. Candidate genes involved in flowering time include known flowering regulators and also genes not previously associated with the control of flowering time; furthermore, the relationship of flowering time and geographic origin indicates likely roles for genes in both photoperiod and autonomous pathways in generating switchgrass flowering time variation.
Grabowski PP, Evans J, Daum C, Deshpande S, Barry KW, Kennedy M, Ramstein G, Kaeppler SM, Buell CR, Jiang Y, Casler MD (2017) Genome-wide associations with

flowering time in switchgrass using exome-capture sequencing data. *New Phytologist* **213**: 154-169.

<http://onlinelibrary.wiley.com/doi/10.1111/nph.14101/abstract>

- **Advances in understanding flowering time in grasses.** In our continuing studies of flowering time in the model grass *Brachypodium*, we have published two new papers: 1) we demonstrate that VRN2 is a repressor of flowering that functions broadly in grasses from rice to *Brachypodium*, and 2) we identified six quantitative trait loci (QTL) that control differences in flowering time. Furthering the molecular-level understanding of the flowering network in the model grass *Brachypodium* improves the potential to manipulate flowering time in bioenergy grass crops to increase biomass yield.

Woods DP, McKeown MA, Dong Y, Preston JC, Amasino RM (2016) Evolution of *VRN2/Ghd7*-like genes in vernalization-mediated repression of grass flowering. *Plant Physiology* **170**: 2124-2135.

<http://www.plantphysiol.org/content/170/4/2124>

Woods DP, Bednarek R, Bouché F, Gordon SP, Vogel JP, Garvin DF, Amasino RM (2017) Genetic architecture of flowering-time variation in *Brachypodium distachyon*. *Plant Physiology* **173**: 269-279.

<http://www.plantphysiol.org/content/173/1/269>

- **Native zips.** In this study we surveyed a set of plants representing the spermatophytes or “seed plants” for the presence of monolignol (ML) ferulate conjugate ester linkages (“zips”) within the lignin polymers of these plants. All gymnosperms tested showed no evidence of zip-lignin, whereas low zip-levels were present in the isolated lignins of many, but not all, angiosperms; further, FMT was shown to be specific for the production of zip-lignin; a new grass-specific FMT was identified, revealing convergent evolution as the route to produce two distinct types of FMT. Identification of plant lines with increased zip-lignin content, likely able to be more easily and economically deconstructed, could provide superior sources of biomass for conversion to biofuels and bioproducts.

Karlen SD, Zhang C, Peck ML, Smith RA, Padmakshan D, Helmich KE, Free HCA, Lee S, Smith BG, Lu F, Sedbrook JC, Sibout R, Grabber JH, Runge TM, Mysore KS, Harris PJ, Bartley LE, Ralph J (2016) Monolignol-ferulate conjugates are naturally incorporated into plant lignins. *Science Advances* **2**: e1600393.

<http://advances.sciencemag.org/content/2/10/e1600393>

- **Formaldehyde stabilization increases lignin monomer yield.** Extracting a soluble and uncondensed lignin substrate during biomass pretreatment could facilitate the production of lignin monomers and be compatible with current biorefining strategies. In this study we used formaldehyde to stabilize lignin during extraction, leading to near theoretical yields of lignin monomers after hydrogenolysis of the extracted product; yields were three to seven times higher than those obtained when using the analogous method without formaldehyde. Our formaldehyde stabilization results suggest that lignin upgrading could be easily integrated into current biorefinery schemes, especially considering that formaldehyde is a relatively inexpensive bulk chemical that can be produced from biomass-derived syngas or methanol, either sourced biologically or from lignin methoxyl groups.

Shuai L, Amiri MT, Questell-Santiago YM, Héroguel F, Li Y, Kim H, Meilan R, Chapple C, Ralph J, Luterbacher JS (2016) Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science* **354**: 329-333.

<http://science.sciencemag.org/content/354/6310/329>

- **Cooperative electrocatalytic alcohol oxidation with electron-proton transfer mediators.** We examined the electrochemical oxidation of alcohols and identified a cooperative catalyst system with 2 components, an organic nitroxyl TEMPO and Cu(bpy). The co-catalyst system represents a unique class of electrocatalysts for alcohol oxidation,

which can offer much faster electrocatalytic rates and lower overpotentials, both of which are crucial in energy-conversion applications.

Badalyan A, Stahl SS (2016) Cooperative electrocatalytic alcohol oxidation with electron-proton-transfer mediators. *Nature* **535**: 406-410.

