

ACCOMPLISHMENT REPORT
Central-East Regional Biomass Research Center
October 2015 to October 2017
Rob Mitchell, Coordinator

Locations Reporting

Ames, IA	Peoria, IL
Lincoln, NE	University Park, PA
Madison, WI	Urbana, IL
Mandan, ND	Morris, MN

(1) Feedstock Development

Competition affects plant performance in breeding and selection nurseries. Breeding for increased biomass yield of switchgrass for use as a bioenergy feedstock was initiated in 1992. Small gains have been achieved, but the rate of progress has been slow, partly because most breeders conduct their selection on spaced plants where the individual plant can be easily visualized and distinguished from others. This research has demonstrated that selection for spaced-plant biomass is highly inefficient at identifying individuals with the best breeding value for realistic swards that would be used for on-farm production. Use of genomic DNA to predict biomass yield of swards would be considerably more efficient than the used of spaced-plant nurseries.

Genomic selection is a promising tool for improving switchgrass for bioenergy. Switchgrass is a relatively high-yielding and environmentally sustainable biomass crop, but further genetic gains in biomass yield must be achieved to make it an economically viable bioenergy feedstock. Genomic selection is an attractive technology to generate rapid genetic gains in switchgrass and meet the goals of a substantial displacement of petroleum use with biofuels in the near future. The accuracy of genomic prediction was as high as 0.62. This value will allow switchgrass breeders to employ genomic prediction in breeding programs, which is expected to triple the rate of genetic gain for biomass yield. If these results are accurate, it will allow us to achieve the 10 ton/acre goal by 2030. Experiments are already in place to employ genomic prediction in the USDA switchgrass breeding programs and to test the accuracy of these results.

Marker-assisted selection could accelerate switchgrass breeding efforts. Single-nucleotide DNA markers associated with flowering time have been identified within broad germplasm panels of switchgrass. These markers either reside within functional genes within the various flowering time pathways or are strongly linked to some of these genes. These markers will be useful for designing primers that can be used to screen large populations of switchgrass seedlings growing in the greenhouse, selecting only those with the latest predicted flowering time before taking them to the field for establishment of breeding nurseries. This marker-assisted breeding approach could significantly reduce the length of time required to develop high-yielding and cold-tolerant late-flowering populations of switchgrass.

Selection site affects plant breeding outcomes. To avoid competition of biomass with food or feed crops, most commercialization proposals suggest that switchgrass should be grown exclusively on marginal lands that are not fit for food or feed production. Currently, nearly all breeding and selection to

create new varieties of switchgrass is conducted on prime farmland, using nitrogen fertilizer, pesticides, and numerous other inputs. This research showed that there were significant changes in rank performance among switchgrass varieties, depending on soil quality (depth and fertility of the top soil) and the use or absence of nitrogen fertilizer. The study concluded that breeders should put at least some of their efforts and resources toward creating selection sites that mimic a low-input and marginal type of land base for use in breeding and selection of new varieties for that type of land. The consequences of failing to do this would not be felt for many years, perhaps decades, but would nevertheless be a proliferation of new varieties that are poorly adapted to the predominant type of environment set aside for switchgrass biomass production.

(2) Feedstock Production

Perennial biofeedstocks improve soil, increase stability. Understanding how perennial herbaceous biofeedstocks alter soil properties, and in turn, how such alterations affect ecosystem services is essential for the development and adoption of improved management practices to promote multifunctional agricultural landscapes. ARS researchers at Mandan, North Dakota quantified changes to soil properties resulting from different perennial biofeedstocks at five sites in central and western North Dakota over a 5-yr period. Perennial biofeedstocks induced changes in soil properties over the study period, with substantial declines in available phosphorus (P) at sites with high initial P and modest increases in soil organic carbon (SOC) at sites with low initial SOC. Accordingly, results highlighted the value of perennial biofeedstocks to remediate nutrient-laden and/or degraded soils. In contrast, other soil properties changed minimally (electrical conductivity) or not at all (soil pH). Such resistance to change can have important implications for continued soil function, and can confer a period of stability against a backdrop of increased salinity and acidification for rainfed cropping systems in the Northern Great Plains.

