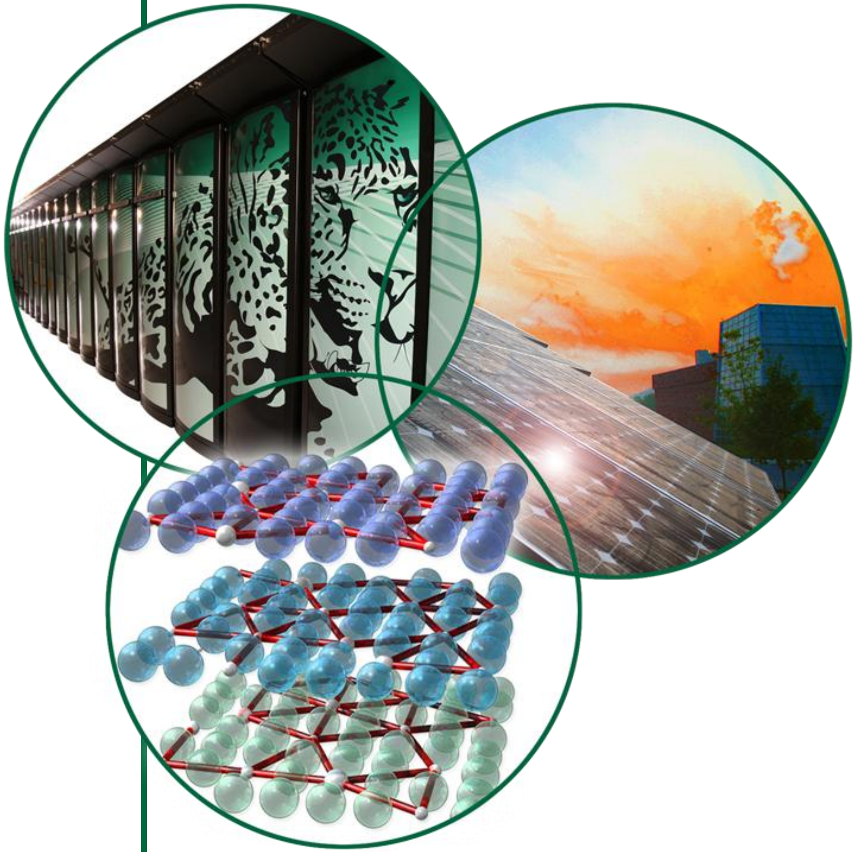
Next Generation Climate and Biomass Research: From Molecular Biology to Earth System Models and Back



Presented by:

Martin Keller

Associate Laboratory Director

Energy and Environmental Sciences

Oak Ridge National Laboratory

April 2011

U.S. DEPARTMENT OF ENERGY

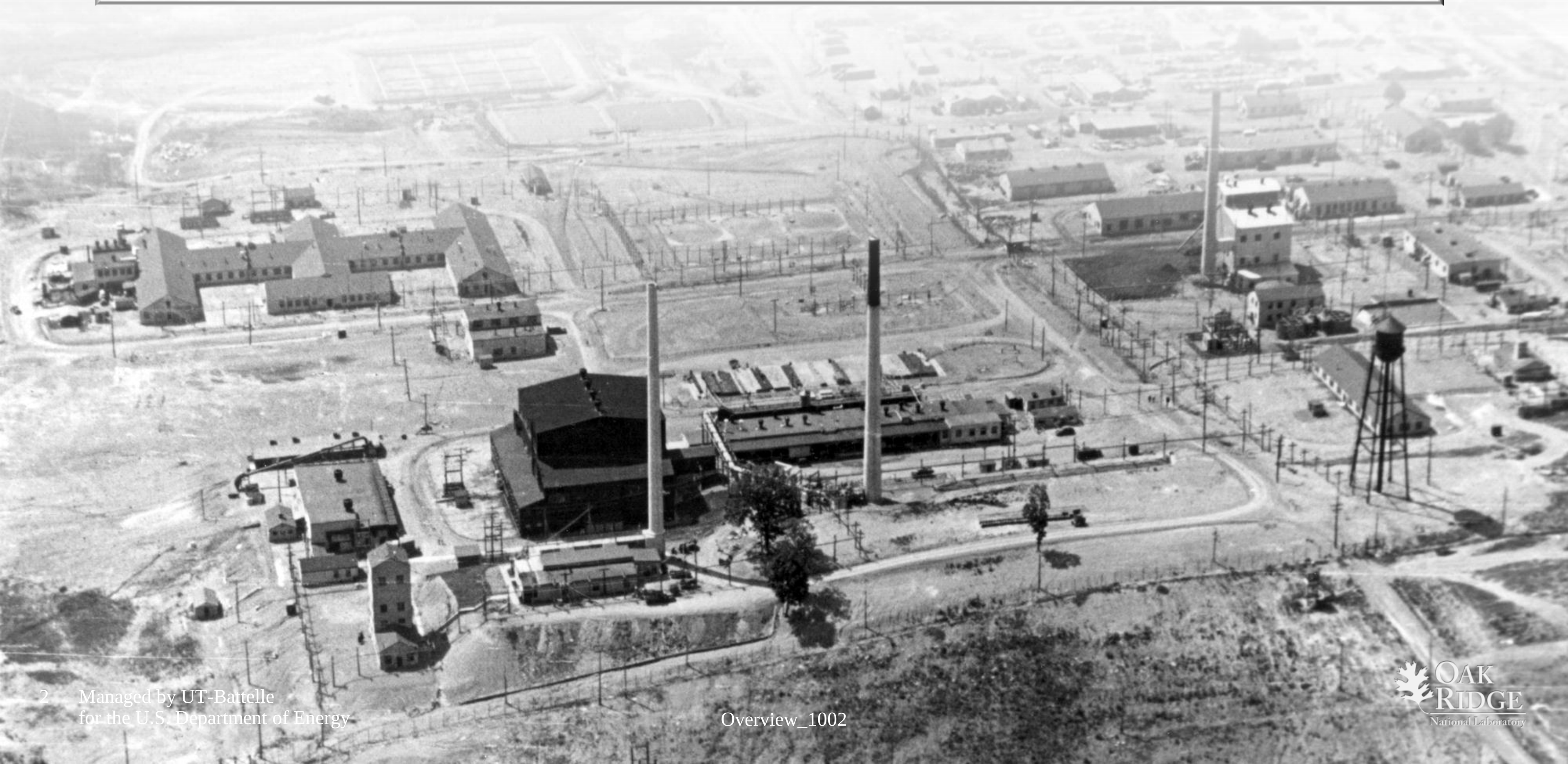
OAK RIDGE NATIONAL LABORATORY

MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

Oak Ridge National Laboratory evolved from the Manhattan Project

ORNL in 1943

The Clinton Pile was the world's first continuously operated nuclear reactor



Today, ORNL is DOE's largest science and energy laboratory

- $1.65B budget
- 4,500 employees
- 4,000 research guests annually
- $500 million invested in modernization

- Nation's largest concentration of open source materials research
- World's most intense pulsed neutron source and a world-class research reactor

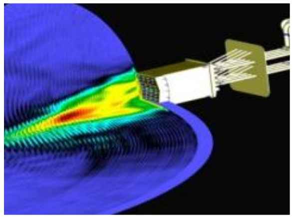
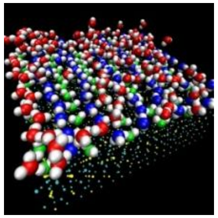
- World's most powerful open scientific computing facility
- Nation's most diverse energy portfolio
- Managing the billion-dollar U.S. ITER project

Delivering science and technology:
We lead major R&D programs for DOE and other customers

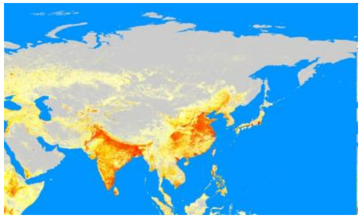
Energy technologies



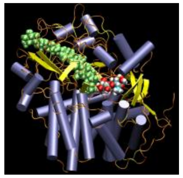
Ultrascale computing

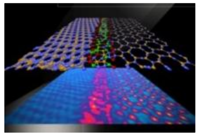
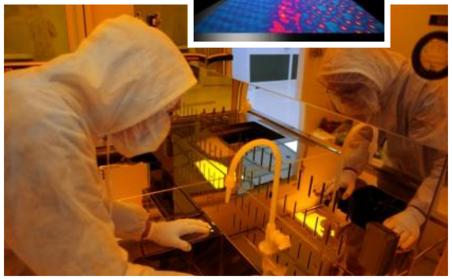




Climate

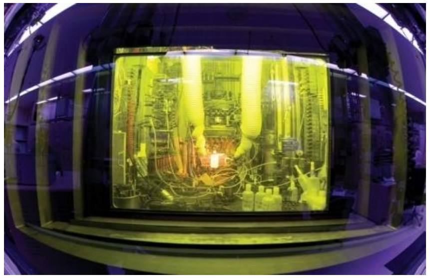
Bioenergy

Materials at the nanoscale

Neutron sciences



Nuclear energy

National security

Oak Ridge National Laboratory

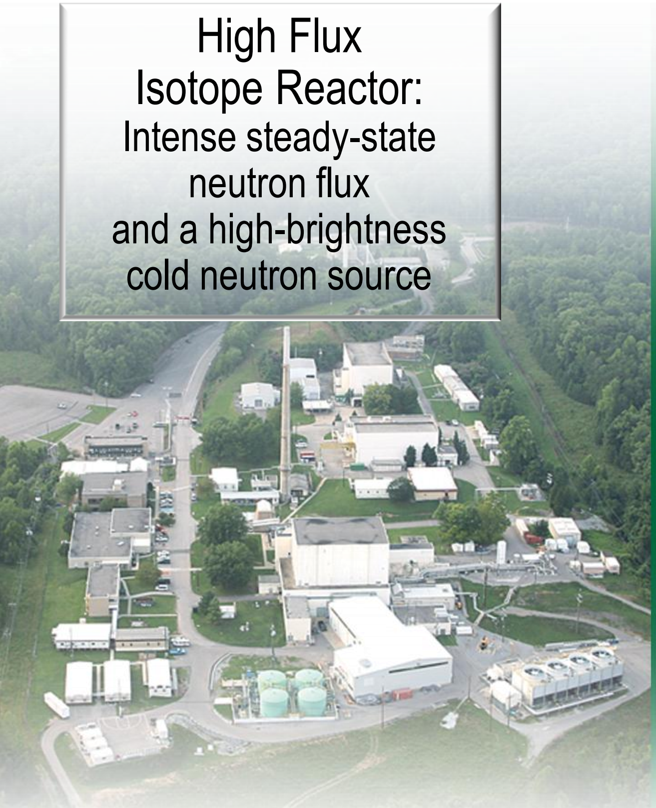
Leading the development of ultrascale scientific computing

- DOE Leadership Computing Facility:
  - World's most powerful open scientific computing facility
  - Jaguar XT upgraded to >2 petaflops
  - Exascale system by the end of the next decade
  - Focus on computationally intensive projects of large scale and high scientific impact
- NSF National Center for Computational Sciences:
  - Kraken upgraded to >1 petaflops
  - World's most powerful academic supercomputer
- NOAA Climate Prediction Center

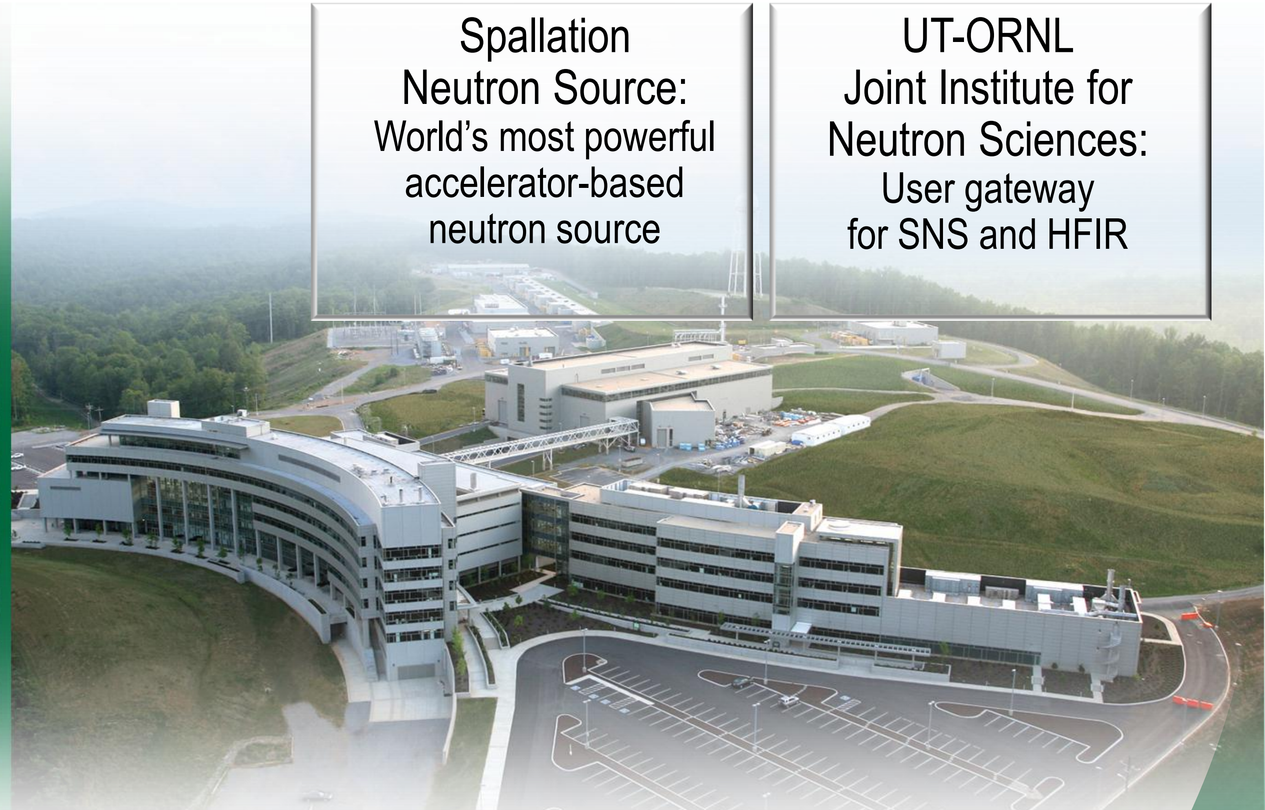
TOP500® SUPERCOMPUTER SITES

The world's most powerful systems for open science

Putting the world's best tools for neutron scattering to work



High Flux
Isotope Reactor:
Intense steady-state
neutron flux
and a high-brightness
cold neutron source



Spallation
Neutron Source:
World's most powerful
accelerator-based
neutron source

UT-ORNL
Joint Institute for
Neutron Sciences:
User gateway
for SNS and HFIR

Delivering neutrons to a growing user community

EESD, an integrated program: From Basic Science to Commercialization

Energy Technologies

Environment

EESD, an integrated program: From Basic Science to Commercialization

Energy technologies

Environment

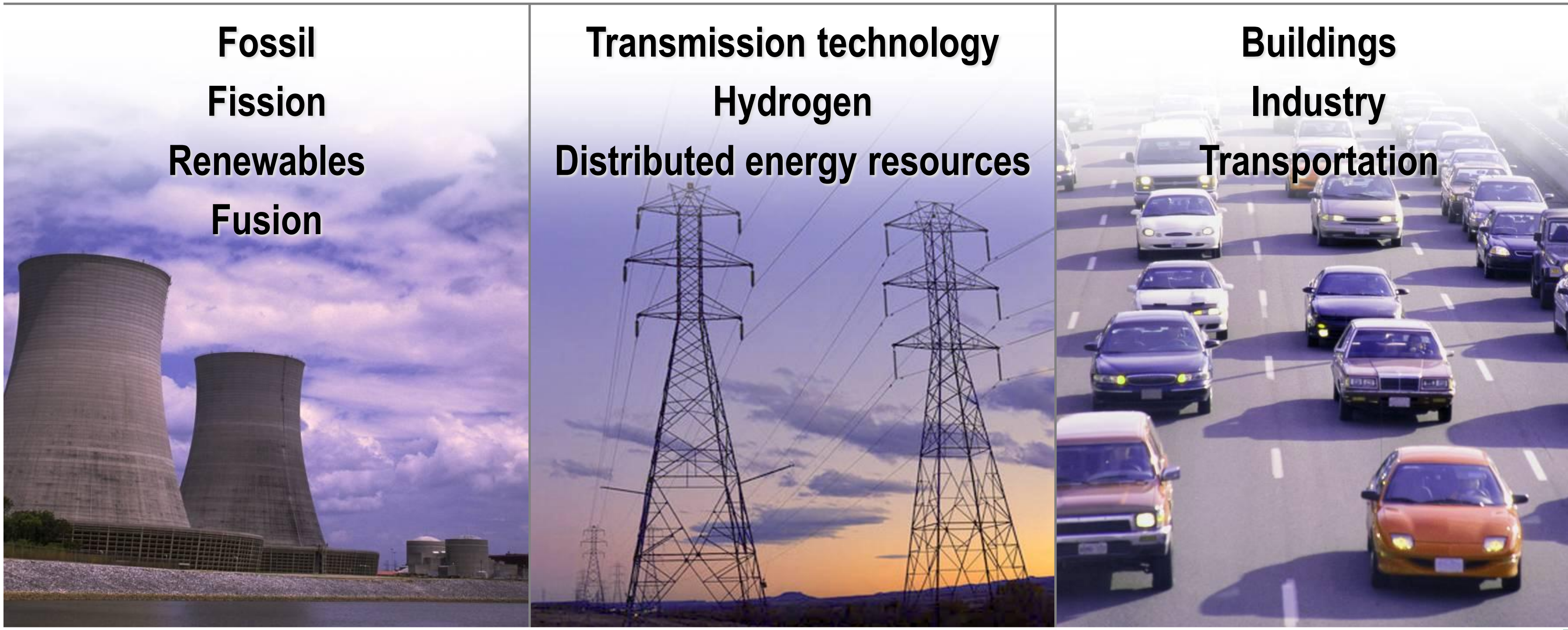
Material sciences

Neutron sciences

Ultrascale computing

Translating science and technology into sustainable energy solutions

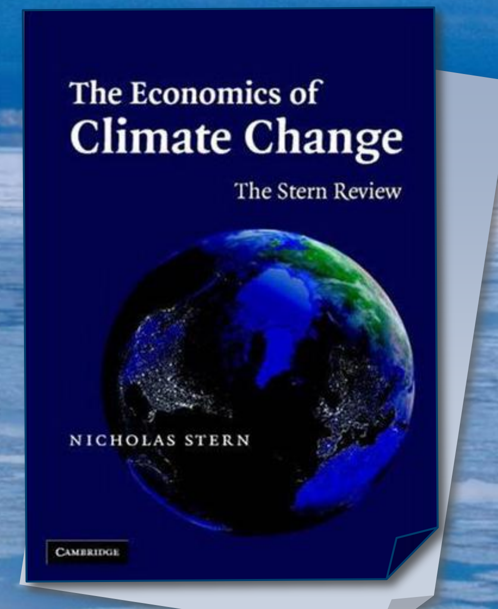
Generation	Distribution	Consumption



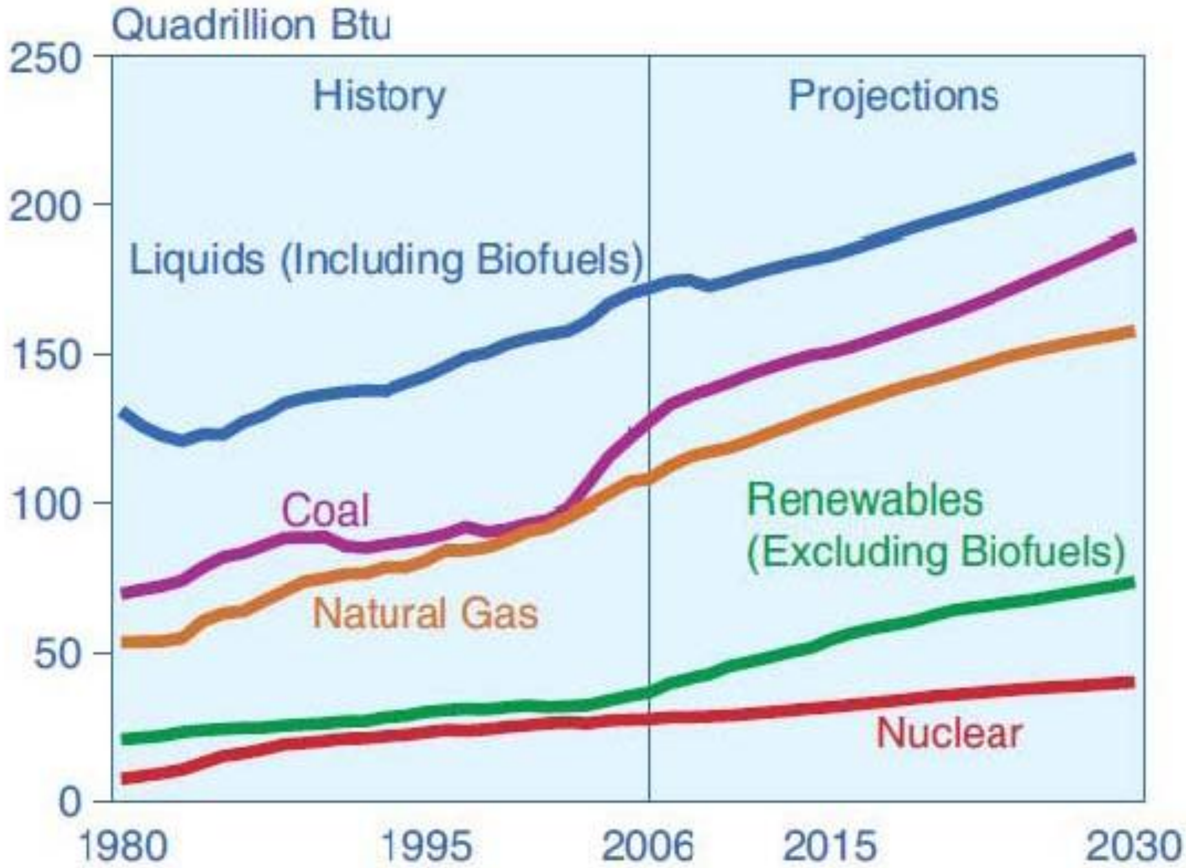
Warming of the climate system is unequivocal

"The costs of stabilizing the climate are significant but manageable"

- **Global atmospheric concentrations of greenhouse gases have increased markedly as a result of human activities since 1750**
- **Hot extremes, heat waves, and heavy precipitation events will continue to become more frequent**
- **Global temperature and sea level will continue to rise for at least a millennium**