<http://www.nature.com/nature/journal/v535/n7612/full/nature18008.html>

- **Evolution of cellulolytic activity in *Streptomyces*.** We compared the phylogenetic diversity of over 1,100 strains of *Streptomyces*, and were able to identify that while plant biomass degrading enzymes (CAZy) are widespread in the genus, key enzyme families were obtained by horizontal gene transfer, and were retained in the most highly cellulolytic strains. This research gave insight into how specialized interactions between microbes and insects has given rise to increased microbial capability to deconstruct plant biomass, and identified the key genes, regulatory circuits, and enzymes used in this process.
Book AJ, Lewin GR, McDonald BR, Takasuka TE, Wendt-Pienkowski E, Doering DT, Suh S, Raffa KF, Fox BG, Currie CR (2016) Evolution of high cellulolytic activity in symbiotic *Streptomyces* through selection of expanded gene content and coordinated gene expression. *PLOS Biology* **14**: e1002475.
<http://dx.doi.org/10.1371/journal.pbio.1002475>
- **Saccharification of thermochemically pretreated cellulosic biomass using native and engineered cellosomal enzymes.** We compared the hydrolytic activity of the most abundant cellosomal enzymes from *C. thermocellum* and investigated the importance of enzyme complexation using a model engineered protein scaffold – rosettasome. Our results indicate that the type of pretreatment can significantly impact the saccharification efficiency of cellosomal enzymes for various consolidated bioprocessing (CBP) scenarios: pretreatments that remove both lignin and hemicellulose can help improve the specific activity of cellosomal enzymes.
Chundawat SPS, Paavola CD, Raman B, Nouailler M, Chan SL, Mielenz JR, Receveur-Brechot V, Trent JD, Dale BE (2016) Saccharification of thermochemically pretreated cellulosic biomass using native and engineered cellosomal enzyme systems. *Reaction Chemistry & Engineering* **1**: 616-628.
<http://dx.doi.org/10.1039/C6RE00172F>
- **Next generation ammonia pretreatment enhances cellulosic biofuel production and separate lignin streams.** We developed a new liquid ammonia pretreatment methodology called Extractive Ammonia (EA) to simultaneously convert native crystalline cellulose I to a highly digestible cellulose III allomorph and selectively extract up to 45% of the lignin from lignocellulosic biomass with near-quantitative retention of all polysaccharides. EA pretreated corn stover yielded a higher fermentable sugar yield compared to AFEX while using 60% lower enzyme loading. It preserves extracted lignin functionalities, offering the potential to co-produce lignin-derived fuel and chemicals in the biorefinery.
da Costa Sousa L, Jin M, Chundawat SPS, Bokade V, Tang X, Azarpira A, Lu F, Avci U, Humpala J, Uppugundla N, Gunawan C, Pattathil S, Cheh AM, Kothari N, Kumar R, Ralph J, Hahn MG, Wyman CE, Seema S, Simmons BA, Dale BE, Balan V (2016) Next-generation ammonia pretreatment enhances cellulosic biofuel production. *Energy & Environmental Science* **9**: 1215-1223.
<http://pubs.rsc.org/en/content/articlelanding/2016/ee/c5ee03051j-!divAbstract>
da Costa Sousa L, Foston M, Bokade V, Azarpira A, Lu F, Ragauskas AJ, Ralph J, Dale B, Balan V (2016) Isolation and characterization of new lignin streams derived from extractive-ammonia (EA) pretreatment. *Green Chemistry* **18**: 4205-4215.
<http://pubs.rsc.org/en/content/articlelanding/2016/gc/c6gc00298f-!divAbstract>
- **Nanoscale structure of biomass.** Using *in situ* imaging at nanoscale resolution and real-time changes during biomass deconstruction process, we were able to summarize the

general structure of the plant cell wall in different plant tissues and plant species, as well as the physicochemical properties of biomass that affect the efficiency of thermochemical pretreatment and enzymatic hydrolysis.

Ding S-Y (2016) Nanoscale structure of biomass. In Marcel Schlaf, Z. Conrad Zhang, eds, Reaction Pathways and Mechanisms in Thermocatalytic Biomass Conversion I, Green Chemistry and Sustainable Technology. Springer Singapore, Singapore, pp 1-14.

http://link.springer.com/chapter/10.1007%2F978-981-287-688-1_1

- **Structural and biochemical characterization of enzymes in the lignin β -aryl ether cleavage pathway from *Sphingobium* sp SYK-6.** Collaborative studies by GLBRC, JBEI, and others structurally and biochemically characterized enzymes in the lignin β -aryl ether cleavage pathway from *Sphingobium* sp SYK-6. With lignin as the most abundant aromatic polymer in nature, these results inform broader lignin valorization efforts that will ultimately enable the development of efficient pathways for the conversion of lignin into renewable aromatics with applications in advanced biofuels and chemicals.