Switchgrass contributions to soil organic carbon, deep soil microbial community composition, and root biomass. More than 50% of the world's soil carbon stocks reside below one foot from the soil surface, but relatively little is known about the importance of rhizodeposit carbon and associated microbial communities in deep soil processes. ARS researchers in Lincoln, NE and Ft. Collins, CO evaluated a lowland and upland switchgrass ecotype for variability in plant root biomass and subsequent carbon cycling as well as soil microbial community abundance and composition. The lowland ecotype had three times the root biomass with coarser root architecture compared with the upland ecotype but the upland ecotype maintained its root biomass throughout an extreme drought event. Root-derived carbon inputs influence soil C processes and switchgrass ecotype interactions with the soil microbial community plays an important role in soil carbon sequestration.

Long-term no-till and stover retention each decrease the global warming potential of irrigated continuous corn. Agricultural production systems can help reduce global warming by storing carbon in soils. Management practices, however, can add greenhouse gases into the atmosphere and counteract the benefit of soil carbon storage. Limited information is available for irrigated row-crop systems. Crop residue removal in high-level production systems such as those under irrigation can supply feedstocks for both livestock and bioenergy. The use of no-till is often recommended as a companion practice for removing crop residues. ARS researchers in Lincoln, NE found that in a long-term irrigated continuous corn system, all management systems were net greenhouse gas producers and so had limited potential to decrease global warming.

ARS researchers also found that there was no global warming benefit to using both no-till and residue retention practices together. Instead using either conservation practice reduced the amount of greenhouse gases released to the atmosphere compared to more conventional practices (disk tillage, residue removal). Although this irrigated corn system did not store carbon, conservation management practices could provide other benefits to producers, such as decreased soil erosion and increased soil health.

Sub-surface soil carbon changes impact biofuel greenhouse gas emissions. Biofuel feedstocks are being developed and evaluated in the United States and Europe to partially offset petroleum transport fuels. Accurate accounting of upstream and downstream greenhouse gas (GHG) emissions is necessary to measure the overall carbon intensity of new biofuel feedstocks. Changes in direct soil organic carbon (SOC) can have a major impact on estimating overall GHG emissions from biofuels. In particular, changes in sub-surface soil depths (>30 cm) could have a large impact on overall GHG emissions from biofuels either positively or negatively that are not always accounted for. ARS scientists in Lincoln, NE showed that corn grain and cellulosic ethanol GHG emissions varied either positively or negatively from baseline GHG emissions depending on SOC changes at near and sub-surface depths. Differences in GHG emission values highlight the importance of accounting for sub-surface SOC stock changes especially in bioenergy cropping systems with high potential for soil C storage.

Corn residue removal affects grain yields between no-till and conventional tillage differently but soil organic carbon stocks are similar. Corn residue demands have increased by the livestock industry for co-feed, bedding, and grazing. ARS scientists in Lincoln, NE collected grain, biomass yield, total soil nitrogen (N), and soil organic carbon (SOC) data over 10 growing seasons to determine the impact of tillage and residue removal rates in an irrigated, continuous corn study. Natural abundance C isotope compositions were used to determine C additions by corn (C4-C) to the soil profile and to evaluate the retention of residual C3-C. Mean grain yields were lower when corn stover was harvested under conventional tillage while grain yields were higher for no-till when corn stover was harvested compared with no stover removal. Changes in SOC and total soil N occurred at near surface soil depths while cumulative (0 to 150 cm) changes were similar between 2001 and 2010. Results support the need to evaluate SOC cycling processes below near-surface soil layers.

Nitrous oxide emissions from grasslands on marginal lands. N₂O is the largest source of greenhouse gas emissions from production of biomass crops and although perennial grasses can provide significant water quality benefits on conservation reserve program (CRP) land, if they are located in wet areas in the landscape, they can lead to high emissions of N₂O. ARS scientists in University Park PA and university scientists found that although N₂O emissions were high in the wet areas of the fields, it was similar in most areas of the landscape compared with previous grassland vegetation present in CRP land, while producing much greater biomass. This finding will inform government agencies developing greenhouse gas mitigation strategies that targeted mitigation strategies can address most emissions.