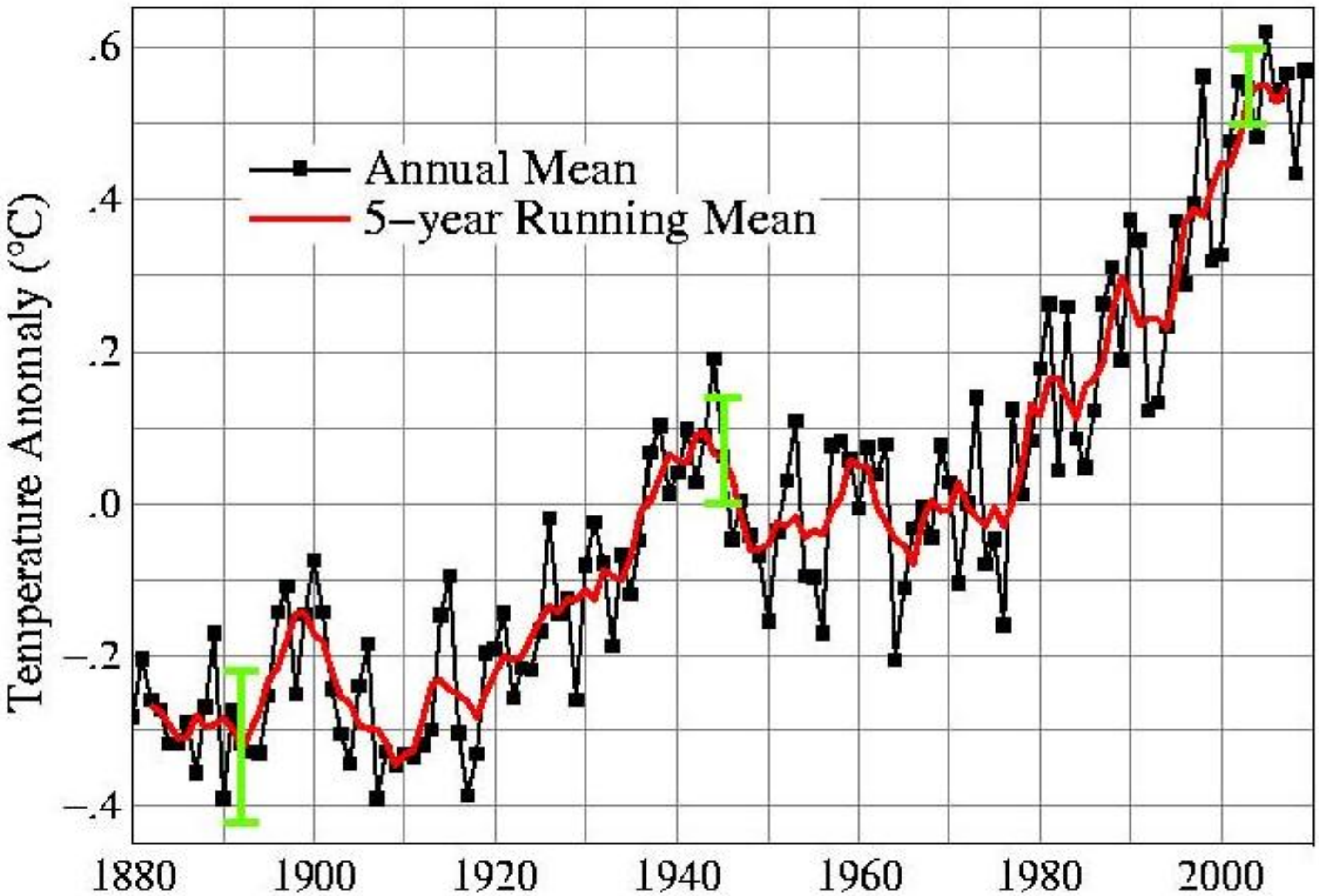


Energy Use: 1980-Present and Projections to 2030



Source: EIA *International Energy Outlook 2009*

Climate Change is real: the temperature record from 1880-2007



Source NASA: http://data.giss.nasa.gov/gistemp/graphs/

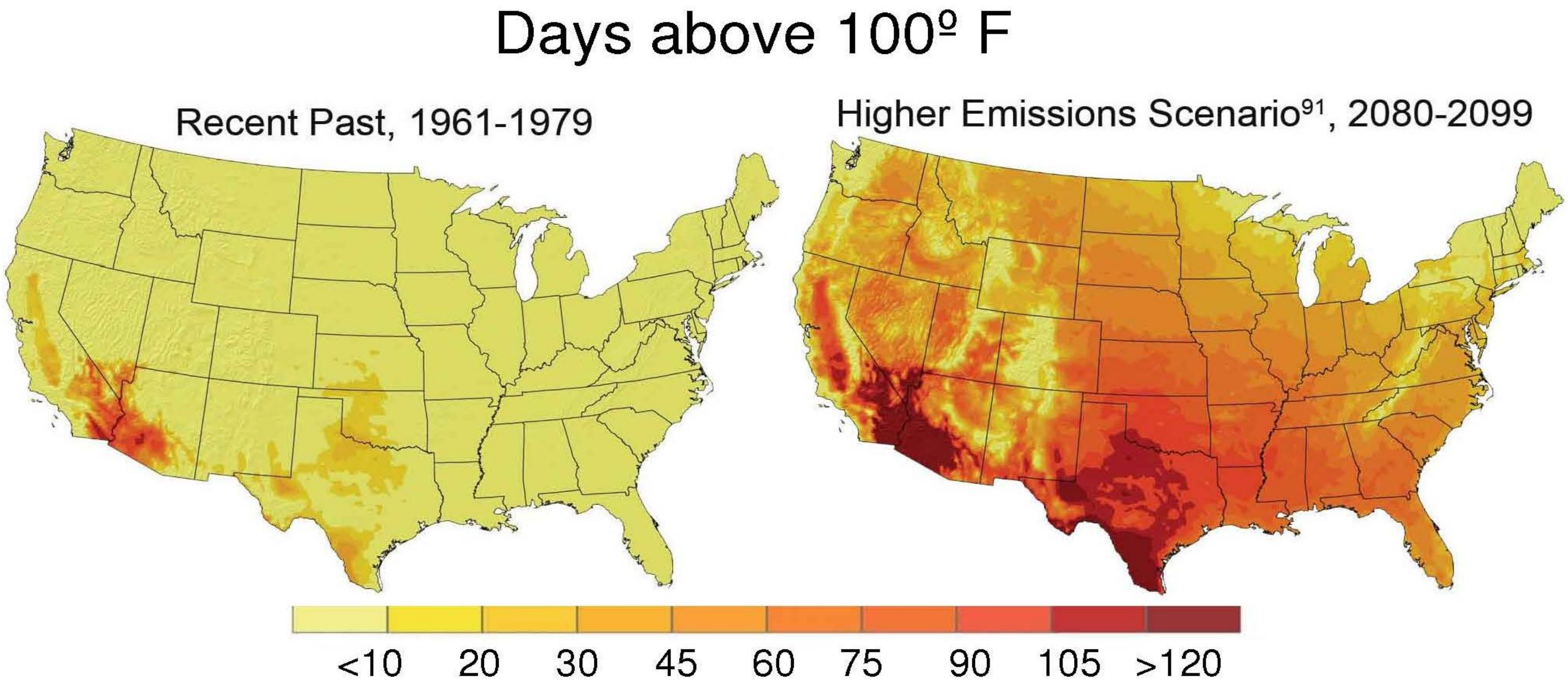
Where was the Carbon going in 2006? (Canadell *et al.*, PNAS 2007)

800 GtC or 380 ppm

+4.1 GtC/yr or + 1.9 ppm/year

Fossil Fuel & Cement	**+7.6 Gt C/year**
Land Use Change	**+1.5 Gt C/year**
Ocean Uptake	**-2.2 Gt C/year**
Terrestrial Uptake	**-2.8 Gt C/year**

If we fail to act, climate could have potentially devastating effects

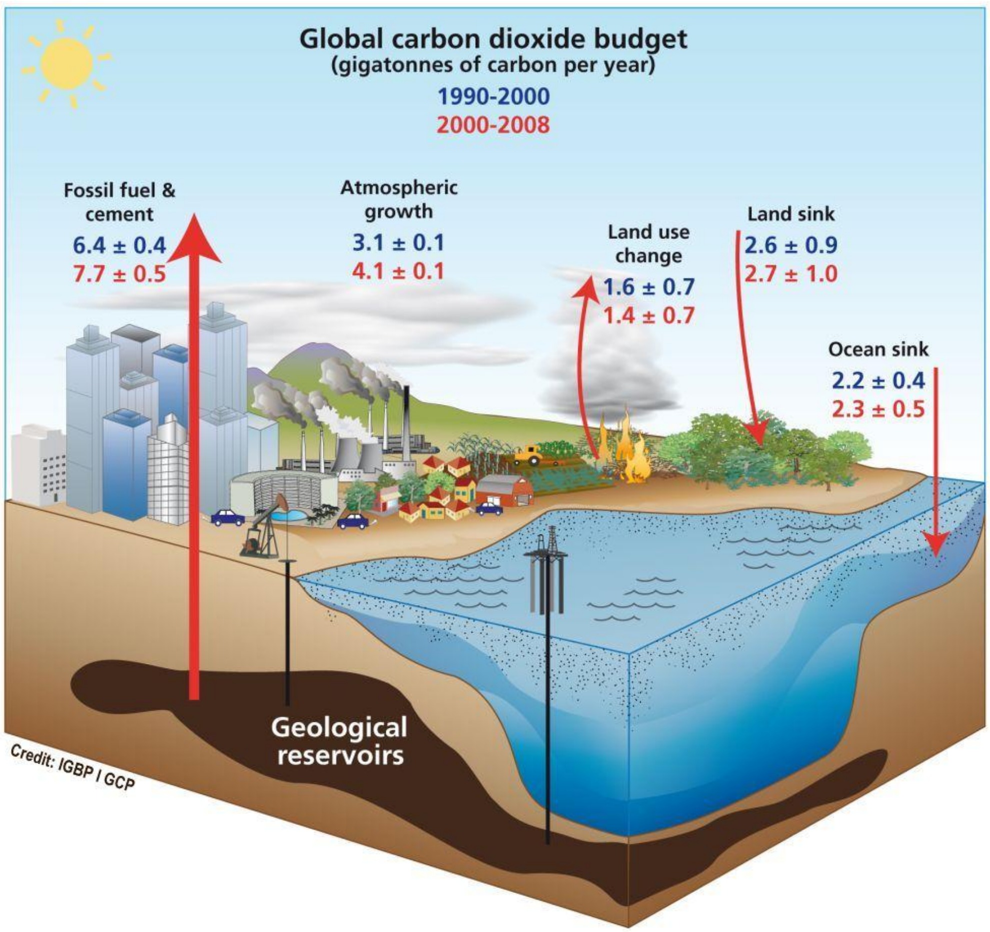


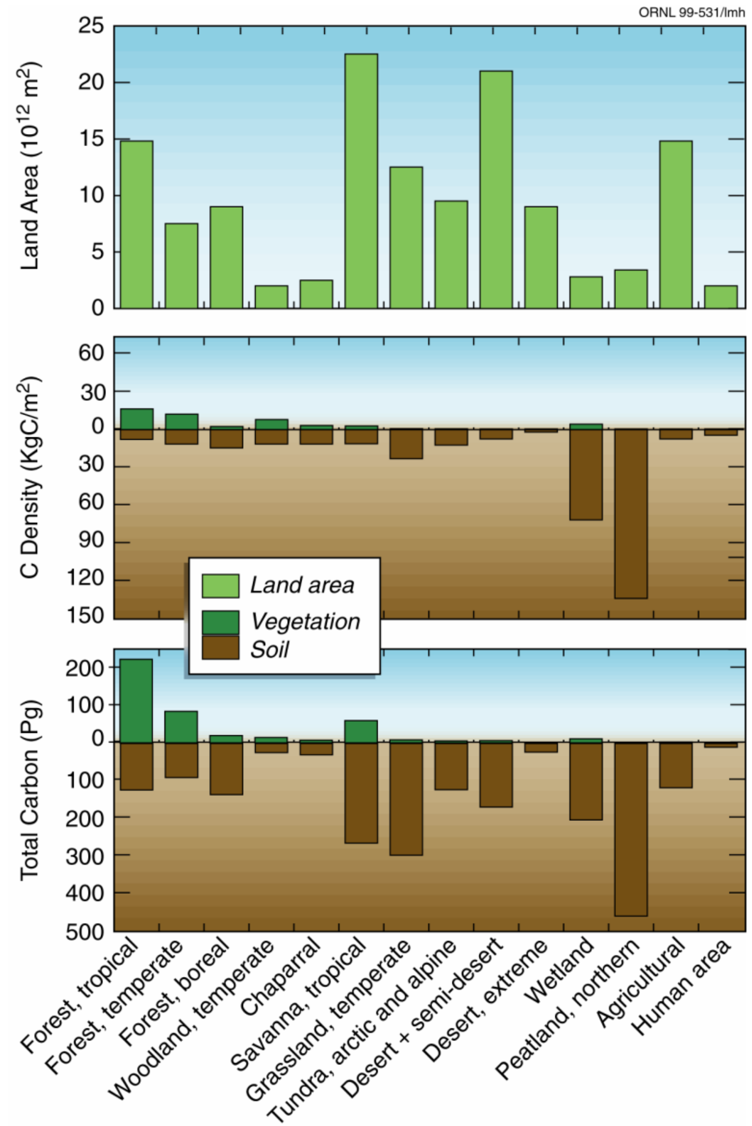
Much of the U.S. would go from 0 - 10 days above 100º F to 45 to 70 days per year above 100º F

Source: NOAA U.S. Global Change Research Program

CCSI Research Themes

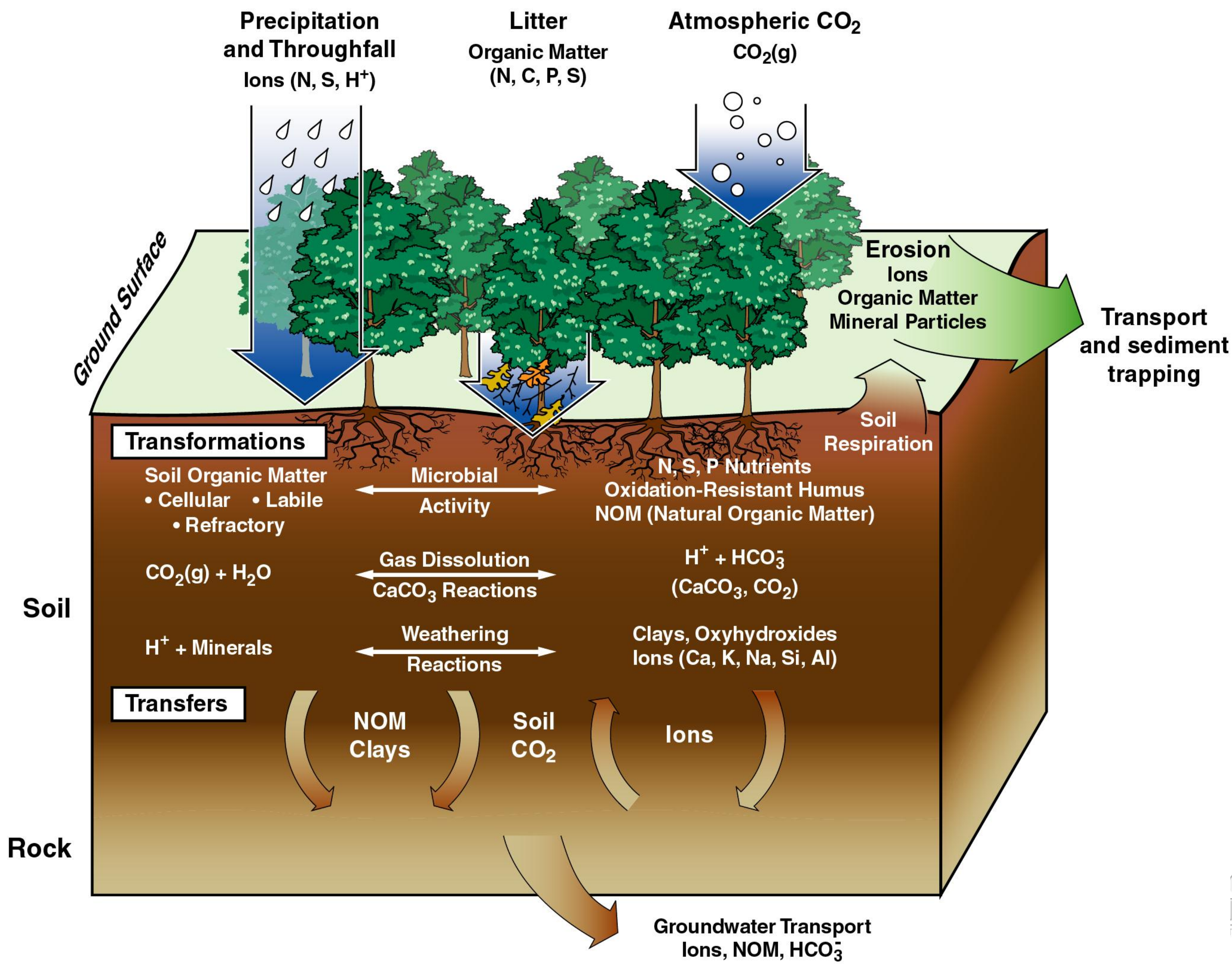
The global carbon cycle is still uncertain and high-latitude and tropical ecosystems are especially important





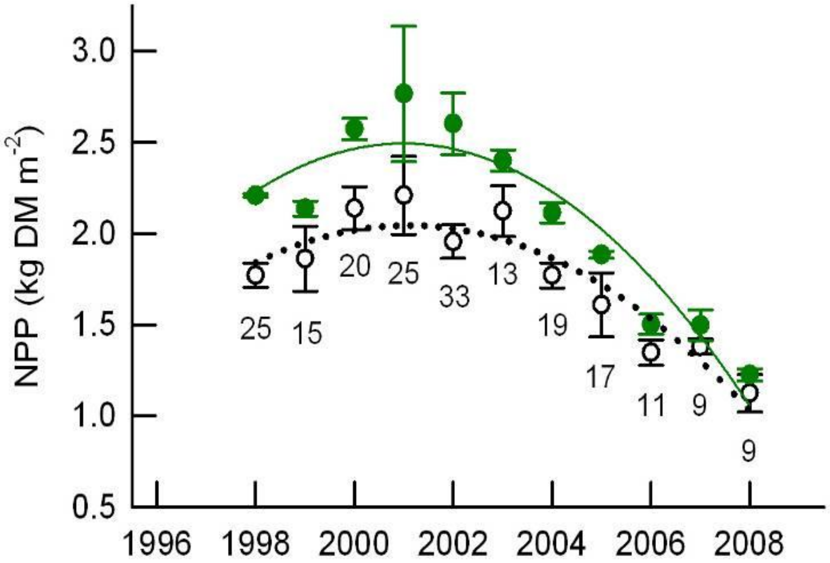
Understanding biogeochemical cycles under a changing climate remains a challenge

ORNL 98-986A/abh



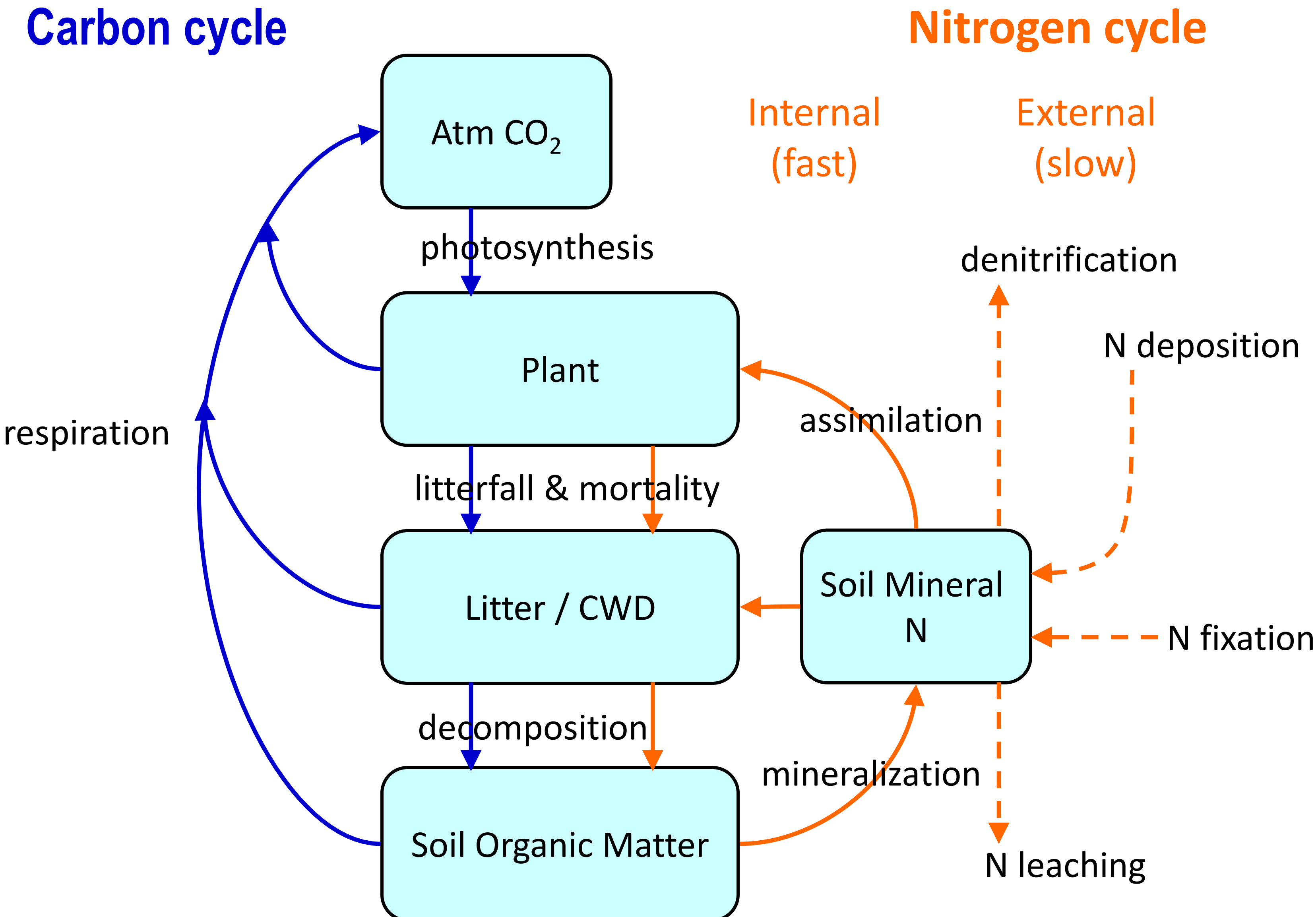
Oak Ridge Experiment on CO_2 Enrichment of Sweetgum