Helmich KE, Pereira JH, Gall DL, Heins RA, McAndrew RP, Bingman C, Deng K, Holland KC, Noguera DR, Simmons BA, Sale KL, Ralph J, Donohue TJ, Adams PD, Phillips GN, Jr. (2016) Structural basis of stereospecificity in the bacterial enzymatic cleavage of β -aryl ether bonds in lignin. *Journal of Biological Chemistry* **291**: 5234-5246.

<http://www.jbc.org/content/291/10/5234.full>

Pereira JH, Heins RA, Gall DL, McAndrew RP, Deng K, Holland KC, Donohue TJ, Noguera DR, Simmons BA, Sale KL, Ralph J, Adams PD (2016) Structural and biochemical characterization of the early and late enzymes in the lignin β -aryl ether cleavage pathway from *Sphingobium* sp SYK-6. *The Journal of Biological Chemistry* **291**: 10228-10238

<http://www.jbc.org/content/291/19/10228>

- **Development of OptSSeq, a synthetic biology tool to identify optimal gene expression elements for ethanol production.** Designing synthetic biofuel pathways with optimized and balanced enzyme levels can be challenging. Using OptSSeq we identified optimal gene expression elements for heterologous ethanol production from a combinatorial library of gene expression signals. OptSSeq is a tool that can be used to tune pathway enzyme levels whose function can be linked to cell growth or survival.

Ghosh, I. N., and Landick, R. (2016) OptSSeq: high-throughput sequencing readout of growth enrichment defines optimal gene expression elements for homoethanogenesis. *ACS synthetic biology* **5**, 1519-1534.

<http://pubs.acs.org/doi/abs/10.1021/acssynbio.6b00121>

- **Identification of the cellular target of ionic liquid toxicity and engineering of a yeast tolerant strain.** Chemical genomics was used to identify yeast gene deletions that confer sensitivity or resistance to imidazolium-based ionic liquids (IIL). Yeast were subsequently successfully engineered for improved IIL tolerance while demonstrating increased sugar to biofuel conversion relative to non-engineered strains. This paper demonstrates the utility of chemical genomics-guided biodesign for development of biocatalysts.

Dickinson, Q., Bottoms, S., Hinchman, L., McIlwain, S., Li, S., Myers, C. L., Boone, C., Coon, J. J., Hebert, A., Sato, T. K., Landick, R., and Piotrowski, J. S. (2016) Mechanism of imidazolium ionic liquids toxicity in *Saccharomyces cerevisiae* and rational engineering of a tolerant, xylose-fermenting strain. *Microbial Cell Factories* **15**, 17.

<https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-016-0417-7>

- **Drought-stressed switchgrass produces compounds inhibitory to microbes.** This study examined how interannual variability in precipitation impacts feedstock deconstruction and biofuel production by microbial catalysts. The results indicate that environmental variation can have significant effects on biomass properties, which can then impact

downstream processes. Understanding how feedstock variability impacts the bioconversion pipeline may lead to the development of predictive models based on known inputs.

Ong, R. G., Higbee, A., Bottoms, S., Dickinson, Q., Xie, D., Smith, S. A., Serate, J., Pohlmann, E., Jones, A. D., Coon, J. J., Sato, T. K., Sanford, G. R., Eilert, D., Oates, L. G., Piotrowski, J. S., Bates, D. M., Cavalier, D., and Zhang, Y. (2016) Inhibition of microbial biofuel production in drought-stressed switchgrass hydrolysate. *Biotechnology for Biofuels* **9**, 237.

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-016-0657-0>

- Yeast evolved for enhanced xylose utilization reveal unexpected interactions between cell signaling and Fe-S cluster biogenesis.** Rapid xylose utilization in yeast was found to be dependent upon genetic interactions among four genes, uncovering a surprising genetic interaction that facilitates aerobic and anaerobic utilization of xylose. Understanding how yeast cells adapt to non-preferential carbon sources uncovers potential genetic engineering strategies that can improve efficiency of biofuel production.

Sato, T. K., Tremaine, M., Parreiras, L. S., Herbert, A. S., Myers, K. S., Higbee, A. J., Sardi, M., McIlwain, S. J., Ong, I. M., Breuer, R. J., Narasimhan, R. A., McGee, M. A., Dickinson, Q., La Reau, A., Xie, D., Tian, M., Piotrowski, J. S., Reed, J. L., Zhang, Y., Coon, J. J., Hittinger, C. T., Gasch, A. P., and Landick, R. (2016) Directed evolution reveals unexpected epistatic interactions that alter metabolic regulation and enable anaerobic xylose use by *Saccharomyces cerevisiae*. *PLoS genetics* **12**, e1006372.