Crop legacy affects N₂O emissions accounting. There are several methods to estimate N₂O emissions used for life cycle assessments (LCAs), however, they have not been evaluated for multiple crop rotations. ARS scientists in University Park, PA, in collaboration with university

scientists, evaluated several methods for estimating N₂O emissions for grain and sweet sorghum rotations in Uruguay which include different legumes. We have found that when the preceding crop is a legume, N₂O emissions are underestimated when the N₂O estimation methods do not account for legacy nitrogen from preceding crops in the rotation, an error which increases in size with the amount of N fixed by the legume. This finding will inform university, industry, and government agencies to evaluate methods used in estimating N₂O emissions of LCAs for crops in complex rotations

Landscape strategies to minimize greenhouse gas emissions: Multiple factors can affect the carbon footprint of biofuels, such as soil type, land use, and management intensity, however there are few examples of available data to calibrate across this range of factors. In collaboration with Colorado State University, ARS Researchers at University Park PA compiled a dataset of switchgrass field trials in the United States with yield, changes in soil carbon, and soil nitrous oxide emissions, spanning a range of climates, soil types, and management conditions to calibrate and validate our model. We found that the switchgrass yields and greenhouse gas emissions varied greatly across a landscape large enough to supply the biorefinery in response to variations in soil type, land use history, and management intensity, providing the biorefinery significant opportunities to minimize the carbon footprint of their feedstock. These results demonstrate the value of this modeling approach to identify strategies to mitigate greenhouse gas emissions and minimize the carbon footprint of the bioenergy feedstock production.

Reducing the carbon footprint of cellulosic ethanol. After producing ethanol from crop residues such as corn stover and straw, a slowly decomposing byproduct remains which is typically burned for energy recovery, but harvesting crop residues can result in decreased crop yields and soil carbon levels. Agricultural Research Service (ARS), Drexel University and Colorado State University scientists compared the current practice of burning this residue, to applying it back to the land. They found that although most studies have recommended burning this material to generate electricity for the biorefinery, applying it to the land instead resulted in ethanol with the lowest greenhouse gas (GHG) footprint, highest levels of soil carbon, and the greatest offset of GHG emissions. This finding could help the industry evaluate the different markets for byproducts produced at the biorefinery, considering both the economic and environmental impacts.

Zero net GHG emissions from Great Plains agriculture is possible: The Great Plains is an important agricultural production region supplying food for the global marketplace. Understanding the historical change in greenhouse gas (GHG) emissions with climate and technology, can help identify solutions for the future reductions in GHG emissions. In collaboration with Colorado State University, University of Colorado, and University of Michigan, ARS Researchers at University Park PA found that the largest sources of GHG emissions prior to 1930 was from plowout of native grasslands, whereas after 1930, it was GHG emissions from agriculture production. In contrast with cool and wet weather, hot and dry weather decreased soil carbon, thereby increasing GHG emissions. Increased adoption of no tillage and slow release nitrogen fertilizers would reduce GHG emissions, potentially achieving zero net GHG emissions if adoption is high enough, without reducing agricultural production.

A head start on minimizing invasions from bioenergy crops. Perennial plant biomass is an increasingly important part of the U.S. renewable energy portfolio; however, widespread production of exotic perennial vegetation has raised concerns about invasions of bioenergy crops into adjoining natural areas. ARS researchers in Urbana, Illinois found that controlled introductions of *Miscanthus x giganteus* and *Miscanthus sinensis* in old-field and floodplain forest habitats did not affect resident plant community composition over a five-year period. Although local *Miscanthus* invasions in the Midwest and Southeast U.S. show a long term potential for this species to develop problematic populations, these results show that there will likely be a lag of several years or more before new invasions cause damage to native plant communities in natural areas. This should provide enough time for bioenergy producers to implement monitoring and eradication plans that minimize invasions from these bioenergy crops.

Corn residue harvest changes soil hydrology and soil aggregation. Reducing or eliminating tillage is recommended when crop residues like corn stalks are harvested. However, information is lacking on whether conservation tillage strategies are sufficient to protect soil resources when residues are aggressively harvested. Researchers at the ARS in Morris, Minnesota and collaborators compared soil aggregation and water infiltration in fields with and without tillage, with no residue or maximum residue harvested. Soil in tilled fields were more exposed to wind and water and had reduced ability to capture rainwater. Fields managed without tillage captured and stored more rainwater when residue covered the soil. This work will aid producers, energy industry, and action agencies to balance the pros and cons of harvesting crop residues such as corn stalks for bioenergy.