- Fine-root production was stimulated by elevated CO_2, especially deeper in the soil, leading to greater carbon input to soil and greater access to mineral nitrogen
- Initial enhancement of net primary productivity was not sustained because of feedbacks through the nitrogen cycle
- Stable isotope analysis indicated that N availability declined faster in plots exposed to elevated CO_2, consistent with model predictions
- Carbon storage in the soil increased in CO_2-enriched plots, including in protected forms
- Successional development of the understory community was accelerated in elevated CO_2

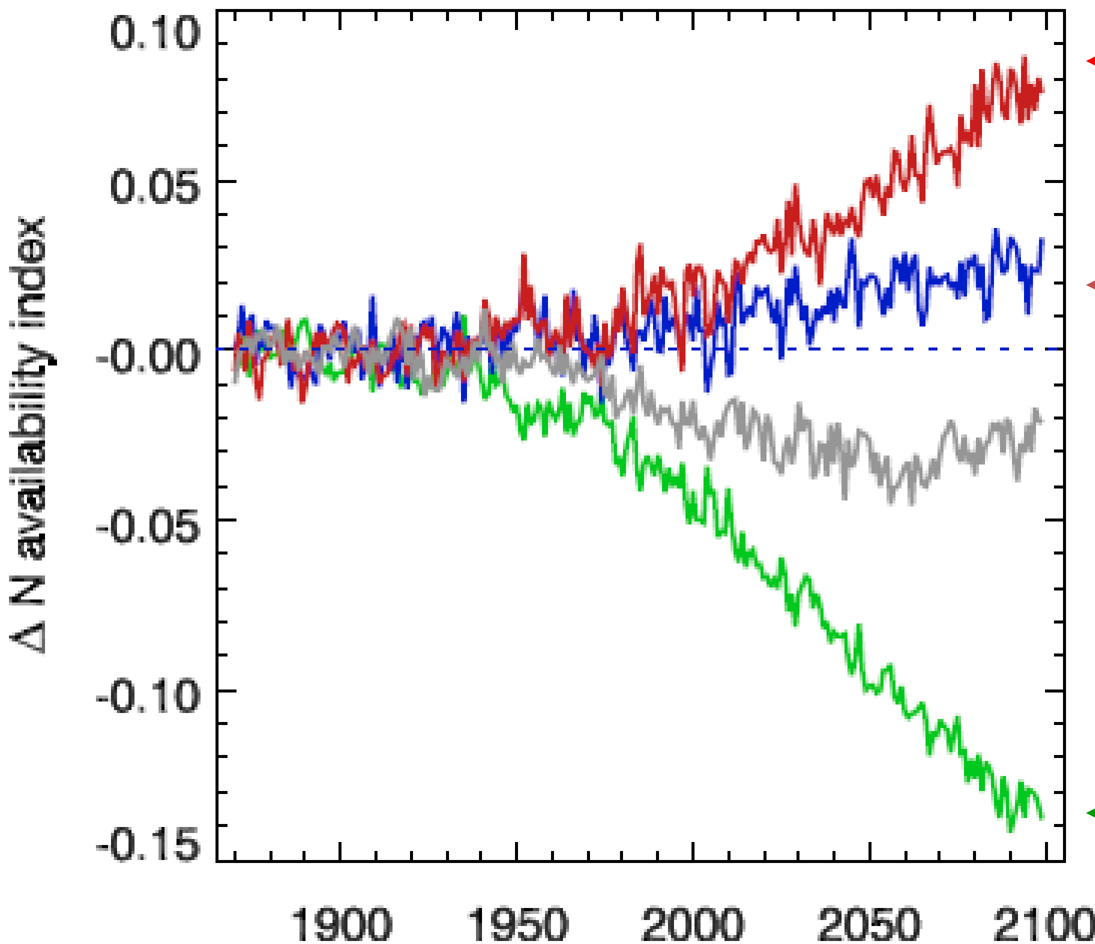


 Managed by UT-Battelle
for the U.S. Department of Energy

It's not just the carbon cycle, either ...



Effects on N availability (actual: potential GPP)



Increased N availability due to climate change

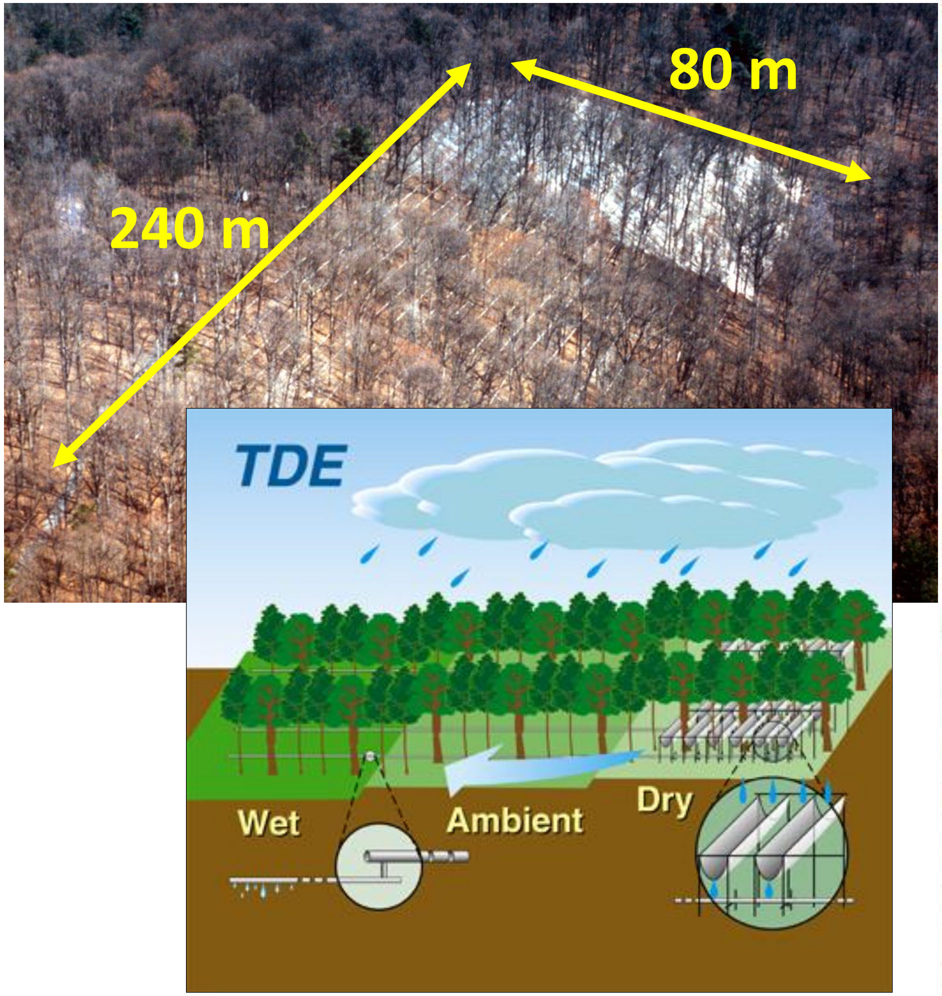
Increased N availability due to anthropogenic N deposition

Net effect (all factors)

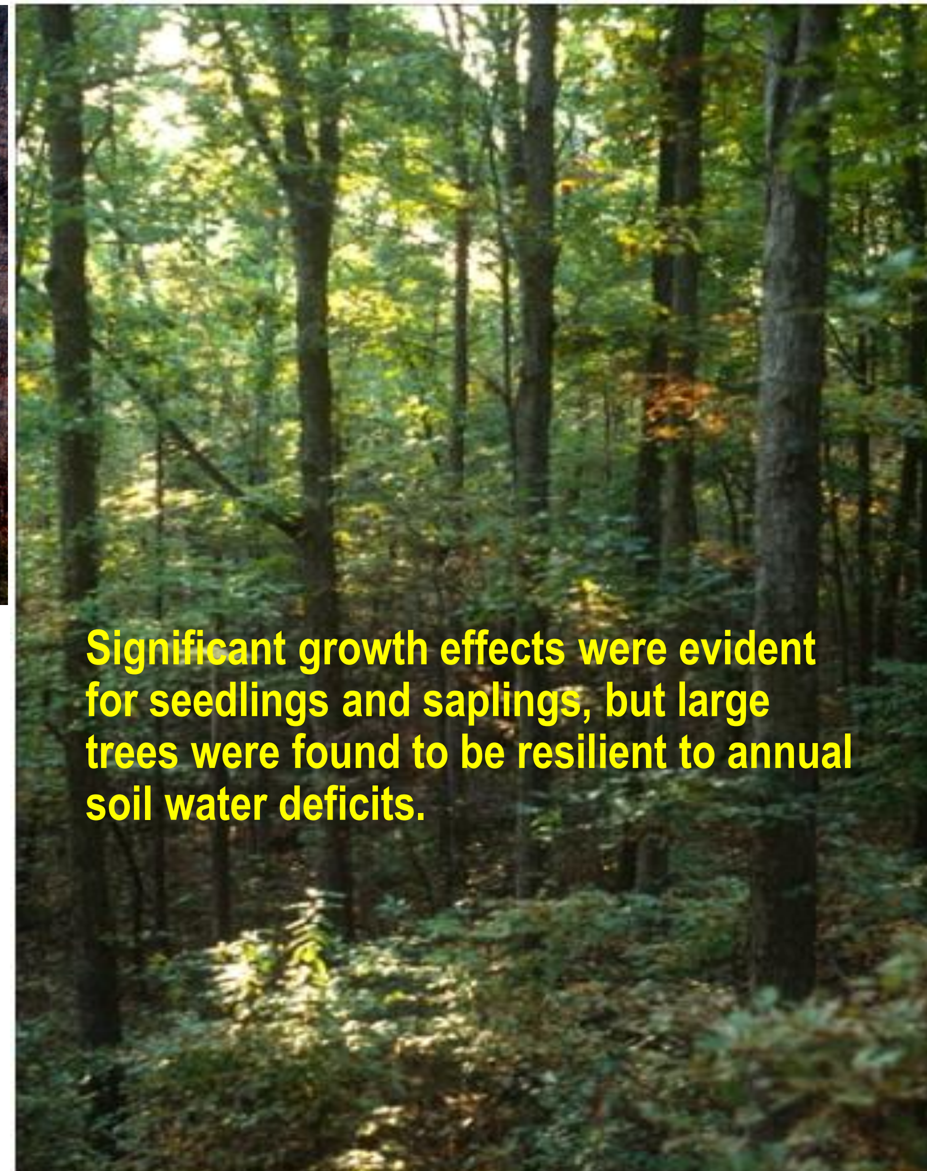
Decline in N availability under increasing CO_2

Thornton and others

Long-term Precipitation Change Experiment (1993 to 2006)

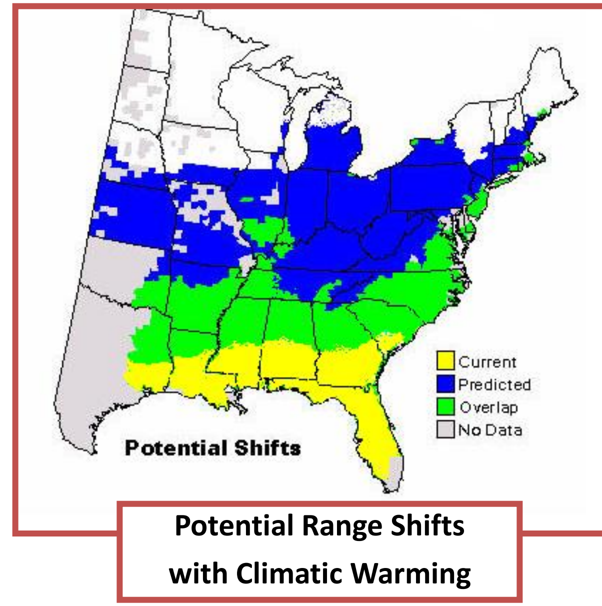


13-years of chronic manipulation (±33%) at realistic ecosystem scales (1.9 ha in three 0.64 ha plots)



Forest responses to a warmer climate

Response and Adjustment in Trees



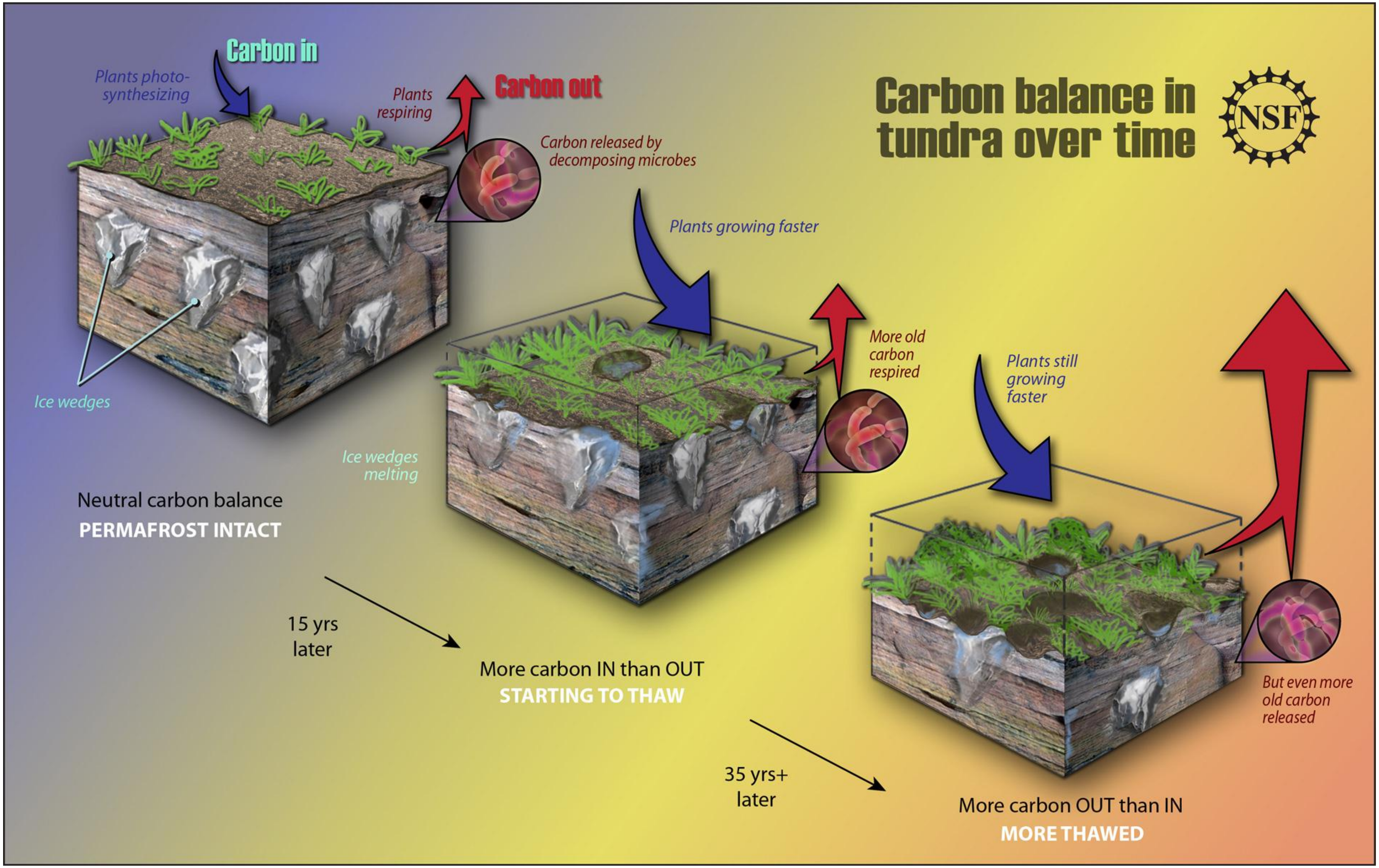
- Provide a mechanistic basis and ecological understanding to inform predictions of forest ecosystem responses to atmospheric warming
- Temperature-controlled Chambers;
  - Ambient, +2°C, +4°C

Conclusions:

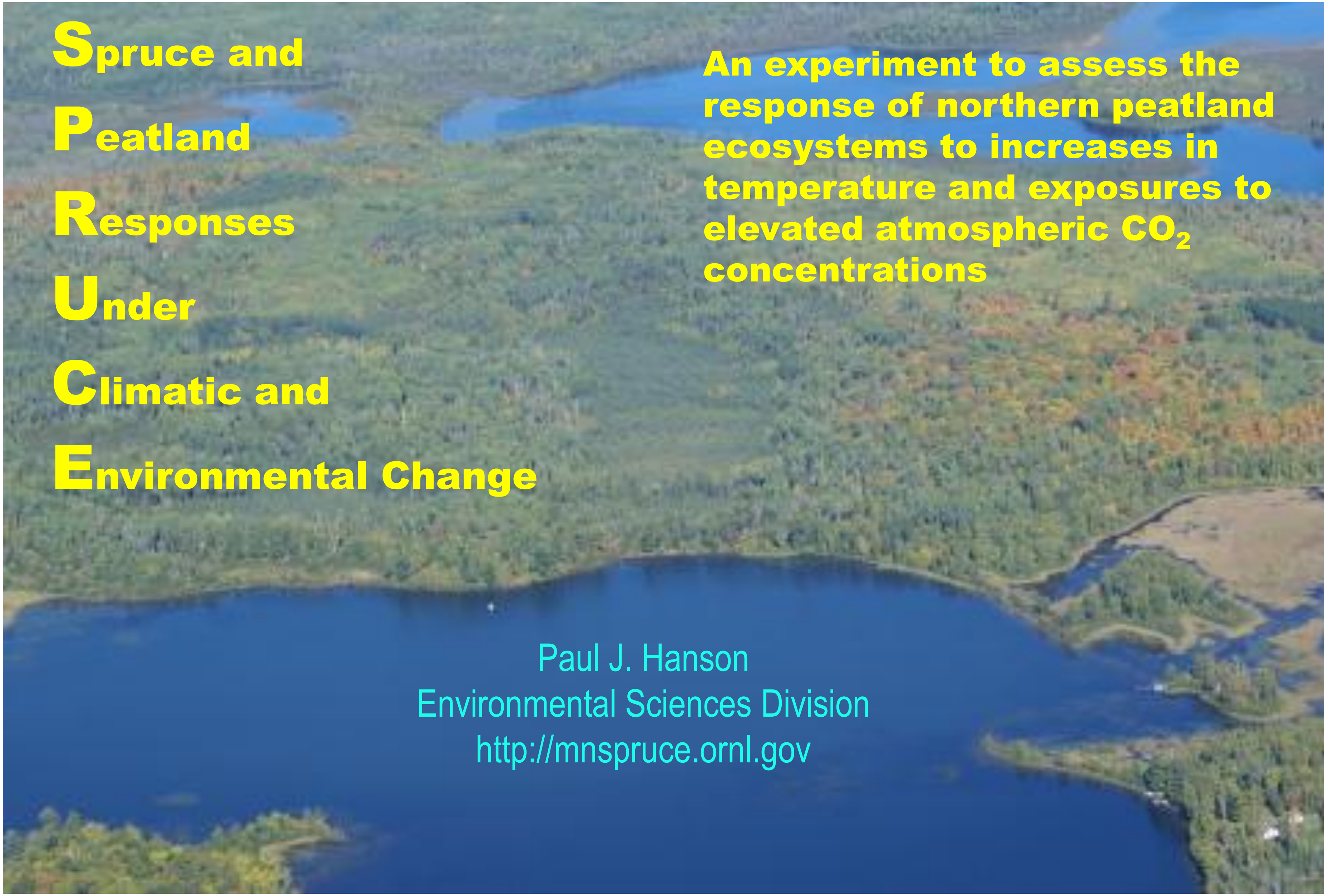
Warming extends the growing season - a benefit

Tissue acclimation minimizes the respiratory loss of carbon from accelerated physiological processes

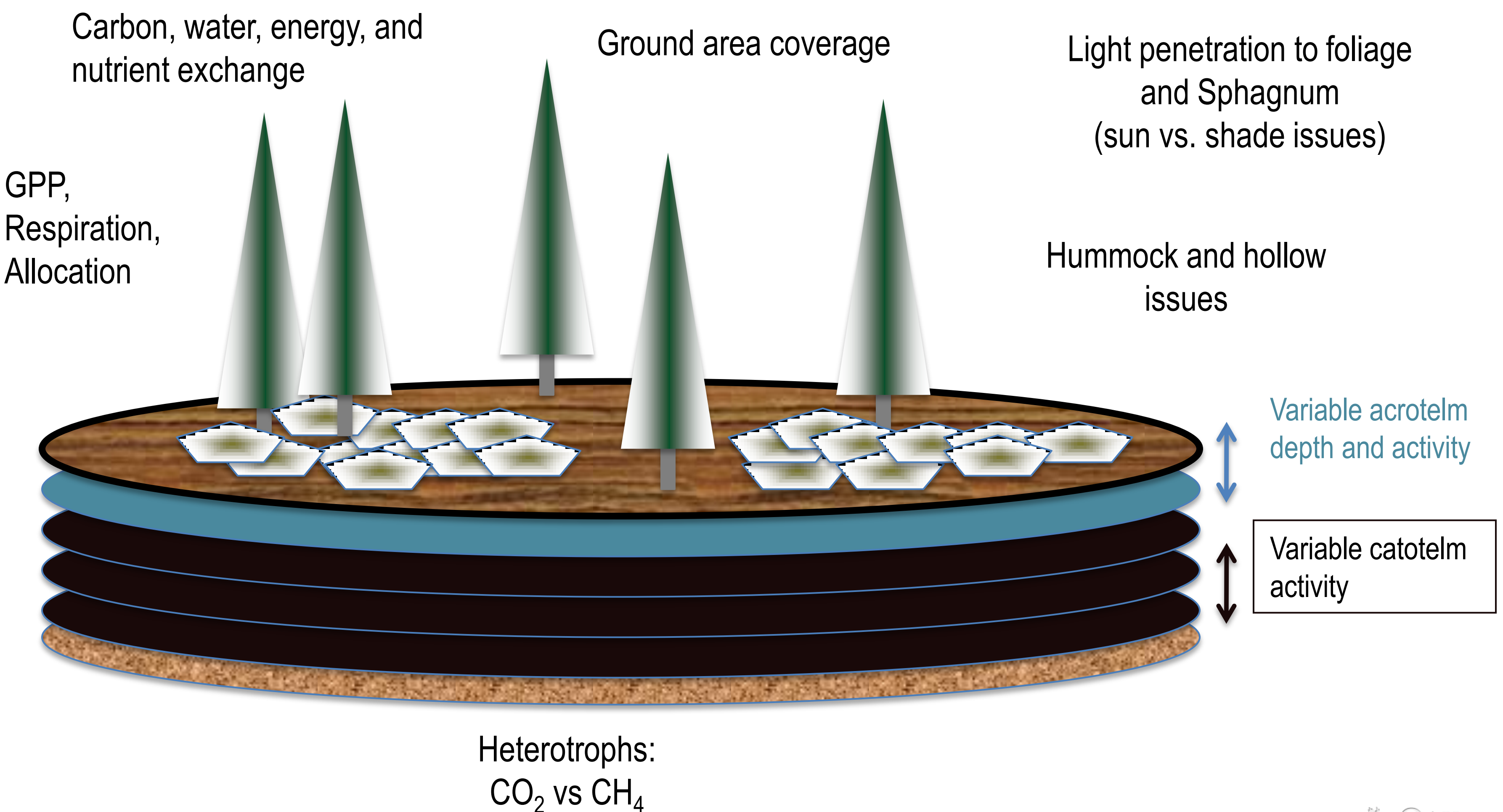
Critical to understand response of high-latitude ecosystems response to climate change