<http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1006372>
- Ecosystem water-use efficiency of annual corn and perennial grasslands.** To examine the interacting influence of dominant plant functional groups (C₃ and C₄) and land-use history on water-use efficiencies WUEs of annual corn and perennial (restored prairie, switchgrass and smooth brome grass) grasslands in the US Midwest, we determined ecosystem-level (eWUE) and intrinsic (iWUE) WUEs using eddy covariance and plant carbon isotope ratios, respectively. We conclude that ecosystem water-use efficiency (eWUE) of planted perennial grasslands is determined to a great extent by the relative abundance of C₃ and C₄ plant species within a given season. These results inform models of the interaction between carbon and water cycles in grassland ecosystems under current and future climate and management scenarios.

Abraham M, Gelfand I, Hamilton SK, Shao C, Su Y-J, Robertson GP, Chen J (2016) Ecosystem water-use efficiency of annual corn and perennial grasslands: contributions from land-use history and species composition. *Ecosystems* **19**: 1001-1012.

<https://link.springer.com/article/10.1007/s10021-016-9981-2>
- Nitrogen fertilization effects on productivity and nitrogen loss in perennial bioenergy cropping systems.** In this study, we explore how nitrogen (N) fertilization impacts N loss pathways and plant productivity in three biomass production systems based on grasses and forbs native to North America; we studied a switchgrass monoculture, a mixture of five grasses, and a mixture of 18 prairie species. We observed a cropping systems-level tradeoff between biomass production and loss of environmentally harmful forms of N, with the native grasses and prairie species mixtures having lower N₂O emissions, NO₃⁻ leaching, and aboveground productivity; our findings hold significant implications for the selection and management of perennial bioenergy cropping systems. Our results show, with respect to nitrogen processing, polycultural biomass production systems can be resilient in the face of annual weather fluctuations, which indicates the environmental and management benefits of these systems should not be overlooked.

Duran BEL, Duncan DS, Oates LG, Kucharik CJ, Jackson RD (2016) Nitrogen fertilization effects on productivity and nitrogen loss in three grass-based perennial bioenergy cropping systems. *PLOS ONE* **11**: e0151919.

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0151919>

- **Cover crops and pest suppression in annual maize bioenergy cropping systems.** The overall goal of our two-year, two-state study was to determine the effects of a winter cover crop system designed to enhance the agronomic performance of a bioenergy cropping system on predatory invertebrate communities and biocontrol services. Our specific objectives were 1) to determine if winter cover crops altered the abundance and diversity of predator communities, and 2) to assess the impact of the resulting predator communities on rates of predation in the subsequent crop. Our results show that planting a rye/pea cover crop in continuous maize systems grown for grain and stover did not alter biocontrol services under the conditions we studied; while we found no significant positive or negative impacts of this cover crop system on biocontrol services, bioenergy cover cropping systems could be managed to increase multiple ecosystem services by altering cover crop identity, or timing of planting and harvest.

Fox AF, Kim TN, Bahlai CA, Woltz JM, Gratton C, Landis DA (2016) Cover crops have neutral effects on predator communities and biological control services in annual cellulosic bioenergy cropping systems. *Agriculture, Ecosystems & Environment* **232**: 101-109.

<http://www.sciencedirect.com/science/article/pii/S0167880916303711>

- **Effect of switchgrass rhizospheres on agricultural soils of the upper Midwest.** Rhizosphere microbial communities play an essential role in determining plant productivity, particularly in agriculturally marginal environments. We compared microbial communities in switchgrass rhizospheres and their associated bulk soils in two regions of the U.S. upper Midwest (Michigan and Wisconsin) with contrasting soil types, and at two site types with differing switchgrass establishment ages and management intensities. Overall, our findings suggest switchgrass rhizospheres systematically stimulate microbial growth and microbial residue turnover.

Liang C, da C. Jesus E, Duncan DS, Quensen JF, Jackson RD, Balser TC, Tiedje JM (2016) Switchgrass rhizospheres stimulate microbial biomass but deplete microbial necromass in agricultural soils of the upper Midwest, USA. *Soil Biology and Biochemistry* **94**: 173-180.

<http://www.sciencedirect.com/science/article/pii/S0038071715004137>

- **Importance of minimizing nitrogen fertilizer use in the biofuel industry.** To evaluate possible climate benefits of cellulosic biofuel crops, we measured soil N₂O emissions and nitrate leaching along a switchgrass high resolution N-fertilizer gradient for three years post-establishment. Results revealed an exponential increase in annual N₂O emissions that each year became stronger; concomitantly, switchgrass yields became less responsive each year to N fertilizer. Minimizing N fertilizer use will be an important strategy for fully realizing the climate benefits of cellulosic biofuel production.