Emission and mitigation from perennial grasses used for biofuel feedstocks. Perennial grasses are a potential bioenergy feedstock. Growing grasses can increase soil carbon, which mitigates greenhouse gas emission because it keeps carbon in the soil instead of in the atmosphere as carbon dioxide. When grasses are grown and harvested for energy they need to be fertilized. Some of the nitrogen in the fertilizer likely will be released as a greenhouse gas – nitrous oxide. Ideally, enough carbon dioxide is changed into beneficial soil carbon to offset any unwanted nitrous oxide emission from fertilizer. ARS researchers in Morris, Minnesota compared nitrous oxide emission and soil carbon storage between grasses grown with legumes or with nitrogen fertilizer, between autumn and spring harvests, and between perennial grasses and a corn-soybean rotation. Results of this study found that beneficial soil carbon increased under perennial grasses, but nitrogen management needs to be refined to optimize grass production while minimizing nitrous oxide released. This work will aid producers, agronomists and energy industry to meet production goals while reducing emissions related to nitrogen fertilization.

Assessed corn residue harvest impacts on soil and water infiltration properties on Minnesota farms. Corn residue may be harvested for many reasons (e.g., animal feed or bedding, bioenergy) and these materials are also critical for protecting soil and hydrological properties. However, information is lacking from on-farm sites to document whether conservation tillage strategies are sufficient to protect soil resources when residues are aggressively harvested. ARS scientist from Morris, Minnesota, in collaboration with university scientists and local producers assessed the short-term effects of harvesting corn residues at on-farm experimental sites. Results of this study suggested that to protect soil and water infiltration properties from degradation all corn residue needs to remain on the soil, even if the field had

little tillage disturbance. This work will aid producers, energy industry, and action agencies to balance the pros and cons of harvesting crop residues such as corn stalks for bioenergy.

Bee nutrition is bolstered by specialty oilseed crops. Intense agriculture in the upper Midwest has led to a lack of annual floral resources important for sustaining the abundance and health of native and domesticated pollinators (e.g., honey bees), which has been linked to their recent decline. Floral pollen is the primary source of protein for sustaining bees. ARS researchers in Morris, Minnesota, demonstrated that several specialty oilseeds that they are studying (e.g., borage, calendula, echium, and cuphea) provide copious amounts of pollen that bolster the abundance and diversity of pollinators. When rotated with traditional crops like corn and soybean, specialty oilseeds provide a vital energy source and protein to sustain pollinators throughout the entire growing season. Results benefit bee keepers, farmers seeking high-value crop alternatives requiring low agricultural inputs, the specialty crop industry, and general public seeking to promote the use of specialty oilseeds for food, fuel, and specialty chemicals.

Including alfalfa in corn production systems reduces N fertilizer application while increasing economic returns. A five-year alfalfa-alfalfa-alfalfa-corn-corn rotation implemented to improve soil health, decrease nitrate-nitrogen (NO₃-N) leaching, and support sustainable harvest of biomass feedstock for bioenergy and/or bio-products reached a point where N availability following three years of alfalfa could be assessed to determine its effect on N fertilizer recommendations for this year's corn crop. Based on the late-spring soil NO₃-N test, fertilizer N application could be reduced from 200 pounds per acre to 90 pounds per acre for first-year corn following three-years of alfalfa. This reduction in the amount of N fertilizer needed not only decreases the potential negative environmental impacts associated with excessive residual N at the end of the growing season, but also provides a substantial economic benefit to producers.

Soil quality/soil health indexing strategies evaluated for Brazilian sugarcane production. Increasing demand for biofuel has intensified land-use change for sugarcane (*Saccharum officinarum*) production in Brazil. Currently there is no globally agreed upon method for assessing soil quality (SQ), so a field-study was conducted at three sites within the largest sugarcane-producing region of Brazil to develop an acceptable SQ index. ARS scientist in Ames, Iowa found that simple, user-friendly strategies were as effective as more complex methods for identifying SQ changes. Therefore, we recommend using a small number of soil indicators (e.g., pH, available phosphorus, available potassium, Visual Evaluation of Soil Structure scores, and soil organic carbon concentration) and proportional weighting to evaluate sugarcane production effects on soil quality in Brazil. We also recommend giving priority to management practices that prevent soil physical or biological degradation within sugarcane producing areas.