Zina Deretsky, National Science Foundation


Spruce and
Peatland
Responses
Under
Climatic and
Environmental Change
An experiment to assess the response of northern peatland ecosystems to increases in temperature and exposures to elevated atmospheric CO_2 concentrations
Paul J. Hanson
Environmental Sciences Division
http://mnspruce.ornl.gov

System Modeling of Biogeochemical Cycles and Organism Responses




Lat. = 47.506234°
Long. = -93.452209°
S
S1
Minnesota
Spruce Experiment
Michigan
Wisconsin
298 km
© 2010 Google
Image USDA Farm Service Agency
© 2010 Europa Technologies
© 2010 Tele Atlas
Google
Chicago
N

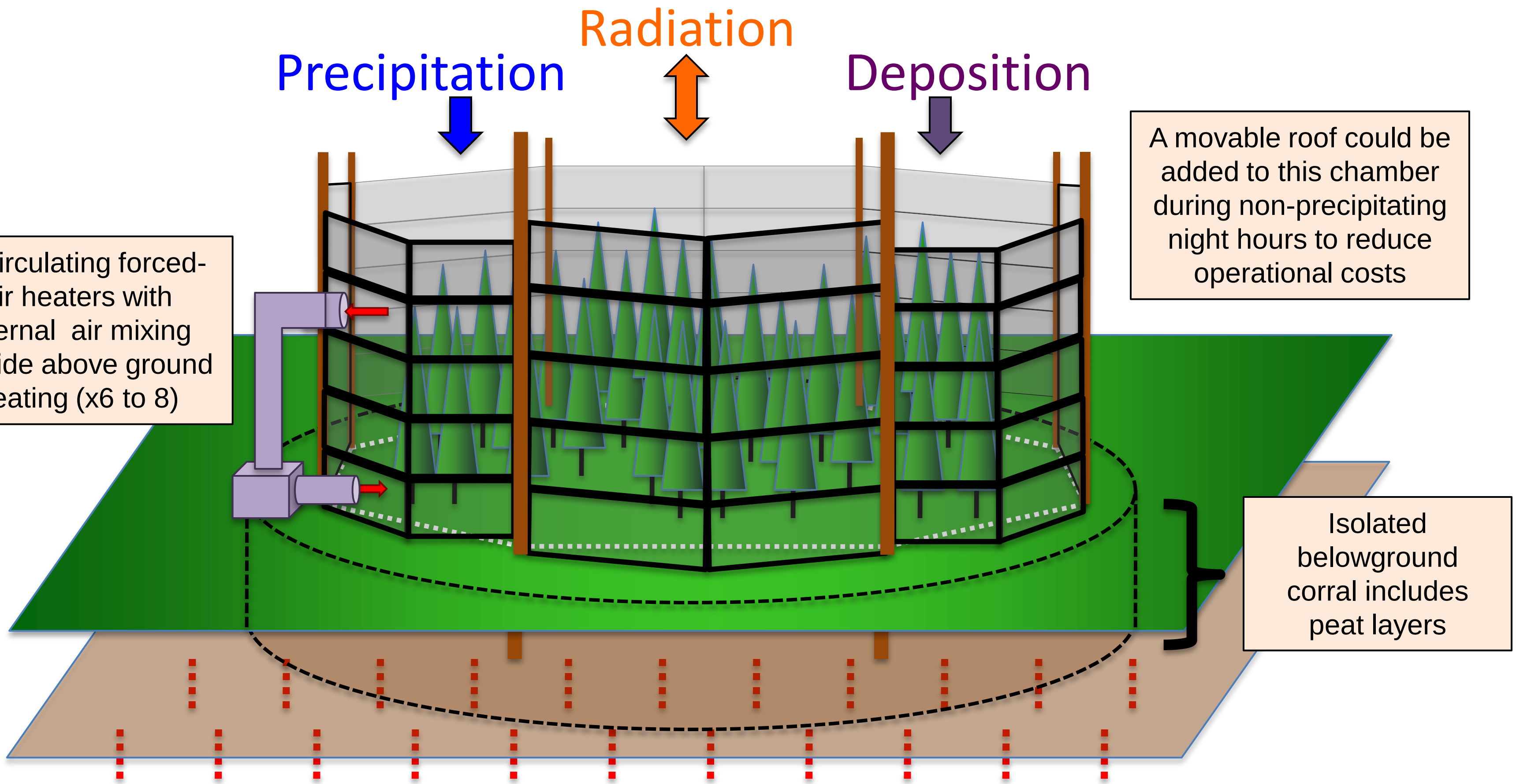
The *Picea – Sphagnum* Ecosystem

- A Critical Ecosystem
  - The experiment will be conducted in a *Picea mariana* [black spruce] – *Sphagnum spp.* forest in northern Minnesota
  - This ecosystem located at the southern extent of the spatially expansive boreal peatland forests is considered vulnerable to climate change and is expected to generate important greenhouse gas feedbacks to the atmosphere under changing future climates

Our Approach to Warming

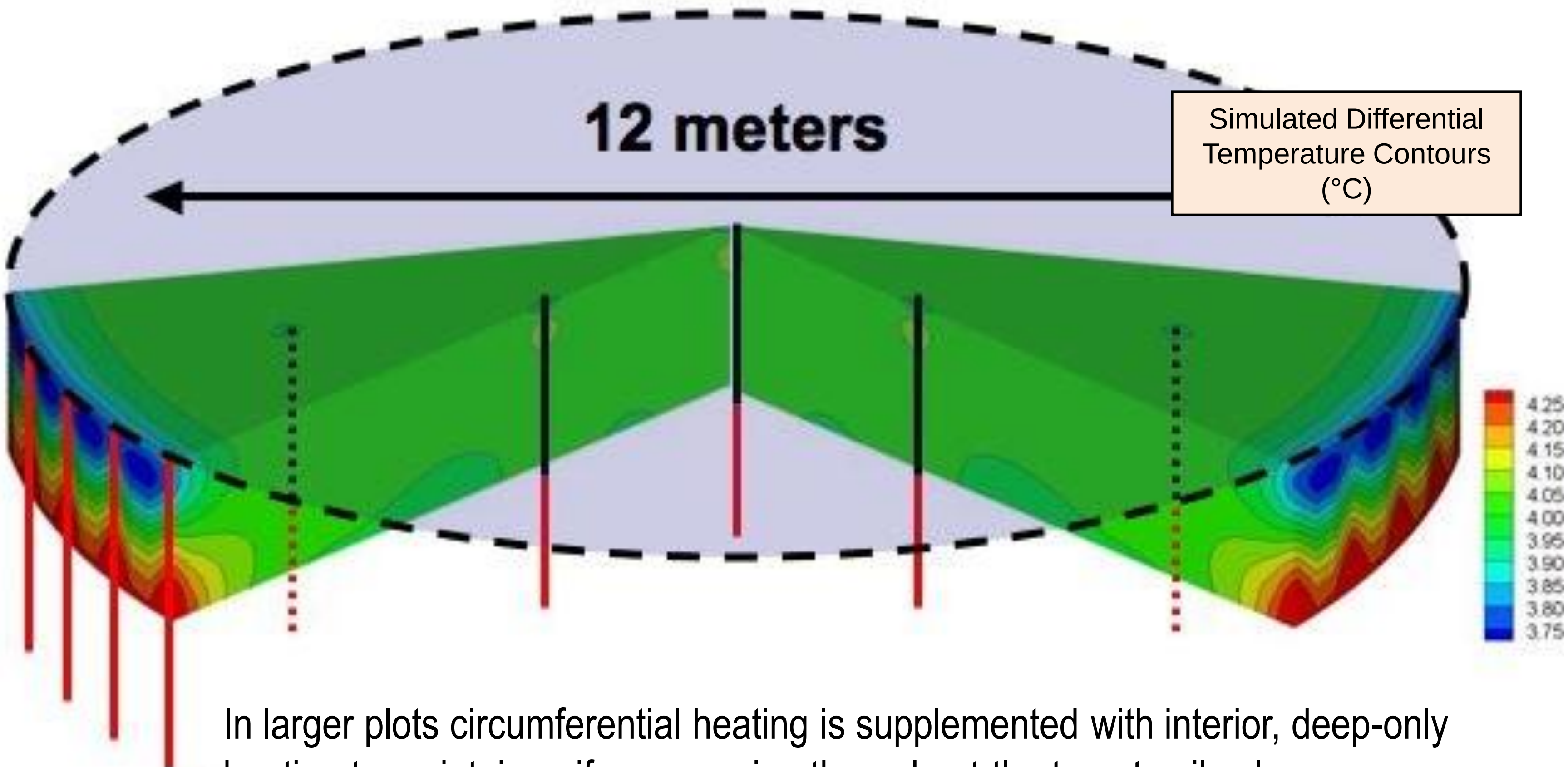
- By 2100 future terrestrial environments may be 4 to 8° C warmer than today (Solomon *et al*. 2007) depending on the location
- An overlooked reality is that mean deep (>1m) soil temperatures will also rise with climate warming
(Huang 2006)
- Experimental systems must be improved to provide the best atmospheric and soil conditions appropriate for characterizing terrestrial ecosystem responses to year 2100 scenarios -- air and soil warming by as much as 8 to 10° C
- Projected climate conditions are beyond those observable in current natural settings or through the use of climate gradients; <u>We can not adequately substitute space for time</u>

Response SFA Experimental Plot



Belowground heating provided by gradual heat inputs from deep heating probes located away from the target biology

Belowground heating designs to accommodate full ecological diversity have been simulated and are being constructed for testing for application to the whole-ecosystem manipulations in the replicated study in the S-1 bog



In larger plots circumferential heating is supplemented with interior, deep-only heating to maintain uniform warming throughout the target soil volume

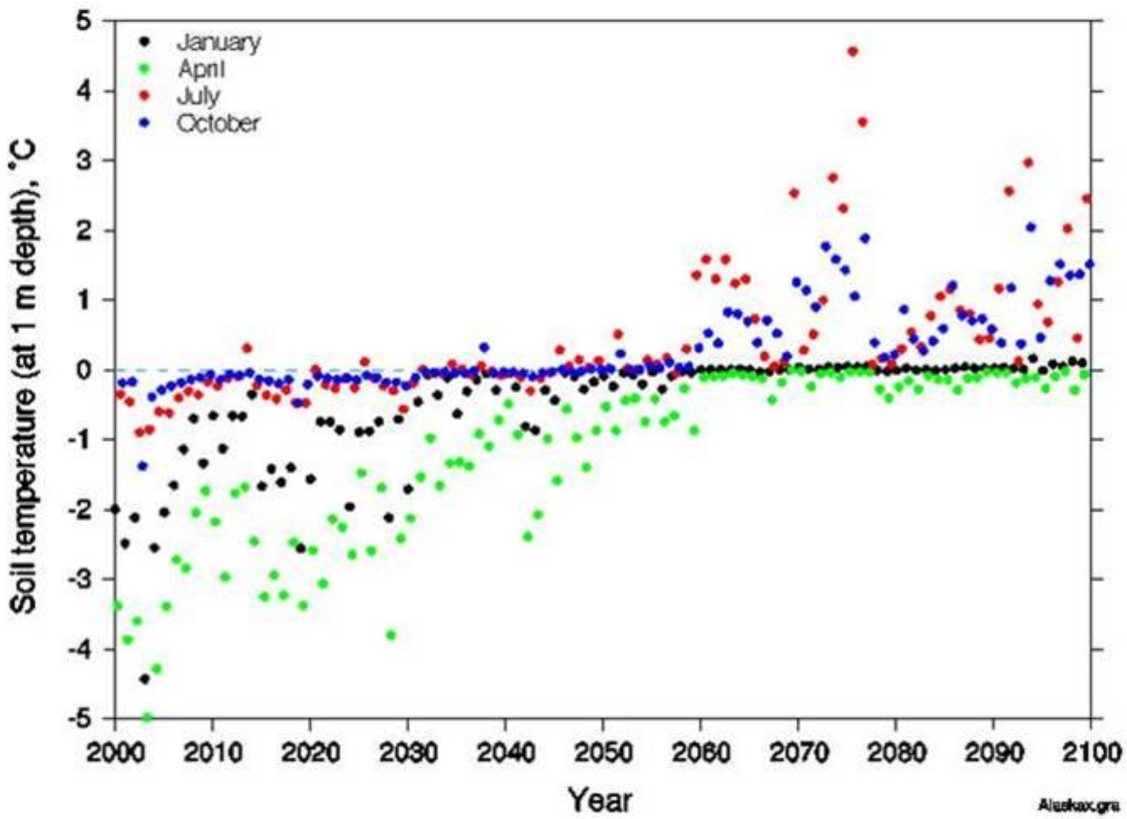

United Rentals

A simulation for a future northern Alaska

An improved, deeper soil profile was added to the Community Land Model (CLM)

In simulations based on the A1B IPCC emissions scenario, within about 50 years permafrost at 1 m depth becomes summer water

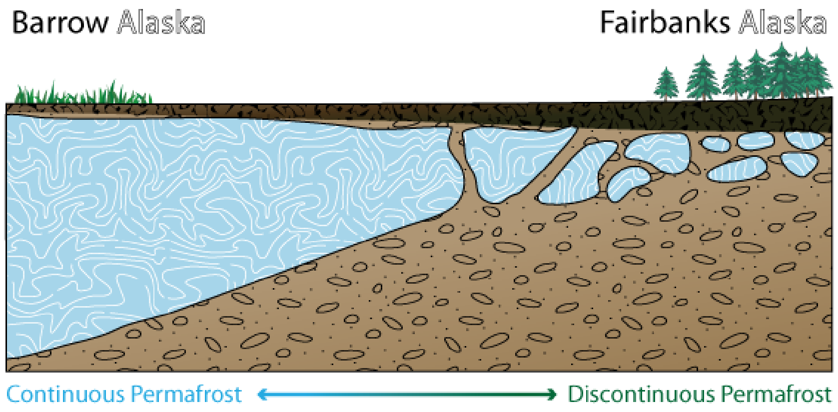
By year 2100 about 80% of "near-surface permafrost" is lost



Data courtesy of Dr. Dave Lawrence [see *Journal of Geophysical Research* 113, F02011, 2008]

The structure (short vegetation) of arctic tundra may make it amenable to the next-generation experiment, but the arctic environment would pose challenges

- Scientifically:
  - High-latitude permafrost contains large stocks of carbon (under both tundra and boreal forest)
  - Past, present, and future warming is greatest at high latitude
  - Warming increases the active layer depth (depth of summer soil thawing) and melts permafrost, which could cause a LARGE net release of CO_2 and/or CH_4 to the atmosphere — a strong positive feedback to warming
  - Warming might reduce albedo (another positive feedback)



Site Selection

Fairbanks, Alaska

Permafrost Research Station

Lat./Long. 64.877N, 147.670W

Ave. temperature: -3.3 ^{o}C

Active layer: 55 to 85 cm

Permafrost: low moisture

Barrow, Alaska

Barrow Environmental Observatory

Lat./Long. 71.277N, 156.619W

Ave. temperature: -12.6 ^{o}C

Active layer: 30 to 50 cm

Permafrost: ice rich

Microbial transformation of carbon buried in permafrost

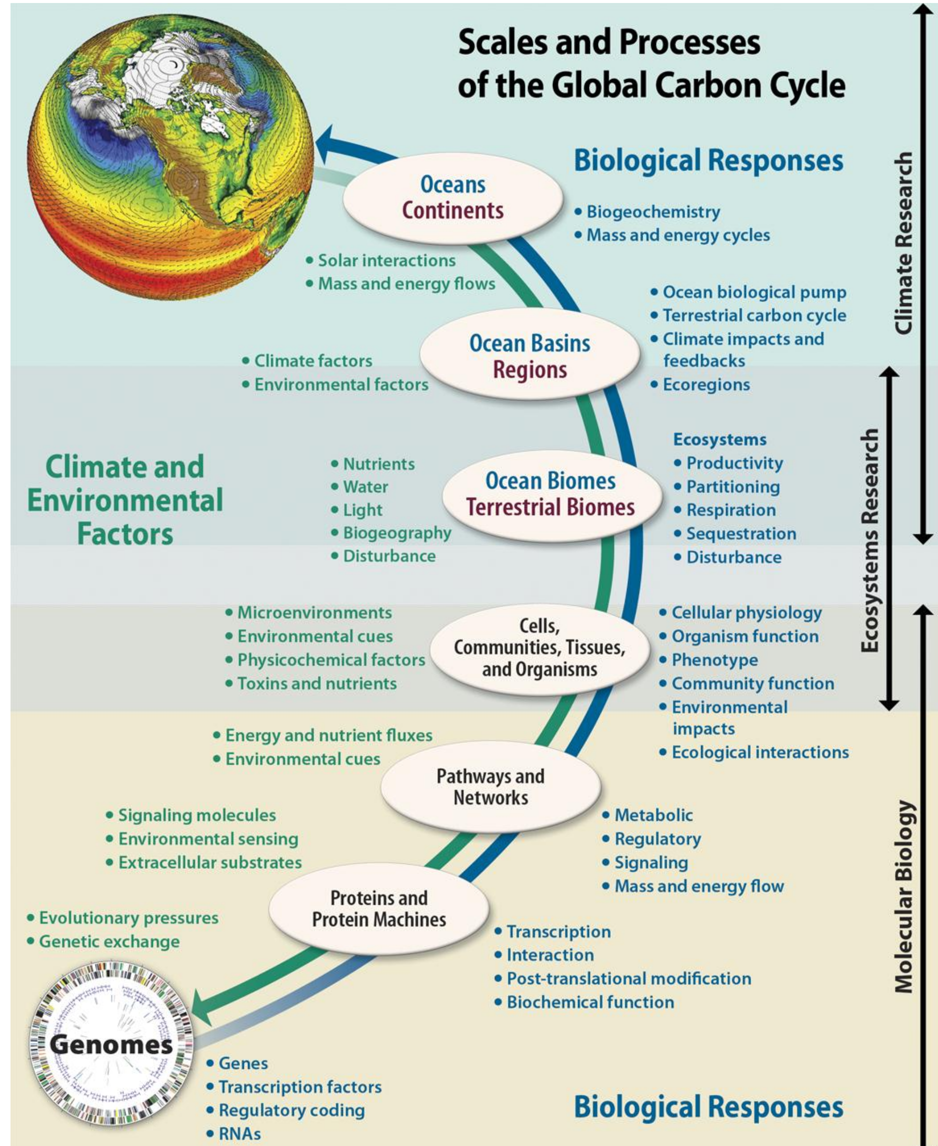
Discriminating between two hypothetical outcomes of permafrost thawing and carbon biodegradation

Opportunities for collaboration:
CARVE - Carbon in Arctic Reservoirs Vulnerability Experiment (Charles Miller, Principal Investigator)

Photos
NGEE-Arctic (http://ngee.ornl.gov)
http://academic.emporia.edu/aberjame/wetland/baltic/baltic.htm

New Scaling and Complexity in Climate Change Science

- **Multiple, interacting factors from molecular to global scales**
  - Nitrogen cycle
  - Feedbacks in critical ecosystems (e.g., boreal, permafrost, tropical forests)
  - Shifts in biological systems at molecular scale with impacts at large scales

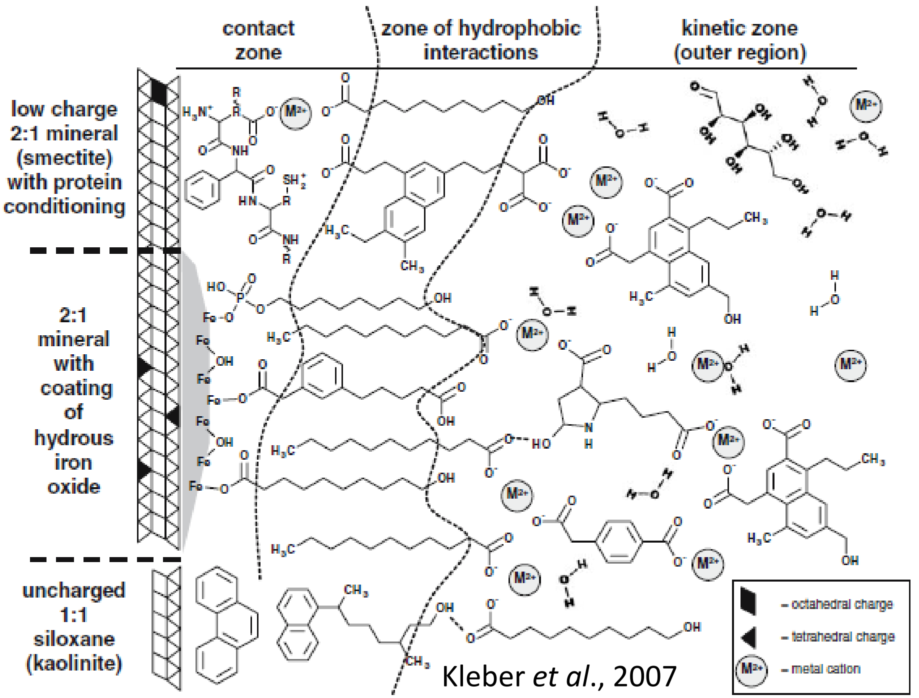


U.S. DOE. 2008. Carbon Cycling and Biosequestration: Report from the March 2008 Workshop, DOE/SC-108, U.S. Department of Energy Office of Science (http://genomicsgtl.energy.gov/carboncycle/).