Ruan L, Bhardwaj AK, Hamilton SK, Robertson GP (2016) Nitrogen fertilization challenges the climate benefit of cellulosic biofuels. *Environmental Research Letters* **11**: 064007.

<http://iopscience.iop.org/article/10.1088/1748-9326/11/6/064007/meta>

Year 10 (Dec. 1, 2016 - Nov. 1, 2017)

- **Altering carbon allocation in hybrid poplar impacts cell wall growth and development.** In this study we test the hypothesis that cell wall attributes and plant development can be altered by augmenting the available pools of soluble sucrose; to do so, we overexpressed *Arabidopsis* galactinol synthase (*AtGolS3*) in hybrid poplar. These

transgenic lines possessed altered cell wall chemistries and fiber properties, traits characteristic of specialized tension wood (lower total lignin content, higher cellulose content, xylem that often contains small vessels and fibers with a unique inner cell wall layer made primarily of cellulose). This transgenic approach, which yields increased cellulose content, altered hemicellulose composition, and reduced lignin content, is an ideal strategy for poplar and other lignocellulosic-derived feedstocks for improved industrial processing, especially for bioenergy applications.

Unda F, Kim H, Hefer C, Ralph J, Mansfield SD (2017) Altering carbon allocation in hybrid poplar (*Populus alba* × *grandidentata*) impacts cell wall growth and development. *Plant Biotechnology Journal* **15**: 865-878.

<http://onlinelibrary.wiley.com/doi/10.1111/pbi.12682/abstract>

- **Suppression of CCR increases the level of “zips” incorporated into maize lignins.** In this study, we examined the features of a lignin biosynthetic mutant in maize that we hypothesized could result in an increase in the levels of more readily cleavable ester bonds in the lignin backbone (“zip-lignin”). Analysis of a maize *ccr1* insertion mutant, in comparison to wild-type, shows reduced total lignin levels, normal growth and development, increased zip-lignin levels, and increased digestibility as measured by glucose and xylose release. The increased monolignol ferulate incorporation into lignins (*i.e.*, higher zip-lignin levels) in *ccr1* mutant maize plants suggests that downregulation of CCR may be a viable biosynthetic strategy for increasing zip levels in other biofuel crops as well.

Smith RA, Cass CL, Mazaheri M, Sekhon RS, Heckwolf M, Kaeppler H, de Leon N, Mansfield SD, Kaeppler SM, Sedbrook JC, Karlen SD, Ralph J (2017) Suppression of *CINNAMOYL-CoA REDUCTASE* increases the level of monolignol ferulates incorporated into maize lignins. *Biotechnology for Biofuels* **10**: 109.

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-017-0793-1>
- **Establishment of a vernalization requirement in *Brachypodium distachyon* requires REPRESSOR OF VERNALIZATION1.** In order to further the molecular-level understanding of the flowering regulatory network to enable manipulation of flowering time and biomass yield, we screened for *Brachypodium* mutants that flower rapidly without vernalization. We identified a new gene, *REPRESSOR OF VERNALIZATION1* (*RVR1*), required for the repression of the *VRN1* gene prior to cold; thus *RVR1* plays a role in establishing a vernalization requirement in *Brachypodium* and is likely to play the same role in other vernalization-requiring pooid grasses.

Woods DP, Ream TS, Bouché F, Lee J, Thrower N, Wilkerson C, Amasino RM (2017) Establishment of a vernalization requirement in *Brachypodium distachyon* requires *REPRESSOR OF VERNALIZATION1*. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 6623-6628.

<http://www.pnas.org/content/114/25/6623>
- **Characterization of vegetative sorghum stem internode development.** Here we conducted a detailed characterization of the development of vegetative sorghum stem internodes to provide a baseline of information on stem anatomy and gene expression that may be useful for improving biomass yield and composition. Transcriptome profiling indicates that dynamic variation in the levels and action of hormones GA, CK, IAA, BR, ethylene, ABA, and JA modulate gene expression and growth during internode growth and development. This study provides detailed microscopic and transcriptomic data useful for identifying genes and molecular pathways regulating internode elongation in response to various developmental and environmental signals.

Kebrom TH, McKinley B, Mullet JE (2017) Dynamics of gene expression during development and expansion of vegetative stem internodes of bioenergy sorghum. *Biotechnology for Biofuels* **10**: 159.