Applying biochar has mixed effects on corn grain yield response. Applying biochar was hypothesized to help mitigate potential negative soil quality effects of harvesting stover for bioenergy production. Studies by ARS scientist in Ames, Iowa and collaborators found that when averaged over the first five years of study on a Clarion-Nicollet-Webster soil association in

Iowa, corn grain yields were increased by 0.49 Mg ha⁻¹ (7.8 bu/acre) after applying 18.4 Mg ha⁻¹ (8.2 tons/acre) of biochar but not harvesting the stover. In contrast, there was no significant effect on corn grain yields when moderate (~1.75 tons/acre) or high (~3.25 tons/acre) rates of stover were harvested. These mixed results suggest that biochar effects on corn grain yield are more complex than originally hypothesized. Field studies are continuing to elucidate soil health and crop growth factors that may be affected by biochar applications.

Harvest management of switchgrass is a critical question for the development of a future bio-based economy. Harvest management can significantly impact both the yield and quality of biomass, in turn, influencing conversion efficiency. In this study, switchgrass cultivars were harvested once annually at upland peak, after killing frost, or post-winter in the spring. Switchgrass biomass yield was greatest at the upland peak harvest, averaging 9.9 Mg/ha with biomass decreasing as harvest was delayed. Similarly, nutrient content in the biomass was greatest at the upland peak harvest with N, P, and K at 69, 77, and 23 kg/ha, respectively. The majority of the N, P, and K in the biomass at the upland peak harvest was absent from the biomass at the post-frost harvest. Delaying the time of harvest until after frost or post-winter increased the concentration of structural carbohydrates from 500 to over 570 g/kg in the biomass and lignin content from 160 to over 200 g/kg. Conversely, delaying harvest time lowered the amounts of ash and soluble sugars. The later harvest times also yielded more sugars following processing with yields increasing over 20% from the first harvest. Delaying harvest time of the switchgrass crop led to greater production of deoxygenated aromatics improving the efficiency of the catalytic fast pyrolysis and bio-oil quality. The findings show that the loss of minerals in the biomass as harvest time is delayed combined with the greater proportion in cellulose and lignin in the biomass has significant positive influences on conversion through fast pyrolysis.

Switchgrass biomass quality. Switchgrass has been identified as a model bioenergy crop by the United States Department of Energy, but nutrients in the harvested biomass reduce the soil nutrients available for future growth of this perennial grass and can cause mechanical problems during conversion to liquid biofuels. Delaying harvest in the fall and winter to decrease nutrient content, however, can decrease the yield due to leaf drop and lodging. Thus, farmers need guidance regarding the best harvest time. ARS researchers in Ames, Iowa and collaborators at Iowa State University investigated the rate of nutrient loss from switchgrass through resorption and leaching by monitoring nutrient levels in plots subject to natural rainfall and plots subject to additional, simulated, heavy rainfall. Additional research is needed to confirm nutrient leaching losses. The current recommendation is for farmers to harvest switchgrass shortly after fall dieback in order to avoid harvest difficulties and loss of biomass feedstock.

Near-surface soil physical quality affected by corn stover harvest and tillage. Excessive removal of corn stover for biofuel and/or bio-product production has raised concerns regarding negative consequences on soil physical quality. The Least Limiting Water Range (LLWR), which is defined as the range in soil water content having minimal limitations to plant growth associated with soil water (matric potential), aeration, and mechanical resistance, was used to evaluate the impact of two tillage practices (chisel plow vs no-tillage) and three levels of corn stover removal (none, moderate, and high) on central Iowa soils. The results confirmed that soil

resistance to penetration determined the lower limit of the LLWR regardless of tillage or stover removal rate, whereas soil aeration controlled the upper limit only at bulk density $> 1.45 \text{ Mg m}^3$ for chisel plow and $> 1.55 \text{ Mg m}^3$ for no-tillage. The LLWR was smallest for no-tillage with moderate or high corn stover removal, indicating poor soil physical condition for plant growth, while the largest LLWR occurred with moderate stover removal and chisel plowing. The LLWR was also more sensitive than available soil water content for detecting tillage and stover removal effects on soil structural degradation. This information helps define sustainable stover removal areas and will be most useful to crop consultants, research soil scientists, and persons interested in biomass feedstock production.