Improving Soil C Modeling

Modeling C "pools" is an *interfacial* problem: C preservation is determined by attachment to minerals

- **Missing processes:**
  - Carbon chemistry
  - Molecular-scale sorption
  - Mineral type
  - Activities of microbes
  - Leaching of dissolved OC

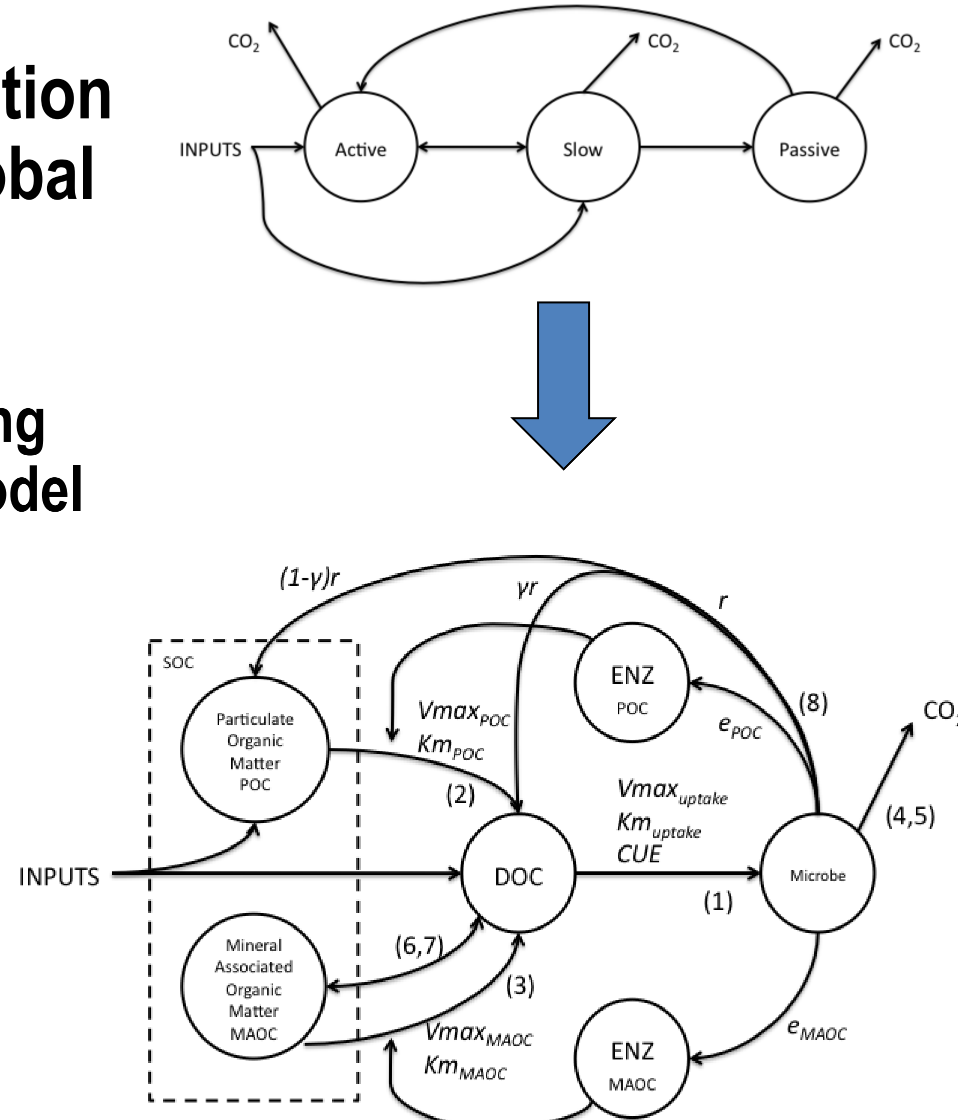


Kleber *et al.*, 2007

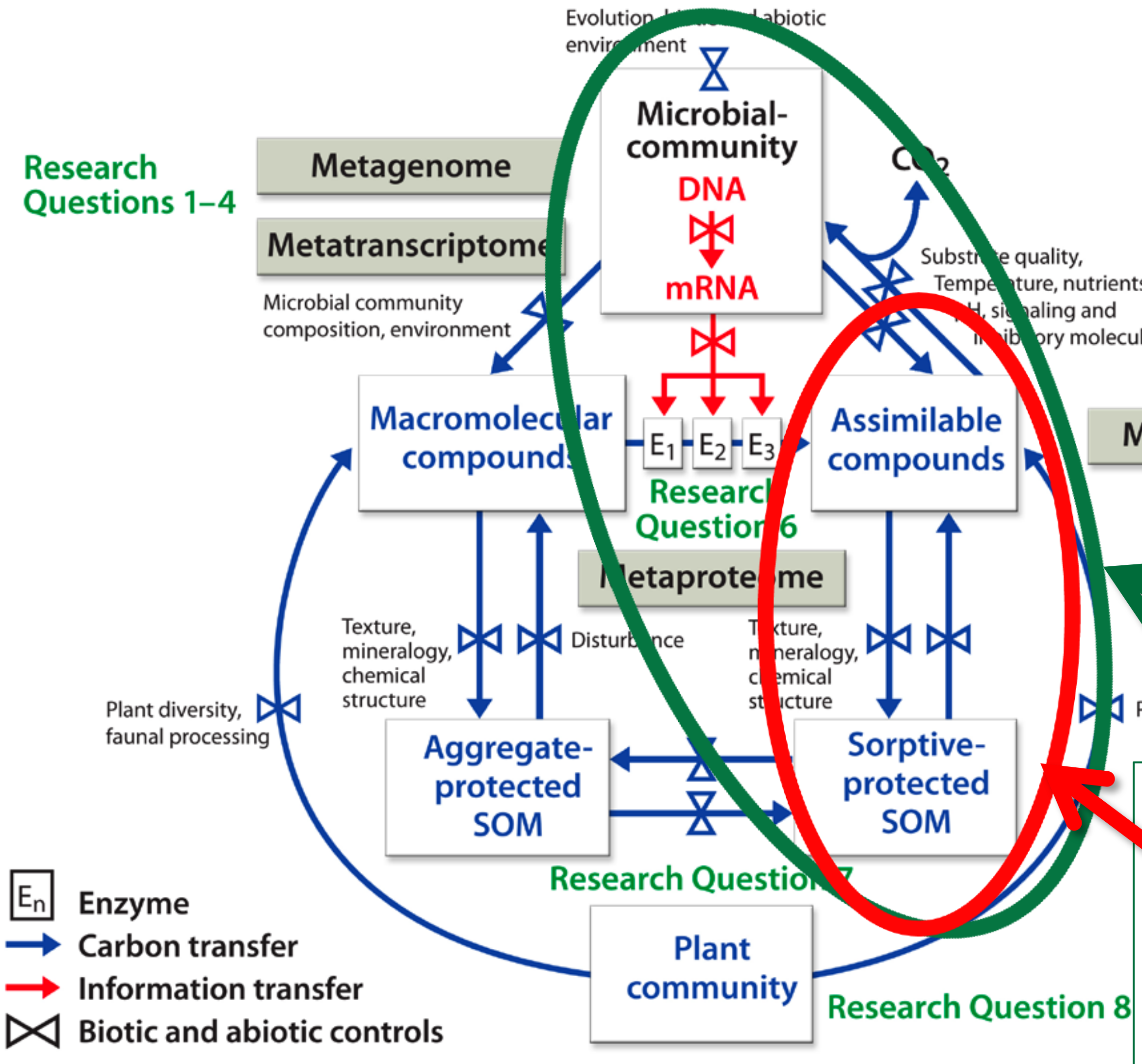
- Currently, we rely upon very limited quantitative databases

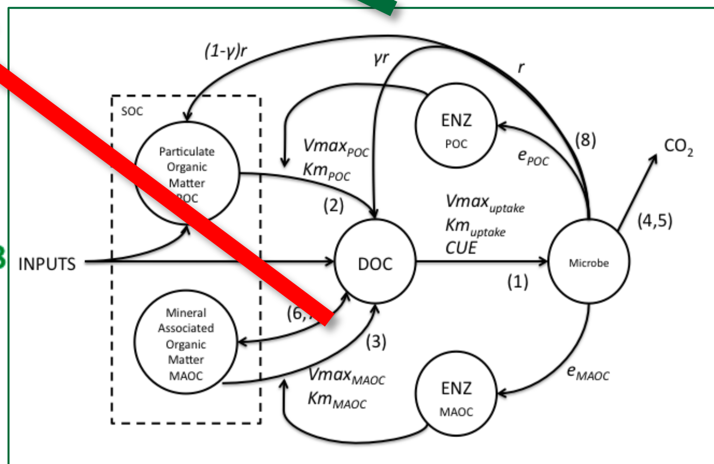
New advances are needed in development of a global soil C simulation model

- **Advance soil OC decomposition models currently used in global models:**
  - **Incorporate recent microbial-enzyme process understanding into a SOC decomposition model**
  - **Concentrate on DOC-MAOC model components and parameters**

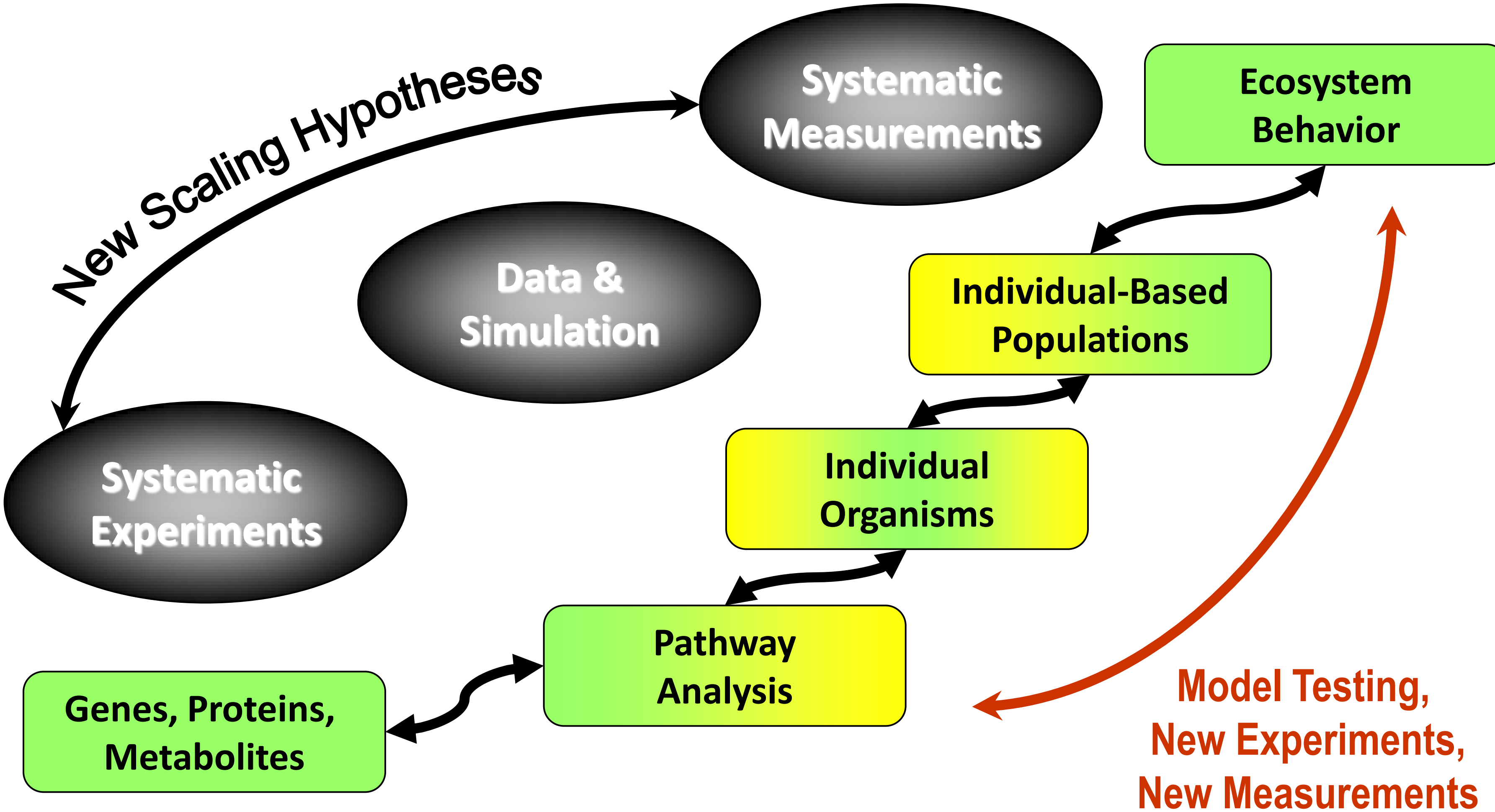


There are important scientific opportunities with this approach that bridge biology and environment

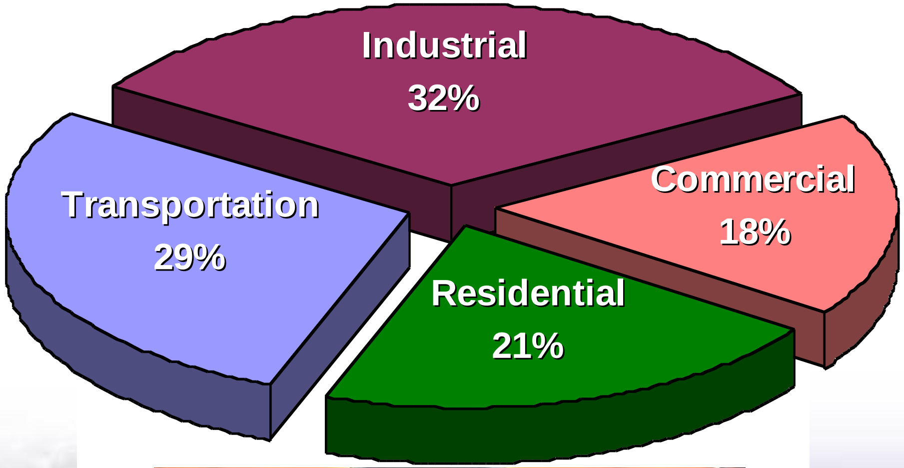


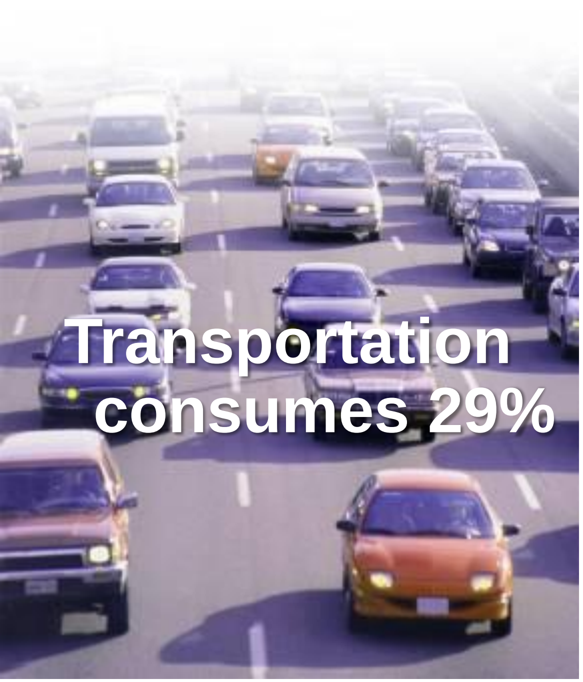


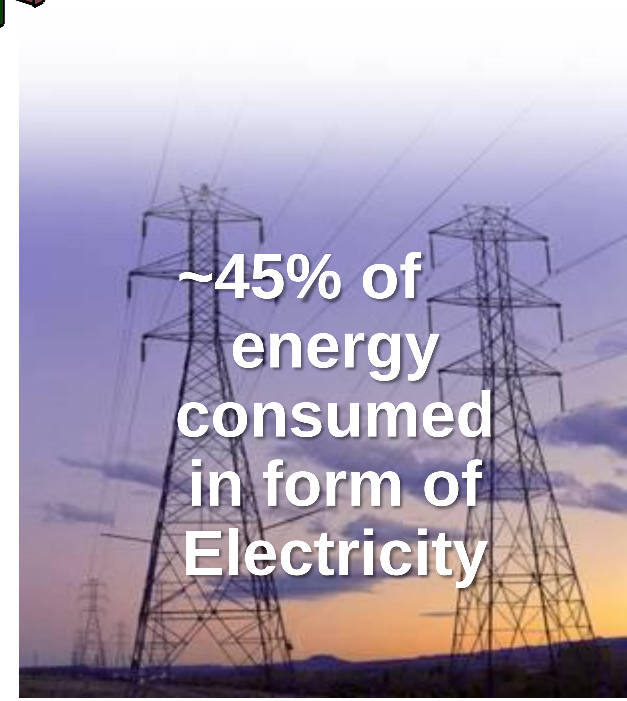
Scaling Ecosystem Dynamics:
New scientific linkages



A Majority of Energy is Consumed in Transportation and Electricity Sectors







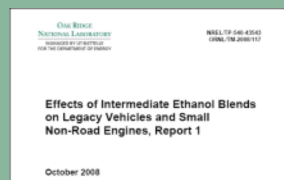
Oak Ridge National Laboratory

Breaking Petroleum's Vise Grip on Transportation, Solving the Carbon Challenge

Scientific Discovery | **Modeling & Simulation** | **Technology Innovation**

Alternative Fuel Sources

Bio-based fuels



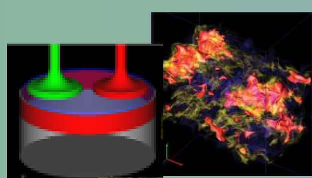
Effects of Intermediate Ethanol Blends on Legacy Vehicles and Small Non-Road Engines, Report 1

October 2008

Compatibility

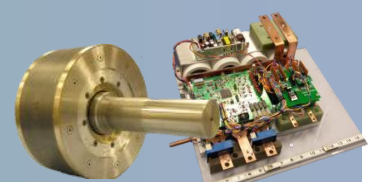
Electrification

Efficient Vehicle Technology



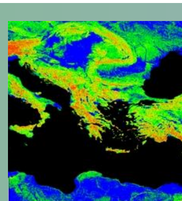
High-efficiency clean combustion

Advanced materials



Energy recovery & management

Optimized Infrastructure



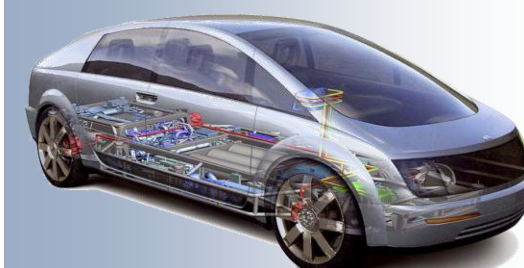
Geospatial information systems

Cognitive radio

Connectivity

Integrated Solutions

- >100 MPGe vehicles
- Diverse, domestic-source fuels
- Highly intelligent, adaptive vehicles and infrastructure
- Mobility and livability
- Affordability
- Safety and security
- Sustainability



Biomass to Fuels and High Value Chemicals

DOE: Office of Science | **DOE: EERE VTP & Biomass** | **Industry**

Bio-derived alcohols, precursors, chemicals

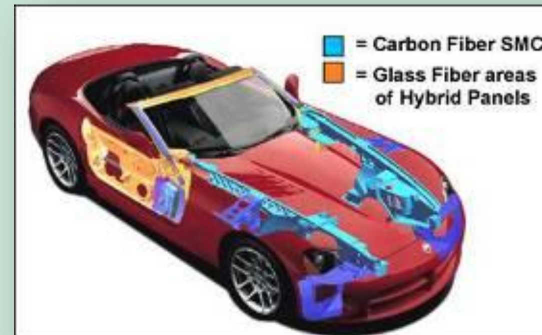
BESC
BioEnergy Science Center

Lignin as low cost feedstock lightweight carbon fiber material

\

Succinic Acid…Renewable and environmentally friendly bioproduct

Intermediate Ethanol blends vehicle evaluation program



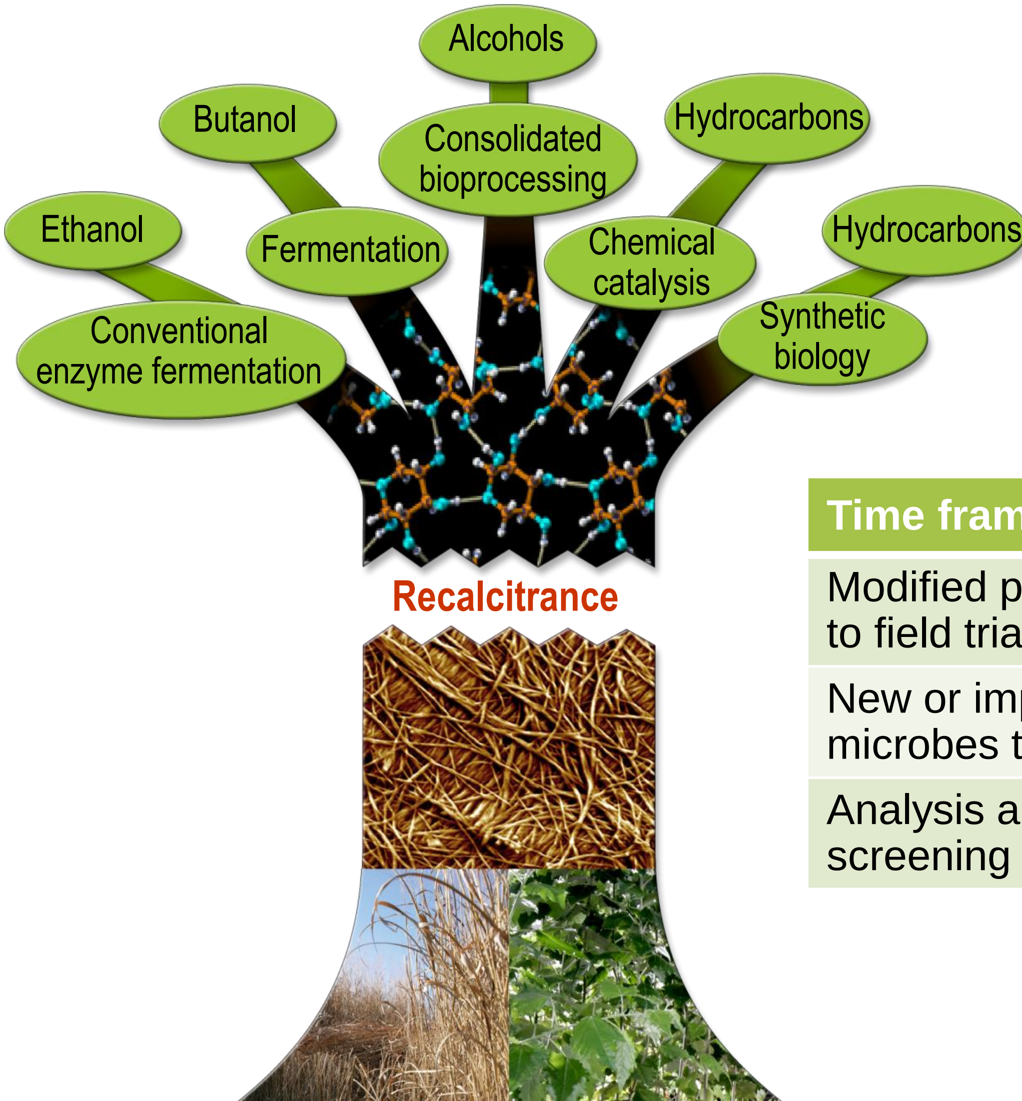
Dual value stream: Ethanol fuel and lignocellulose byproduct is sustainable

Bio-derived chemical (de-icer, solvent applications), commercial plant online 2009

Bio amber

Biomass research yields domestic fuel, high-value chemicals

Access to the sugars in lignocellulosic biomass is the current critical barrier



Time frame	Planned	Actual
Modified plants to field trials	Year 5	Year 4
New or improved microbes to development	Year 4–5	Year 3–4
Analysis and screening technologies	Year 3 on	Year 2 on