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-017-0848-3>

- **Identification of new lignin monomers in palm lignins.** Recent studies indicate that lignification is a flexible mechanism and that plants are capable of using a variety of phenolic compounds for the formation of lignin polymers. In two studies this year, we report the occurrence of new classes of polyphenolic compounds in the lignins of palm species. The incorporation of nonconventional monomers, not usually present in the lignins of other plants, can open up new ways to design and engineer the lignin structure to produce polymers and plant-based biomaterials with altered properties.

del Río JC, Rencoret J, Gutiérrez A, Kim H, Ralph J (2017) Hydroxystilbenes are monomers in palm fruit endocarp lignins. *Plant Physiology* **174**: 2072-2082.

<http://www.plantphysiol.org/content/early/2017/06/06/pp.17.00362>

Karlen SD, Smith RA, Kim H, Padmakshan D, Bartuce A, Mobley JK, Free HCA, Smith BG, Harris PJ, Ralph J (2017) Highly decorated lignins in leaf tissues of the Canary Island date palm *Phoenix canariensis*. *Plant Physiology* [DOI: 10/1104/pp.17.011172].

<http://www.plantphysiol.org/content/early/2017/09/11/pp.17.01172>
- **Transcriptional analysis of flowering time in switchgrass.** Our objective was to identify flowering time gene candidates that could be employed to manipulate flowering time in upland, cold-tolerant switchgrass cultivars, in order to increase total biomass yield for these northern accessions. Orthologs of known flowering time genes had either ecotype- or flowering time-specific patterns, allowing for the possible separation of flowering time traits from other traits used in breeding or transgenic manipulation efforts. The candidate genes presented here may be used to guide switchgrass improvement through marker-assisted breeding and/or transgenic or gene editing approaches.

Tornqvist C-E, Vaillancourt B, Kim J, Buell CR, Kaeppler SM, Casler MD (2017) Transcriptional analysis of flowering time in switchgrass. *BioEnergy Research* **10**: 700-713.

<https://link.springer.com/article/10.1007/s12155-017-9832-9>
- **Development of rapid bioconversion with integrated recycle technology for ethanol production using Extractive Ammonia.** As was done previously with AFEX, we applied the rapid bioconversion with integrated recycle technology (RaBIT) process on the Extractive Ammonia (EA) pretreatment for conversion of corn stover (CS) to ethanol at high solids loading, recycling enzymes via recycling of the unhydrolyzed solids, and shortening the enzymatic hydrolysis and fermentation times to 24 h. We found that ethanol productivity was enhanced 2-fold and the enzyme loading was reduced by 30%, suggesting that the integration of RaBIT with EA enables a very substantial decrease in enzyme loading.

Jin M, Liu Y, da Costa Sousa L, Dale BE, Balan V (2017) Development of rapid bioconversion with integrated recycle technology for ethanol production from extractive ammonia pretreated corn stover. *Biotechnology and Bioengineering* **114**: 1713-1720.

<http://onlinelibrary.wiley.com/doi/10.1002/bit.26302/full>
- **Lignocellulosic pretreatment in a fungus-cultivating termite.** In our study to characterize the natural lignocellulosic degradation system in fungus-cultivating termites, we demonstrate that lignin depolymerization takes place during the rapid passage through the pH-neutral gut of young termite workers in a process that is striking in its speed and efficiency at destroying the traditionally-considered most recalcitrant C-C bonded lignin structural units. This process releases the plant polysaccharide component, thereby facilitating degradation of the substrate by processes subsequently occurring via the fungus-comb microbiome. Thus, natural systems for lignin degradation/pretreatment are

far beyond what was previously recognized and are potential sources of novel ligninolytic agents, enabling more efficient plant cell wall utilization.

Li H, Yelle DJ, Li C, Yang M, Ke J, Zhang R, Liu Y, Zhu N, Liang S, Mo X, Ralph J, Currie CR, Mo J (2017) Lignocellulosic pretreatment in a fungus-cultivating termite. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 4709-4714.

<http://www.pnas.org/content/114/18/4709.full>

- **Fine-tuned identification of glycoside hydrolase specificity on plant cell walls.** We used glycome profiling and NIMS to characterize cell-wall enzymatic hydrolysis in non-pretreated and AFEX-pretreated corn stover and switchgrass using three *Ruminiclostridium thermocellum* enzymes. The three enzymes fall into three classes of glycosyl hydrolases, and results showed that the GH5 and GH11 enzymes had a broader specificity than G10. In addition to fine-tuned, quantitative analysis of enzyme specificities, these methods can help functionally annotate carbohydrate-active enzyme databases that are organized according to genomic data.

Walker JA, Pattathil S, Bergeman LF, Beebe ET, Deng K, Mirzai M, Northen TR, Hahn MG, Fox BG (2017) Determination of glycoside hydrolase specificities during hydrolysis of plant cell walls using glycome profiling. *Biotechnology for Biofuels* **10**: 31.