Combining rye cover crops with corn stover harvest may increase sustainable feedstock supplies and maintain soil resources. Two field studies (2008 to 2014 and 2011 to 2016, respectively) with moderate (~50%) and high (~90%) corn stover harvest on 0-2% slopes in central Iowa were conducted. Corn grain yields ranged from 7.55 to 10.80 Mg ha^{-1} with no treatment by year interaction or reduction in yield due to the rye cover crop. The second study, which included soybean in the rotation and for which the rye cover was harvested as additional feedstock, resulted in corn grain yields, moderate and high stover removal, rye biomass, and soybean grain yields that averaged 9.72, 3.99, 4.98, 4.00, and 2.90 Mg ha^{-1} , respectively. Using Agricultural Marketing Service (AMS) cornstalk bioenergy market prices, stover harvest would net US \$11.47, \$14.47, and \$16.47 Mg^{-1} for moderate, high, and high + cover crop harvest strategies. Therefore, incorporating cover crops could enhance stover removal feasibility from environmental and economic perspectives in central Iowa.

Lignification of corn stover following humic product application depends on drought severity. Across four years of measurements in two corn fields outside Ames, Iowa, ARS scientists found that plant material became increasingly woody, that is, showed enhanced lignification, following humic product application in droughty growing seasons. Corn, along with some other crops, becomes woodier during drought stress, but application of the humic product significantly enhanced this response under droughty conditions, with lignification increasing by 9 to 28% compared to corn plants receiving no humic product. The degree of lignification depended on the drought level: lignification increased with humic product application by only about 5% in the year having mild drought stress, no lignification occurred in a year with no drought stress, and increased crop lignification did not occur in lower parts of the fields having more access to water. These results are consistent with the recent hypothesis that humic products promote crop economic yield primarily by strengthening crop tolerance of environmental stresses. This information provides product users with evidence that humic products can help maintain corn growth during droughty conditions.

(3) Conversion and Co-products Utilization

Conversion of Switchgrass to bio-ethanol: An established field of Liberty switchgrass located in central Wisconsin was harvested in 2014 and 2015 and processed for production of ethanol. ARS scientists at Madison, WI and Peoria, IL determined the field yields were 3,510 – 4,960 liters/hectare. By way of context, corn grown on good land with a harvest of 200 bushels/acre

would yield 5,300 liters/hectare. Liberty is the newest ARS released cultivar bred for biomass production. The yields far exceeded those of other popular switchgrass cultivars (Summer and Kanlow). This is a rare example of field to fermentation integrated study and the first using Liberty. Results from the study help to establish switchgrass as a viable industrial crop and are critical for farmers and ethanol processors considering production of advanced biofuels in the northern US.

Converting switchgrass and corn stover sugars into biodiesel and bio-jet fuel without costly enzymes. ARS researchers in Peoria, Illinois, worked with industrial partners to demonstrate direct conversion of extracted plant sugars into oils to be used as biodiesel or bio-jet fuel. This new process will produce a renewable intermediate for biodiesel or bio-jet fuel while being cost competitive with petroleum-based oils; because sugars from plant fibers using enzymes drive up processing costs. The actual savings of this technology could reduce cellulosic ethanol production costs by 16 to 20 percent.

Switchgrass is difficult to convert into liquid fuels because most of its energy is locked in its cell walls. Cell walls are complex structures made up of many sugars locked into a concrete-like matrix called "lignin". Traditional plant breeding and selection for increased fermentability of switchgrass cell walls has been effective to improve the yield of ethanol in a fermentation system. This research aimed to identify individual genes responsible for the improvements in fermentability. Four genes involved in the synthesis of lignin were sequenced using advanced sequencing methods, yielding 183 DNA variants. Of these, 29 DNA variants were found to be strongly associated with the improved fermentation of switchgrass biomass. These DNA variants will be studied further to determine if and how they can be used in a genomics-assisted breeding program by USDA switchgrass breeders.

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