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The BioEnergy Science Center

U.S. DEPARTMENT OF ENERGY

A multi-institutional DOE-funded center performing basic and applied science dedicated to improving yields of biofuels from cellulosic biomass

- Samuel Roberts Noble Foundation
- National Renewable Energy Laboratory
- Brookhaven National Laboratory
- Cornell University
- University of Minnesota
- Washington State University
- University of California–Riverside
- North Carolina State University
- Virginia Polytechnic Institute
- University of California–Los Angeles

322 People in 19 Institutions

- Oak Ridge National Laboratory
- University of Georgia
- University of Tennessee
- Dartmouth College
- West Virginia University
- Georgia Institute of Technology
- ArborGen, LLC
- Ceres, Incorporated
- Mascoma Corporation

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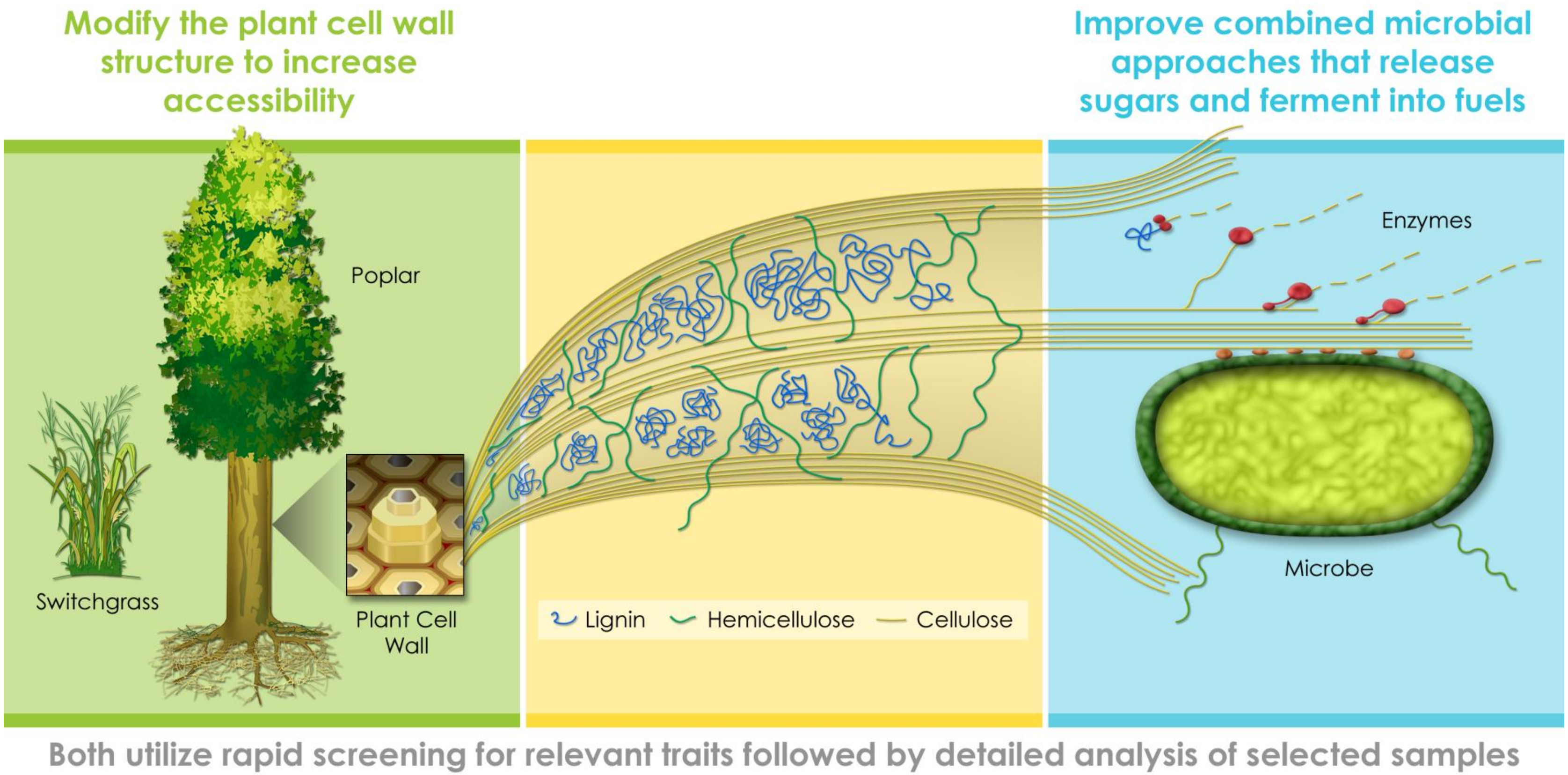
A two-pronged approach to increase the accessibility of biomass sugars



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Genetic block in lignin biosynthesis in switchgrass increases biofuel yields

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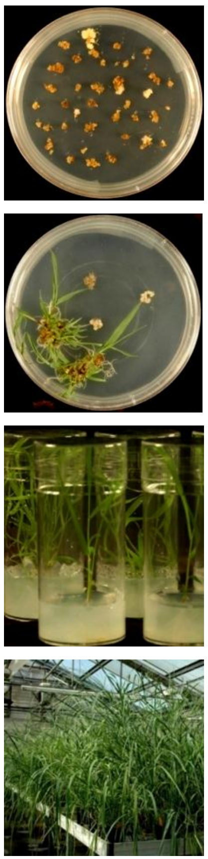
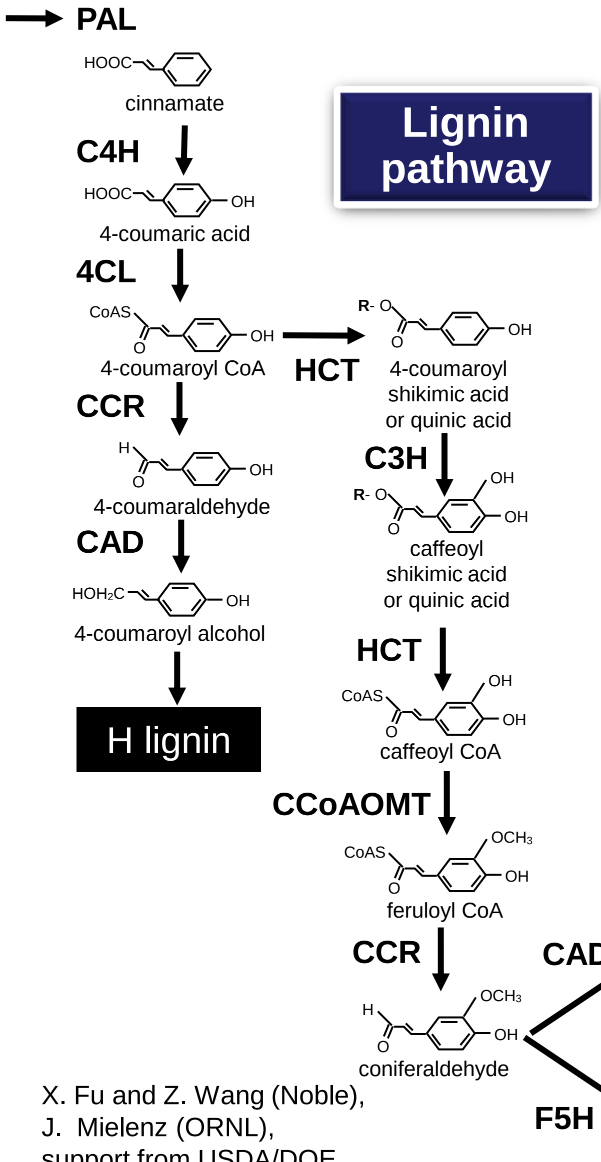
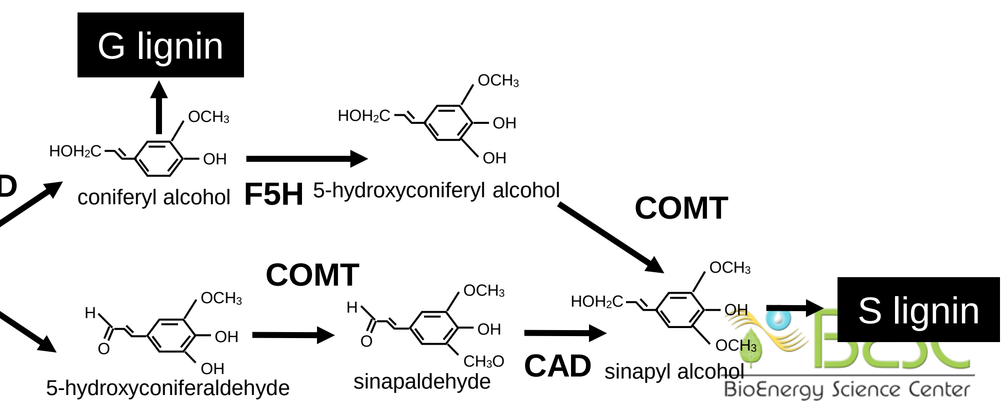
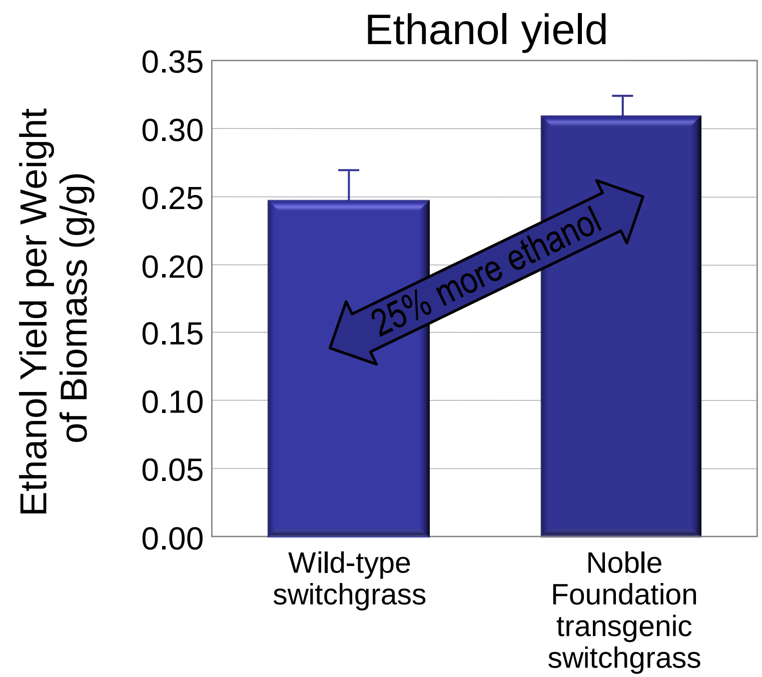
Agrobacterium-mediated transformation of switchgrass









THE SAMUEL ROBERTS NOBLE FOUNDATION

X. Fu and Z. Wang (Noble),
J. Mielenz (ORNL),
support from USDA/DOE

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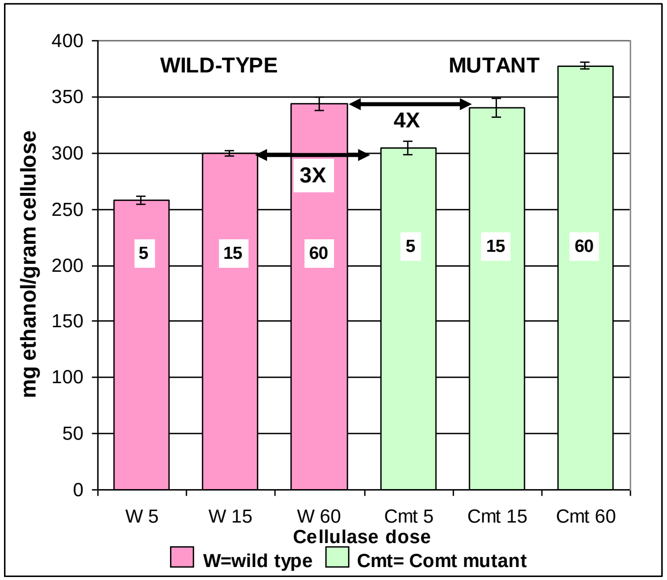
Genetic block in switchgrass lignin pathway reduces enzyme costs by 3-4 fold



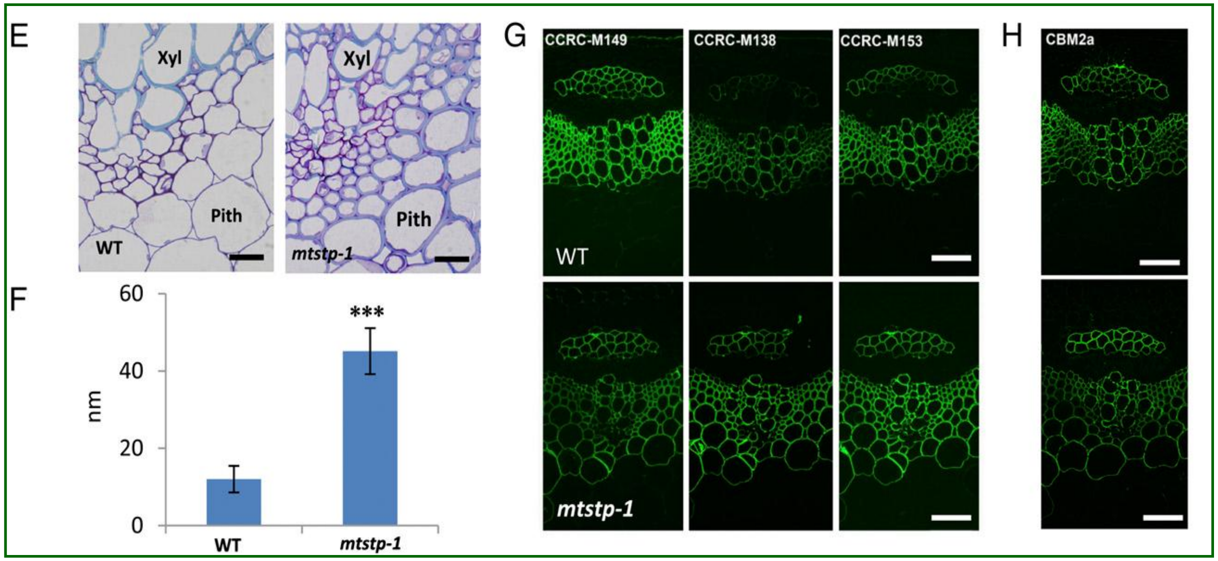
Switchgrass lignin mutants have reduced recalcitrance

- Enzymes used in biomass ethanol cost 32¢ per gallon (2008 OBP Report)
  - DOE Goal is to reduce enzyme costs by 4X
- New results show mutant Noble Foundation switchgrass yields the same ethanol yield as wild-type with 3-4-fold less enzyme dosage
  - Enzyme costs reduced to 8-11¢ per gallon, near the DOE goal
- Also mutant switchgrass produces more ethanol with milder pretreatment conditions
  - Additional cost savings will results
  - Synergy of lower enzyme and milder pretreatment being tested

BioEnergy Science Center

Mutation of Key TF Increases Pith Cell Wall Thickness

- Mutants with secondary cell wall thickening in pith cells leading to an ~50% increase in biomass density in stem tissue of the *Arabidopsis* mutants.
- Repression of TFs that activate secondary wall synthesis were confirmed by in vitro assays and in plant transgenic experiments.



Phenotypic analysis of the Mtstp-1 mutant in *Medicago*.
(E) Light microscopy of pith cell walls in WT and mutant.
(F) Quantification of cell wall thickness of the WTand mutant sections.
(G and H) Detection of xylan and cellulose by immunohistochemistry using monoclonal antibodies against distinct xylan epitopes (G) and a carbohydrate-binding module that binds crystalline cellulose (H) in stem sections. Antibody and CBM names are indicated. (Scale bar: E, 20µm;G and H, 10µm.)

- The discovery of negative regulators of secondary wall formation in pith opens up the possibility of significantly increasing the mass of fermentable cell wall components in bioenergy crops.

Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants
H Wang, U Avcib, J Nakashimaa, MG Hahn, F Chena, and RA Dixon (Noble, CCRC-UGa); PNAS (2010)

BESC leverages high-performance computing and neutron capabilities

BESC shares samples and insights with another BER project to develop lignocellulosic biomass-relevant analyses using neutrons and simulation

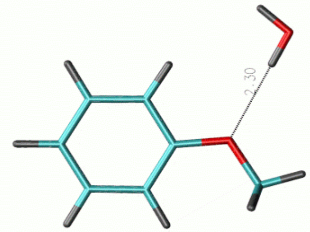
Building simulation models of plant cell walls

1 nm

1 μm

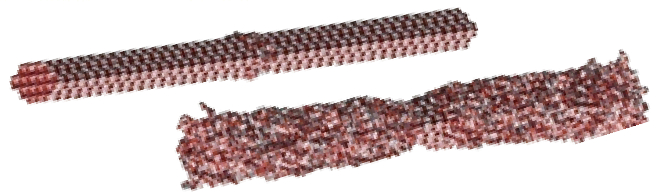
Length scale

Atomic



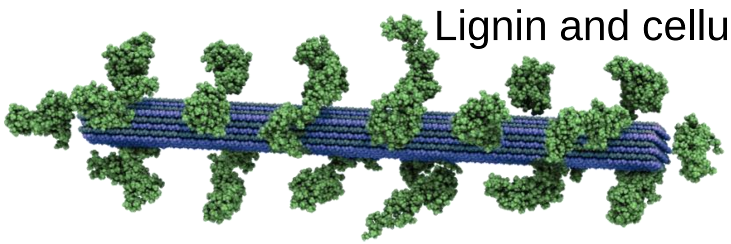
Model compounds: force field of lignin

Molecular



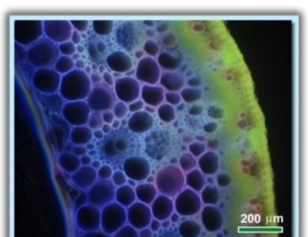
Cellulose

Supra-molecular



Lignin and cellulose

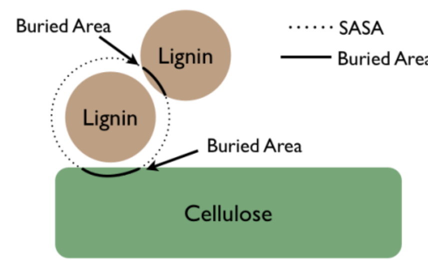
Cellular (future)



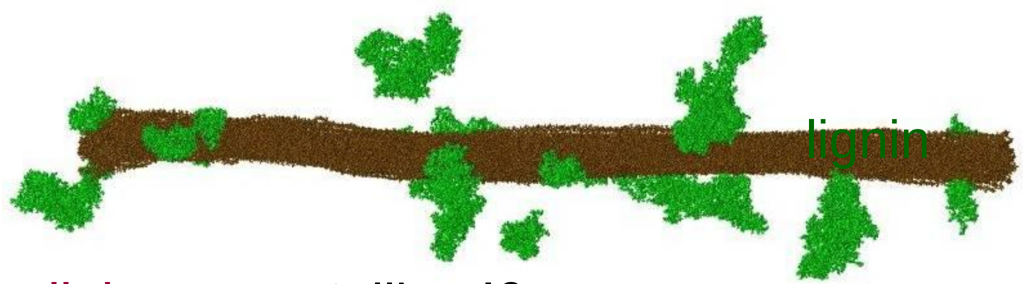
Switchgrass stem cross section

Lignin Aggregation and Precipitation onto Cellulose

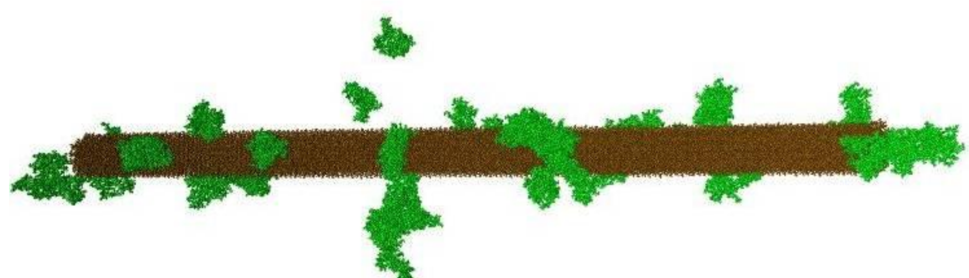
- Biomass recalcitrance after acid pretreatment: lignin aggregation & reprecipitation onto cellulose.
- We studied the effect of cellulose crystallinity on lignin reprecipitation.
- Lignin Forms Larger Interface (closer affinity) with Crystalline Cellulose vs. Semi-Crystalline



cellulose: semi-crystalline



cellulose: crystalline Iβ



Snapshot of the 2 lignin-cellulose models at the end of the Molecular Dynamics simulations

B. Lindner, L. Petridis, R. Schulz and J.C. Smith (in prep. ,2011)

Mining variation to identify key genes in biomass composition and sugar release

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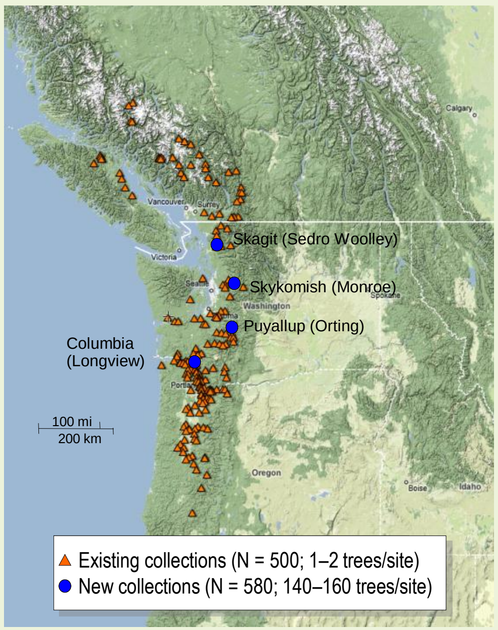
Collected ~1300 samples for *Populus* association and activation-tag study

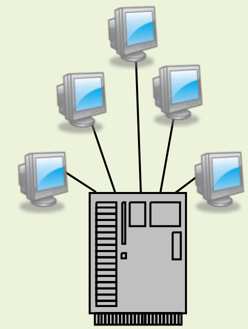


High-throughput screening pipeline

- Create genetic marker map to identify allelic variation
- Identify marker trait association

Sugar release assay

Cell wall biosynthesis database



Establish common gardens for association and activation-tag populations with thousands of plants