<http://dx.doi.org/10.1186/s13068-017-0703-6>

- **Discovery of mutations that increase bacterial lipid production.** Microbial lipids are a potential replacement for petroleum-based fuels but achieving high yields is challenging. High-lipid (HL) production bacterial mutants were isolated and shown to have increased lipid accumulation with alterations to the bacterial cell envelope; two HL mutants were shown to accumulate ~60% of the lipids extracellularly, potentially aided in recovery of product. Knowledge of the mechanisms that govern microbial lipid production can reveal new strategies to increase lipid yield and increase the economic viability of alternatives to fuels or chemicals currently derived from petroleum.

Lemmer, K. C., Zhang, W., Langer, S. J., Dohnalkova, A. C., Hu, D., Lemke, R. A., Piotrowski, J. S., Orr, G., Noguera, D. R., and Donohue, T. J. (2017) Mutations that alter the bacterial cell envelope increase lipid production. *mBio* **8**, e00513-00517.

<http://mbio.asm.org/content/8/3/e00513-17>

- **GVL-based deconstruction strategy triples the fraction of biomass converted to high-value products.** This study sought to determine the yield of high-value products from the primary components of lignocellulosic biomass to assess the economic viability of an integrated biorefinery. Results show that up to 80% of the biomass was obtained as high-value products from the three major components of lignocellulosic biomass (cellulose, hemicellulose, and lignin). Technoeconomic analysis indicates that the technology can be cost-competitive with a current petroleum refinery.

Alonso, D. M., Hakim, S., Zhou, S., Won, W., Hosseinaei, O., Tao, J., Garcia-Negron, V., Motagamwala, A. H., Mellmer, M. A., Huang, K., Houtman, C. J., Labbe, N., Harper, D. P., Maravelias, C., Runge, T., and Dumesic, J. A. (2017) Increasing the revenue from lignocellulosic biomass: maximizing feedstock utilization. *Science Advances* **3**, e1603301.

<http://advances.sciencemag.org/content/3/5/e1603301>

- **Increased ability to identify and predict essential genes from bacterial Tn-seq libraries.** Here we developed a computational tool to identify essential genes under a range of conditions, based on the frequency of random transposon insertions across the genome. Results show that the tool was able to accurately predict the essentiality of many known genes and identified genes not previously considered to be essential. Combining genome-scale experimental and computational methods can provide insight into enzymes and pathways involved in synthesis of biofuels and biochemicals.

Burger, B. T., Imam, S., Scarborough, M. J., Noguera, D. R., and Donohue, T. J. (2017) Combining genome-scale experimental and computational methods to identify essential genes in *Rhodobacter sphaeroides*. *mSystems* **2**, e00015-00017.
<http://msystems.asm.org/content/2/3/e00015-17>

- **GVL plus co-solvent system for biomass deconstruction is economically feasible and cost-competitive.** The GVL method is a promising biomass-agnostic, renewable technology for biomass deconstruction. Technoeconomic analysis of the use of a system containing GVL, water, and organic co-solvent showed that recovery of high-yielding sugar streams and lignin is comparable to other methods. The estimated cost of GVL-derived ethanol had an ~10-12% decrease in minimum selling price per gasoline equivalent compared to other benchmarked processes.

Won, W., Motagamwala, A. H., Dumesic, J. A., and Maravelias, C. T. (2017) A co-solvent hydrolysis strategy for the production of biofuels: process synthesis and technoeconomic analysis. *Reaction Chemistry and Engineering* **2**, 397-405.
<http://pubs.rsc.org/en/content/articlelanding/2017/re/c6re00227g> - !divAbstract

- **Yeast hybridization and adaptive evolution leverages traits relevant to biofuel production.** Interspecies yeast hybrids were generated and adaptively evolved for enhanced biofuel production from biomass. Traits such as enhanced xylose utilization and hydrolysate toxin tolerance contributed to more robust performance in the hybrids relative to natural isolates. This study demonstrates the role and relevance of studying natural isolates to identify useful biofuel traits for biocatalyst design. **Peris D, Moriarty RV, Alexander WG, Baker E, Sylvester K, Sardi M, Langdon QK, Libkind D, Wang Q-M, Bai F-Y, Leducq J-B, Charron G, Landry CR, Sampaio JP, Goncalves P, Hyma KE, Fay JC, Sato TK, Hittinger CT (2017)** Hybridization and adaptive evolution of diverse *Saccharomyces* species for cellulosic biofuel production. *Biotechnology for Biofuels* **10**: 78.
<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-017-0763-7>

- **Hotspots of soil N₂O emission enhanced through water absorption by plant residue.** Here we quantify physical and hydrological soil characteristics that lead to strikingly accelerated N₂O emissions in plant residue-induced hotspots. Results reveal a mechanism for microscale N₂O emissions: water absorption by plant residue that creates unique micro-environmental conditions, markedly different from those of the bulk soil (moisture levels within plant residue exceeded those of bulk soil by 4–10-fold and led to accelerated N₂O production via microbial denitrification). Understanding and modeling hotspot microscale physical and hydrologic characteristics is a promising route to predict N₂O emissions and thus to develop effective mitigation strategies and estimate global fluxes in a changing environment.