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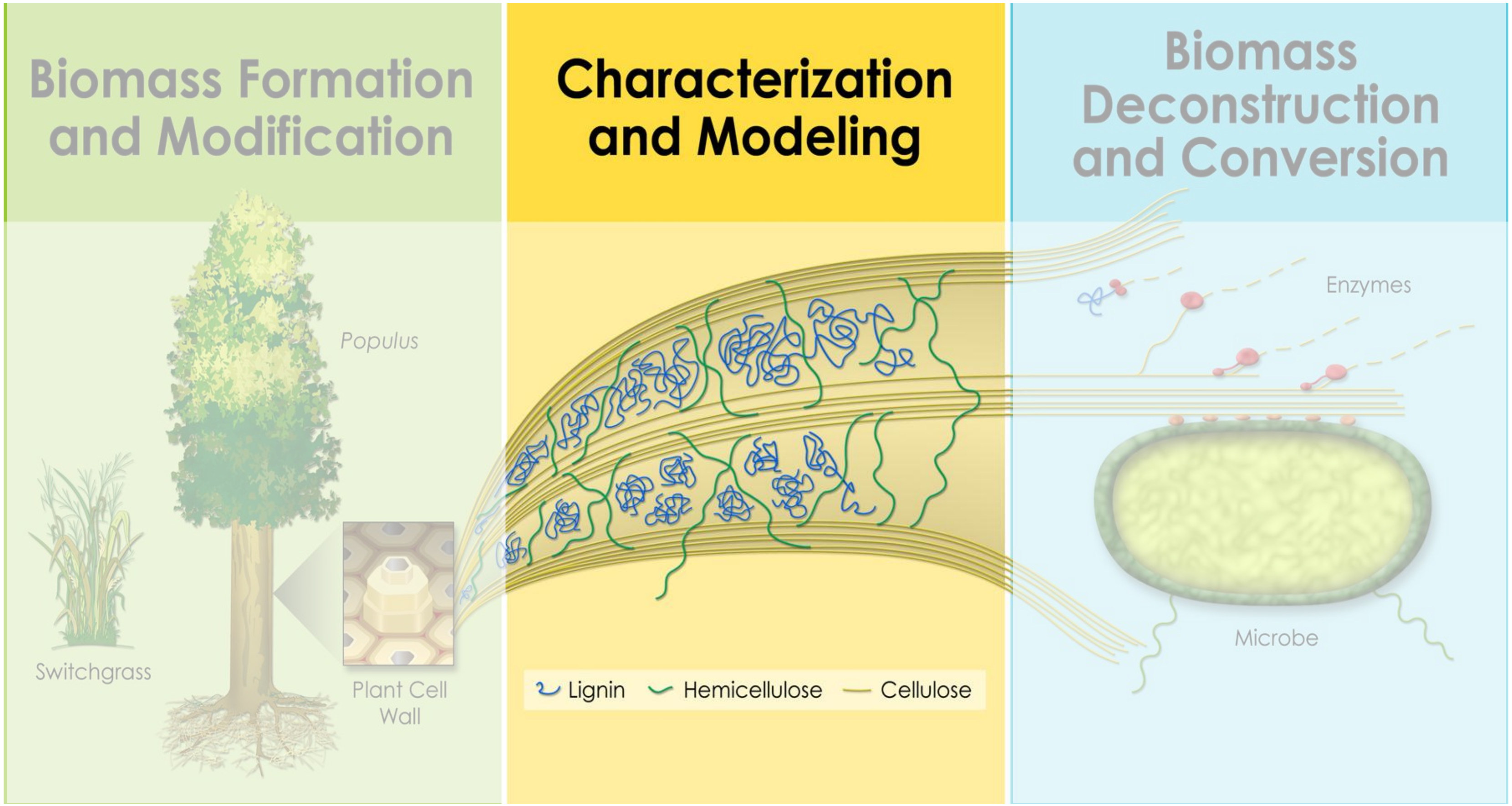
Strategy Part 2: Biomass Recalcitrance Measure, Understand, and Model



High-throughput characterization pipeline for the recalcitrance phenotype

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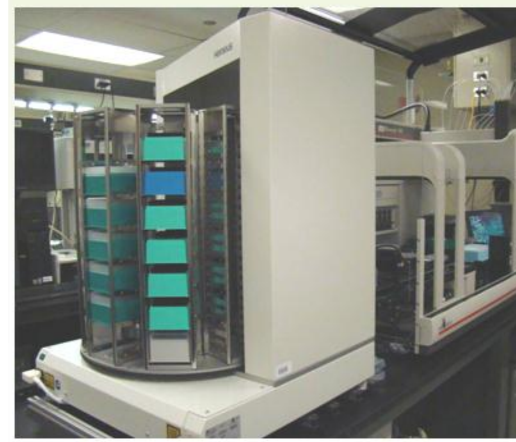
Screening thousands of samples

Composition analytical pyrolysis, IR, confirmed by wet chemistry

Pre-treatment new method with dilute acid and steam

Enzyme digestibility sugar release with enzyme cocktail



Detailed chemical and structural analyses of specific samples

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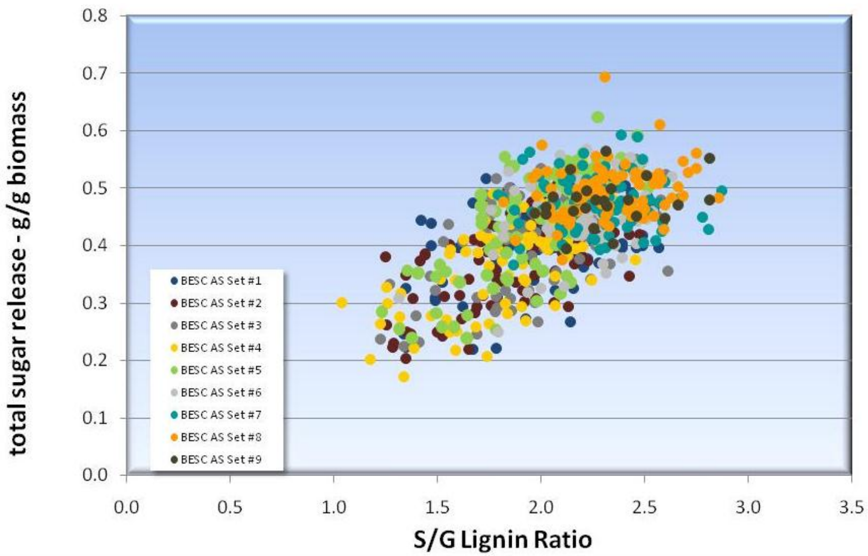
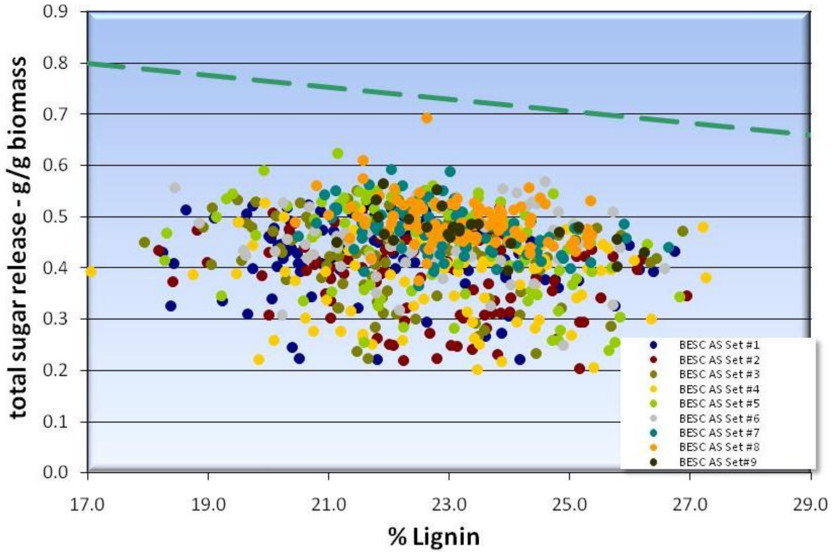
High-throughput screening to analyze natural *Populus* trees

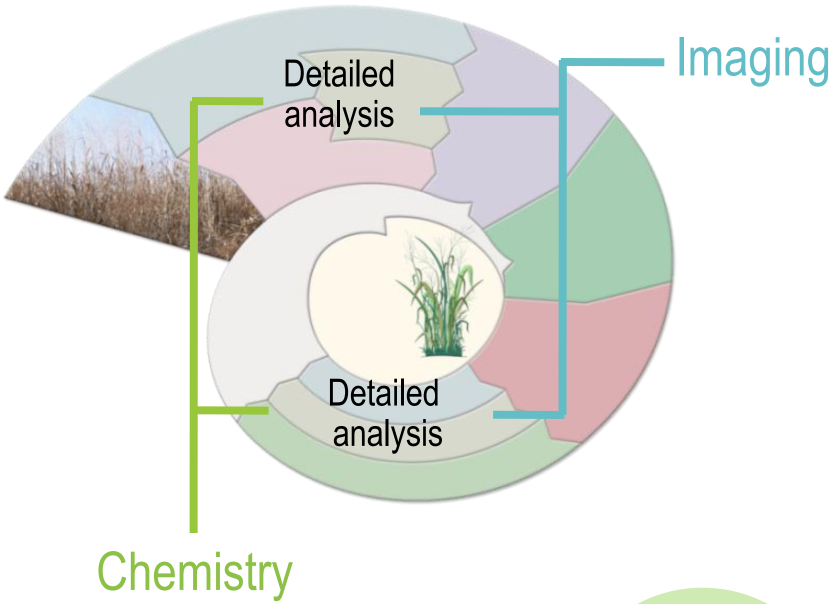
- Screening of 1200 natural *Populus* trees
- Hot water as pretreatment only
- Sugar release varies from 25% to >90% of theoretical value





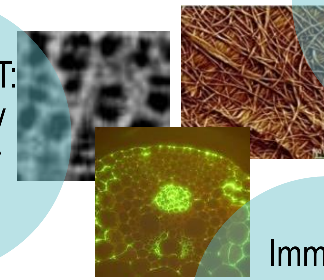
Environmental vs genetic?

Detailed analysis of specific samples inform cell-wall chemistry and structure

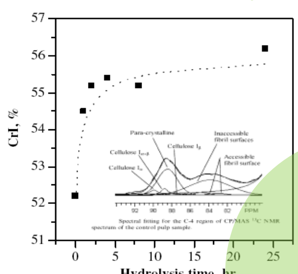


Bio-ultraCAT: 3-D density of *Populus* cell walls

AFM of switchgrass showing cellulose microfibrils

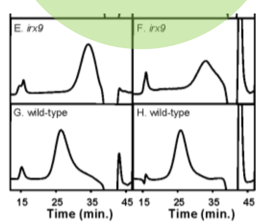


Immuno-localization using wall antibodies on switchgrass

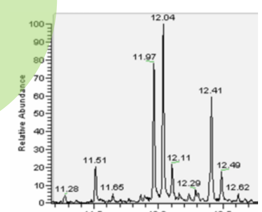


NMR for cellulose crystallinity

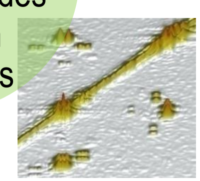
Fractionation and chromatography



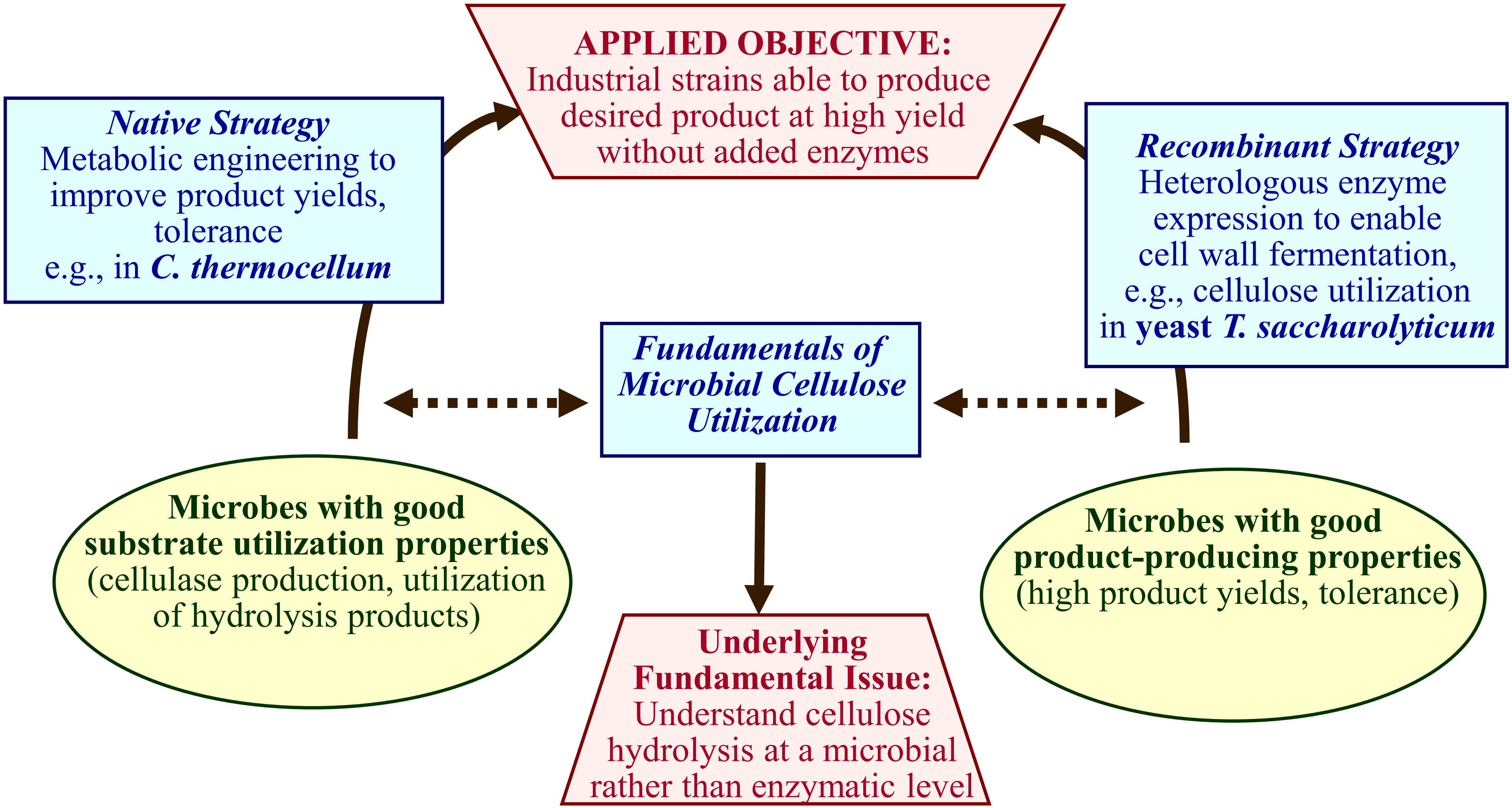
Mass spectrometry for key metabolites



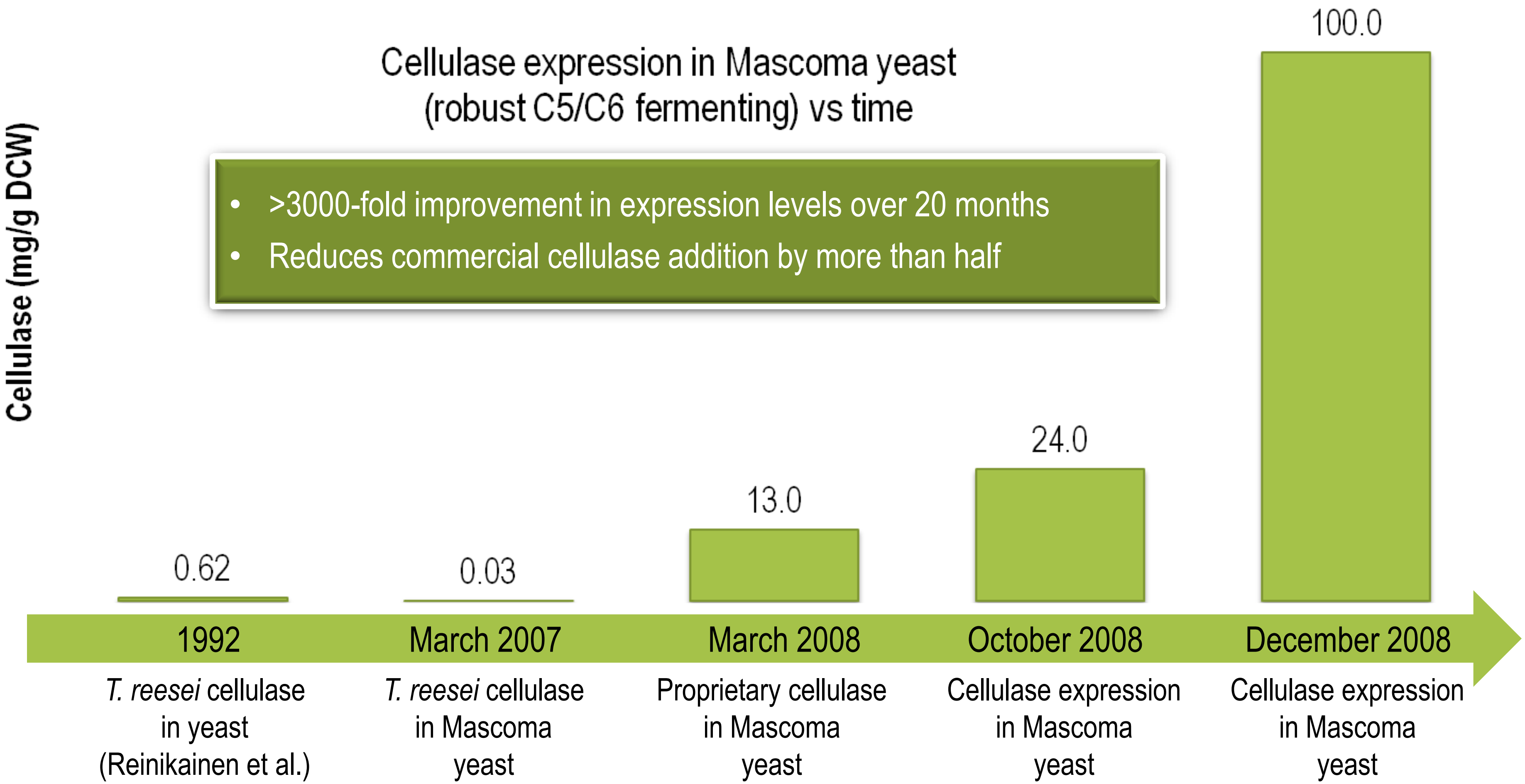
2D ^{1}H-NMR sees altered bonds in polysaccharides and lignin in biomass



CBP Organism Development Strategies and Related Fundamentals



CBP Organism Development Yeast



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Enzymatic and Microbial Hydrolysis
A fundamentally different relationship between microbes and cellulose

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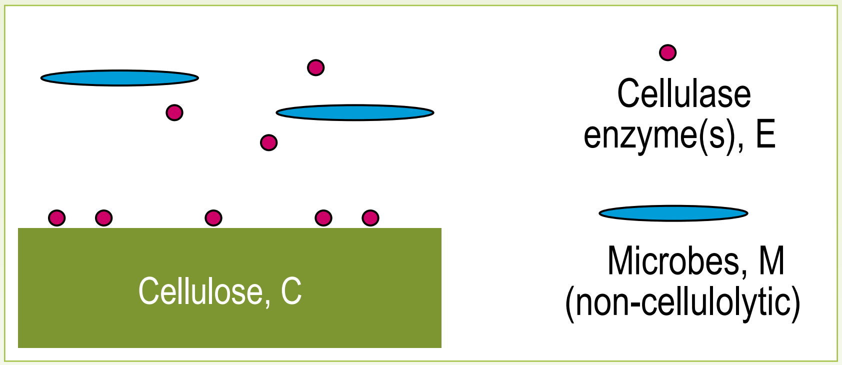
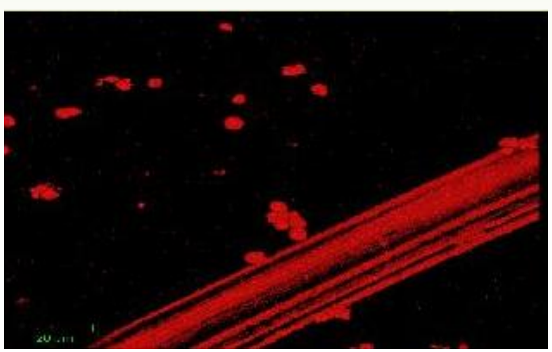
Enzymatic hydrolysis (classical approach)

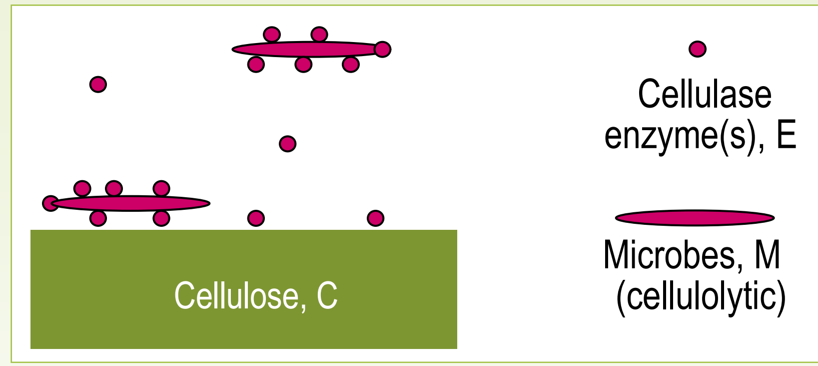


- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound and free
- Cells may or may not be present

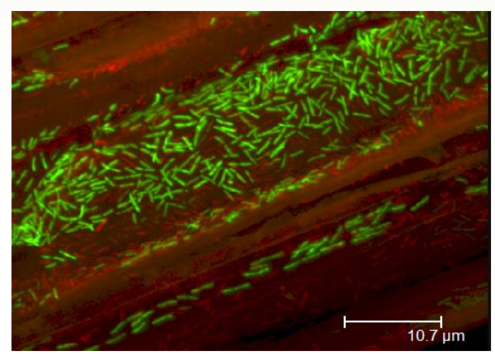


Yeast, enzymes with biomass (Dumitrache and Wolfaardt)

Microbial hydrolysis (CBP)



- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound and free
- Cells both bound and free