Kravchenko AN, Toosi ER, Guber AK, Ostrom NE, Yu J, Azeem K, Rivers ML, Robertson GP (2017) Hotspots of soil N₂O emission enhanced through water absorption by plant residue. *Nature Geoscience* **10**: 496-500.
<http://www.nature.com/nggeo/journal/v10/n7/full/ngeo2963.html>

- **Cover crop root contributions to soil C in a no-till corn bioenergy cropping system.** Here, we examine the relative contributions of cover crop root and shoot to soil carbon (C) in order to determine whether belowground cover crop C could help offset the deleterious effects of residue removal in a no-till continuous corn bioenergy cropping system. We labeled cereal rye (*Secale cereale*), a common winter cover crop, *in situ* with ¹³CO₂, tracked inputs from rhizodeposits during the growing season, and tracked root and shoot inputs into different soil C pools over the following 2 years. Our results suggest that including winter cover crops in annual rotation could increase biofuel feedstocks directly and indirectly; aboveground cover crop residues could contribute to biofuel feedstocks and

belowground cover crop inputs could offset the C removal associated with the use of main crop stover as a biofuel feedstock.

Austin EE, Wickings K, McDaniel MD, Robertson GP, Grandy AS (2017) Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. *Global Change Biology – Bioenergy* **9**: 1252-1263.

<http://onlinelibrary.wiley.com/doi/10.1111/gcbb.12428/full>

- **Cellulosic biofuel contributions to a sustainable energy future: choices and outcomes.** Understanding the effects of management choices is key for realizing the climate and other environmental benefits provided by cellulosic bioenergy crops. Here, we synthesize recent empirical research that targets sustainability concerns to identify potential solutions for managing the land use-related trade-offs of cellulosic biofuels. We organize our conclusions to articulate seven emerging principles that are relevant globally to the sustainability of cellulosic biofuel crop production.

Robertson GP, Hamilton SK, Barham BL, Dale BE, Izaurrealde RC, Jackson RD, Landis DA, Swinton SM, Thelen KD, Tiedje JM (2017) Cellulosic biofuel contributions to a sustainable energy future: choices and outcomes. *Science* **356**: pii: eaal2324.

<http://science.sciencemag.org/content/356/6345/eaal2324>

- **Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA.** Fine roots play a key role in the global carbon (C) cycle because much of the C accumulating in soil is the result of fine root production and turnover. Here we explore the effect of plant community composition and diversity on fine root production in surface soils and plant biomass allocation to fine roots in six perennial cropping systems in composition and diversity planted as biofuel feedstocks. Findings demonstrate a greater potential for diverse biofuel cropping systems to allocate C belowground to fine roots as compared to monocultures, with potential implications for soil C sequestration.

Sprunger CD, G. OL, Jackson RD, Robertson GP (2017) Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. *Biomass and Bioenergy* **105**: 248-258.

<http://www.sciencedirect.com/science/article/pii/S0961953417302283>

- **Soil depth and crop determinants of bacterial communities under ten biofuel cropping systems.** Biofuel-cropping systems, projected for large land areas, can potentially change their soil microbiome and the ecosystem services they catalyze. We determined the bacterial community composition and relevant soil properties for samples collected after six crop years at 0–10 cm, 10–25 cm, 25–50 cm, and 50–100 cm under corn, switchgrass, *Miscanthus*, and restored prairie, as well as 0–10 cm under six additional candidate biofuel crops in replicate side-by-side plots. We found that soil depth, as a proxy for changes in soil biogeochemical, edaphic, and structural properties, was a strong factor in shaping bacterial community structure under a wide range of cropping systems; crop type was a secondary driver and was significant at soil depths above 50 cm after 6 years, with switchgrass' effects deeper.

Zhang B, Penton CR, Xue C, Quensen JF, Roley SS, Guo J, Garoutte A, Zheng T, Tiedje JM (2017) Soil depth and crop determinants of bacterial communities under ten biofuel cropping systems. *Soil Biology and Biochemistry* **112**: 140-152.

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