C. thermocellum on poplar (Morrell-Falvey and Raman, ORNL)

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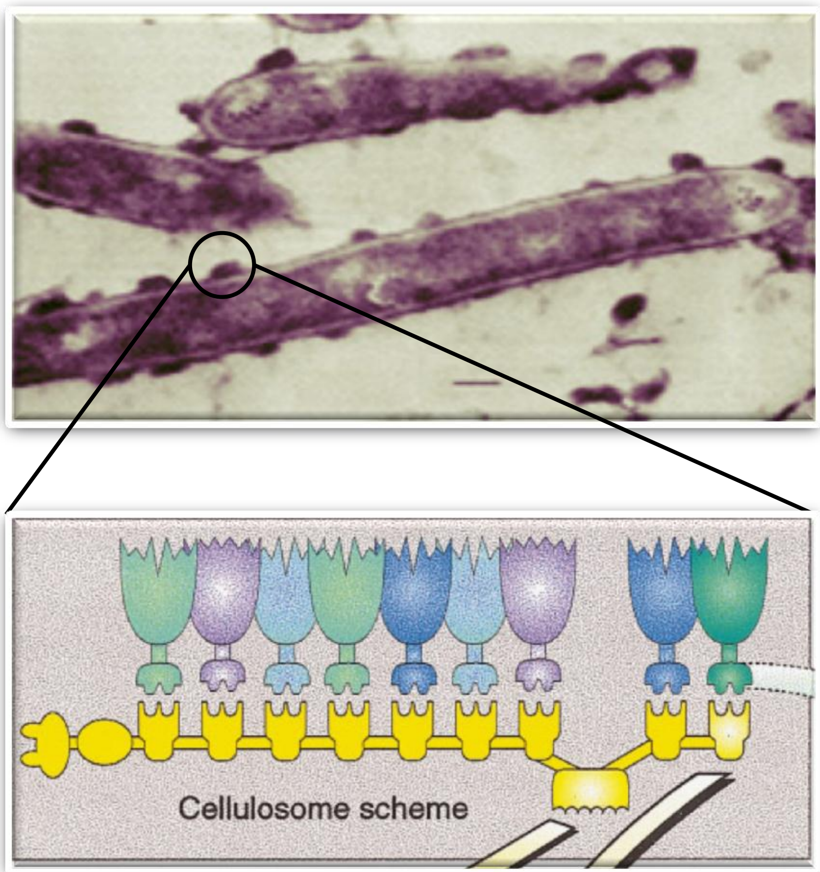
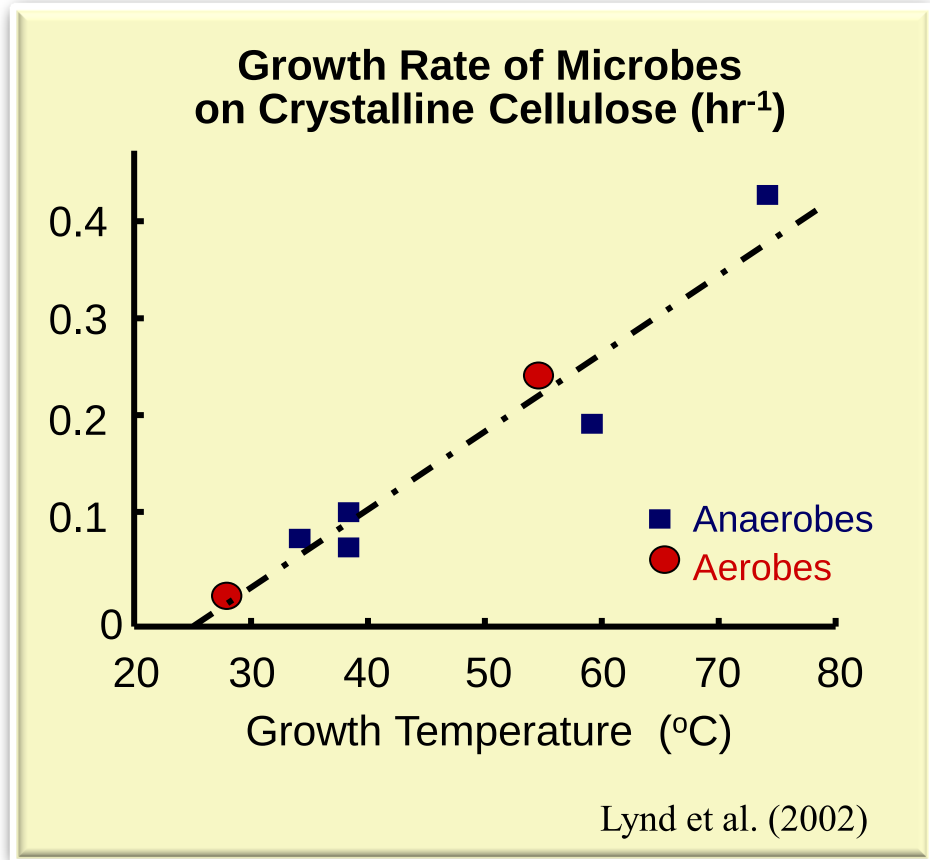
C. thermocellum as a model system

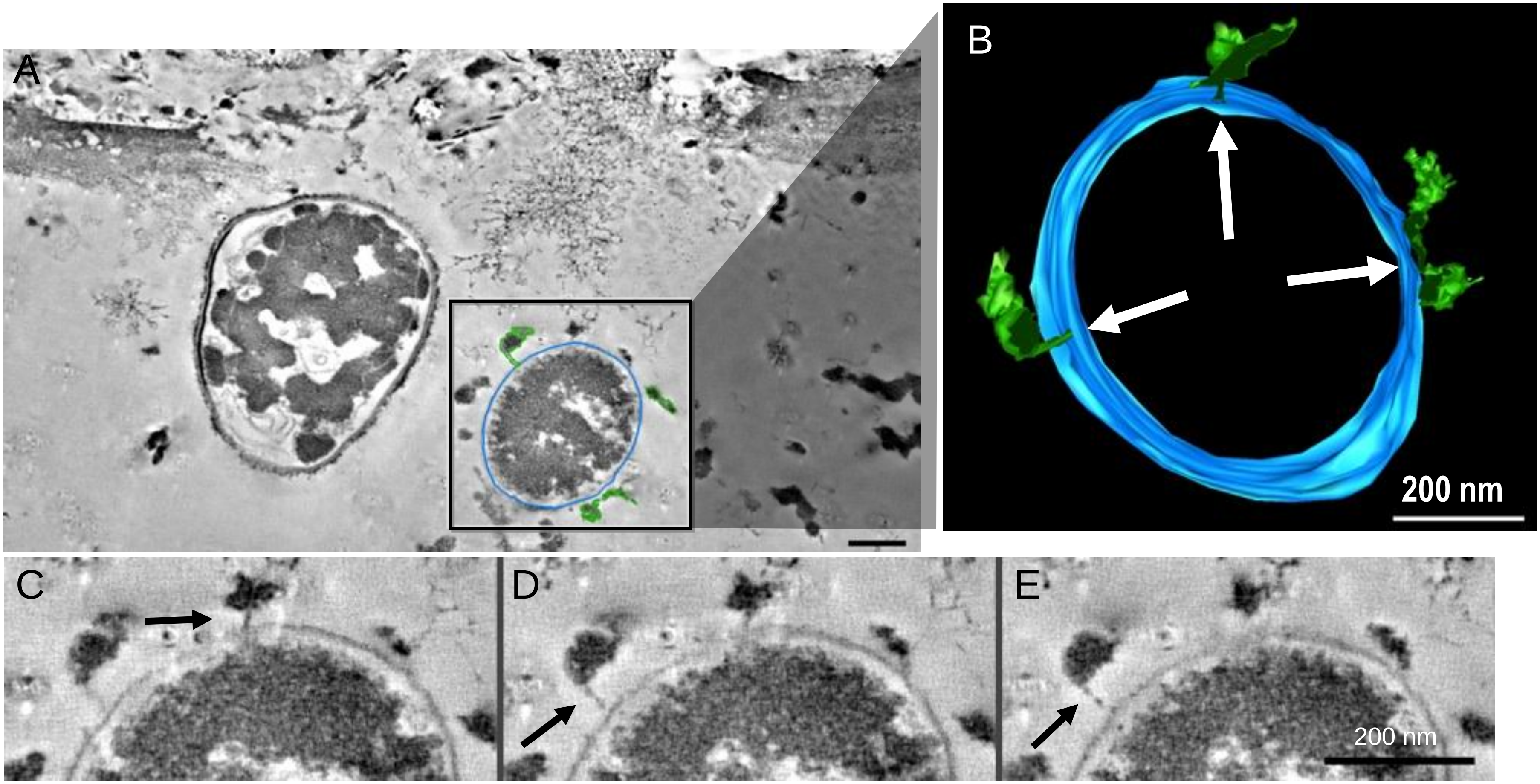
Cellulose hydrolysis mediated by a “cellulosome” complex with over 70 distinct proteins.





One of the highest growth rates on cellulose among described microbes, but does not ferment pentoses, grows poorly on glucose, makes unwanted fermentation products → requires genetic modification.

3D Electron Tomography of *C. cellulolyticum*



Tomogram slices and surface rendered segmentation of bacterial cells and tethered cellulosomes. C–E: Serial slices taken every ~8 nm through tethered cellulosomes. These tethers are seen at one end of most polycellulosmes found near the bacterial cell surface and are ~5 nm in diameter and up to 50 nm in length.

Development of Genetic Tools for Thermophiles

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T. saccharolyticum

Genetic system now fully developed: Transformation, shuttle vectors, gene deletion, removable markers, suicide vector integration, regulated promotors (Shaw, PNAS, 2008; J. Bact., 2009; AEM, 2010)

Natural competence: Recently demonstrated in 13 *Thermoanaerobacterium* and *Thermoanaerobacter* species, shown to involve type 4 pili, ComEA, ComE (Shaw, AEM, 2010)

C. thermocellum

Much more difficult, lagging relative to *T. saccharolyticum*

But emerging

A major impediment in the past (Taylor et al., 2009), now largely removed

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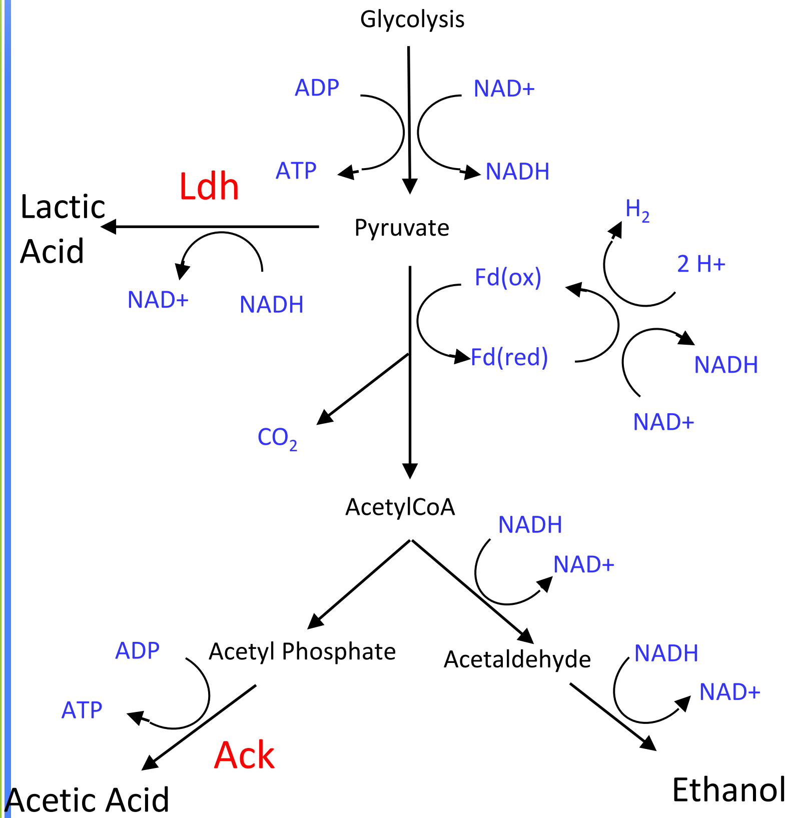
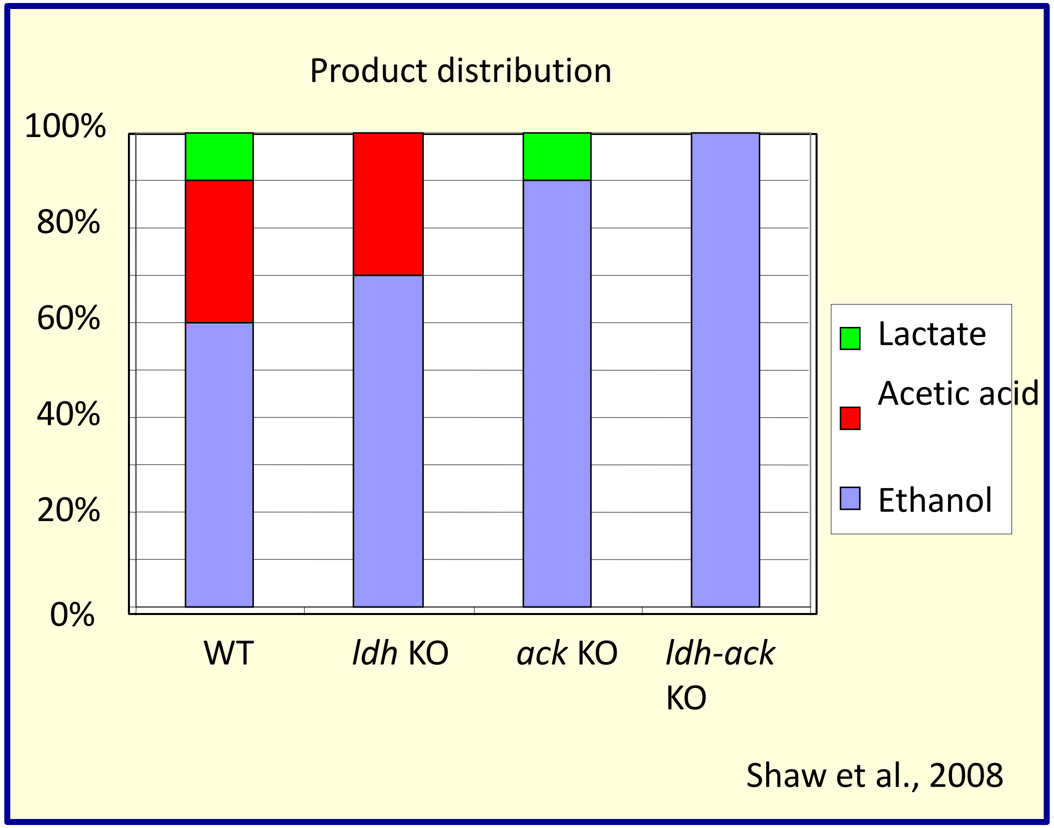
T. saccharolyticum: Knockout of genes associated with lactic & acetic acid production achieved resulting in near-theoretical ethanol yields (Shaw et al., PNAS, 2008)





Lee Lynd, Laboratory; Mascoma Corp.

Genetic tools for *C. thermocellum*

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Gene	Locus	Description	Type of modification	Lead author/affiliation	Publication status
celS	Cthe2089	Cellulosomal GH48	deletion	D. Olson[1]	Accepted
celY	Cthe0071	Non-Cellulosomal GH48	deletion	J. Lo[1]	Accepted
cipA	Cthe3077	Cellulosomal scaffoldin	deletion	D. Olson[1]	In preparation
cipADdocII	Cthe3077	Domain that attaches CipA to cell surface	deletion	A. Guss[1]	In preparation
ech	Cthe3019-3024	Ech hydrogenase	deletion	S. Tripathi[2]	In preparation
hfs	Cthe0425-0428	Hfs hydrogenase	deletion	S. Tripathi[2]	In preparation
ldh	Cthe1053	Lactate dehydrogenase	deletion	S. Tripathi[2] and A. Argyros[2]	In preparation
Gene D01	CtheD01	Central metabolism gene	deletion	A. Argyros[2]	In preparation
pta	Cthe1029	Phosphotransacetylase	deletion	S. Tripathi[2] and A. Argyros[2]	Accepted
rnf	Cthe2430-2435	Ferredoxin oxidoreductase	deletion	S. Tripathi[2]	In preparation
spo0A	Cthe0812	Sporulation initiation factor	deletion	A. Argyros[2]	In preparation
Gene D02	CtheD02	Central metabolism gene	deletion	A. Guss[1]	In preparation
Gene D03	CtheD03	Central metabolism gene	deletion	A. Guss[1]	In preparation
adhE	Cthe0423	Bi-functional aldehyde/alcohol dehydrogenase	overexpression	A. Guss[1]	In preparation
pyrF	Cthe0951	orotidine 5'-phosphate decarboxylase	Deletion and overexpression	S. Tripathi[2]	Accepted
hpt	Cthe2254	hypoxanthine phosphoribosyltransferase	Deletion and overexpression	A. Argyros[2]	In preparation
cat	From pNW33N	Chloramphenicol acetyltransferase	Heterologous expression	D. Olson[1]	Accepted
kan	From pIKM1	Kanamycin resistance gene	Heterologous expression	D. Olson[1]	Accepted
neo	From pUB110	Kanamycin resistance gene	Heterologous expression	D. Olson[1]	In preparation
tdk	From *T. saccharolyticum*	Thymidine kinase	Heterologous expression	S. Tripathi[2]	In preparation
Gene M01	Thermophilic anaerobe	Central metabolism gene	Heterologous expression	D. Olson[1]	In preparation
Gene M02	Thermophilic anaerobe	Central metabolism gene	Heterologous expression	A. Argyros[2]	In preparation
Gene M03	Thermophilic anaerobe	Central metabolism gene	Heterologous expression	A. Argyros[2]	In preparation

[1] Dartmouth College
[2] Mascoma Corporation

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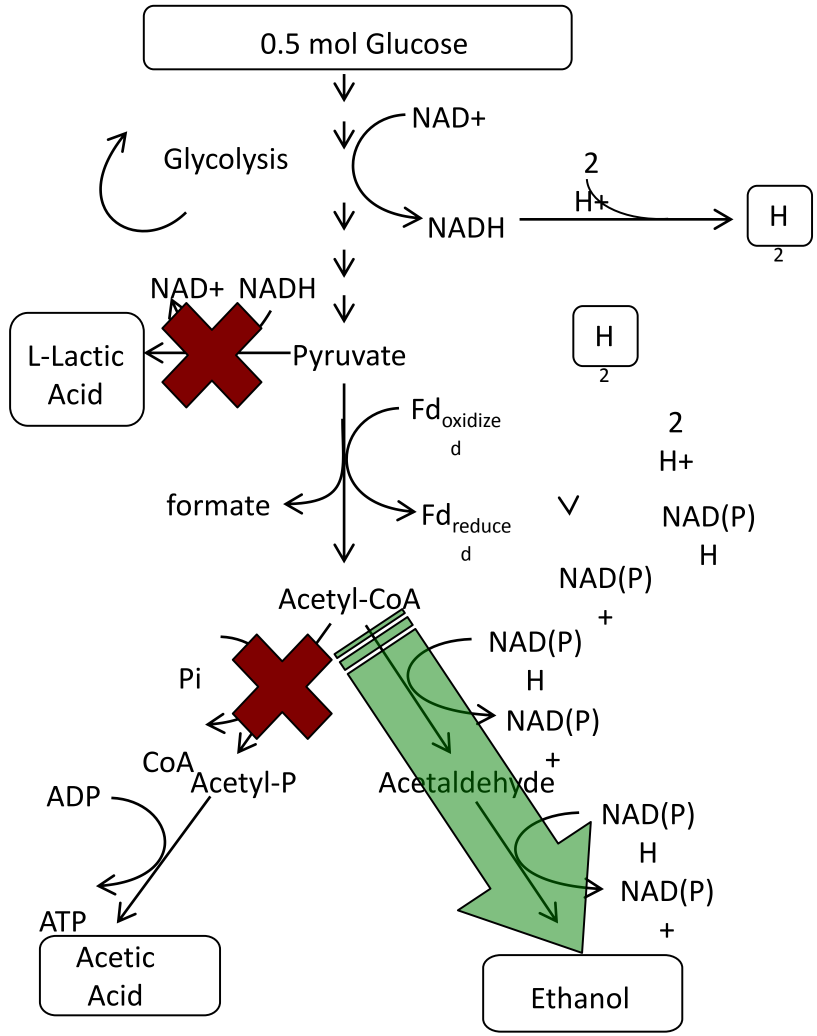
C. thermocellum central metabolism knockouts

M1726
Δ *hpt*Δ*spo0A*

M1629
Δ *hpt*Δ*spo0A* Δ*ldh*

M1725
Δ*hpt*Δ*spo0A* Δ*ldh*Δ*pta*

M1630
Δ*hpt*Δ*spo0A* Δ*pta*



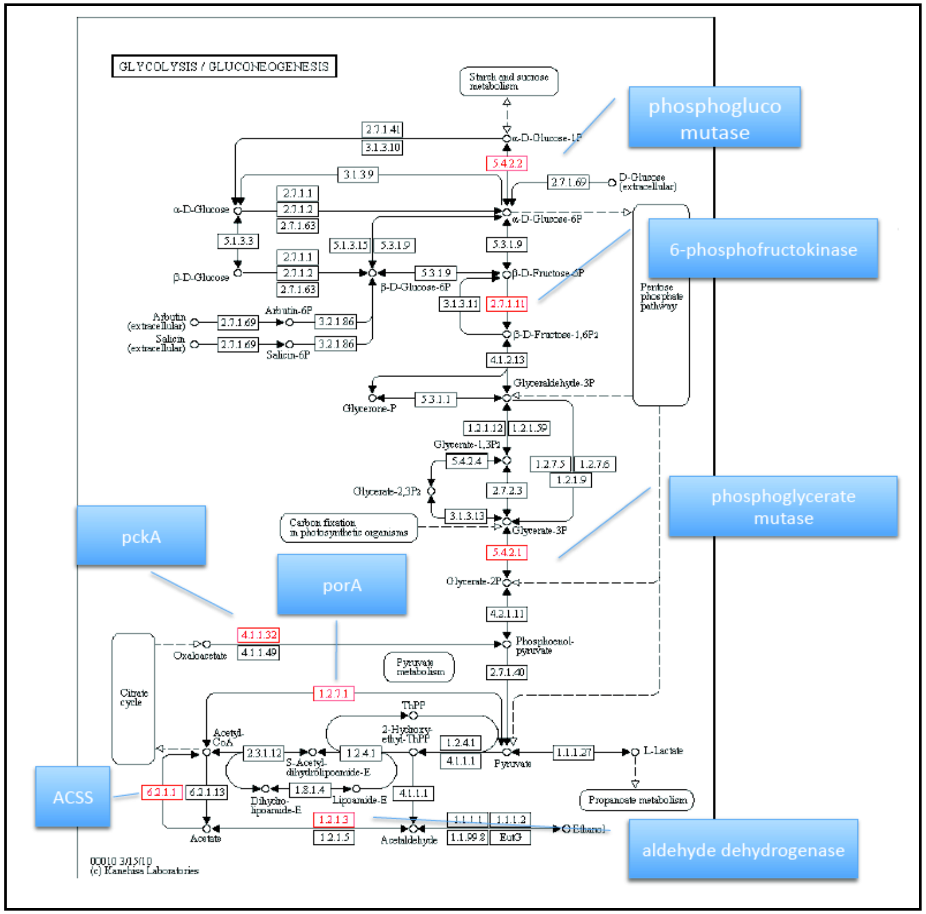
Mascoma Corporation
Lee Lynd, Dartmouth

C. thermocellum: After a much larger effort, an Ack-, Ldh- double knockout mutant was obtained (Argyros et al., unpublished)

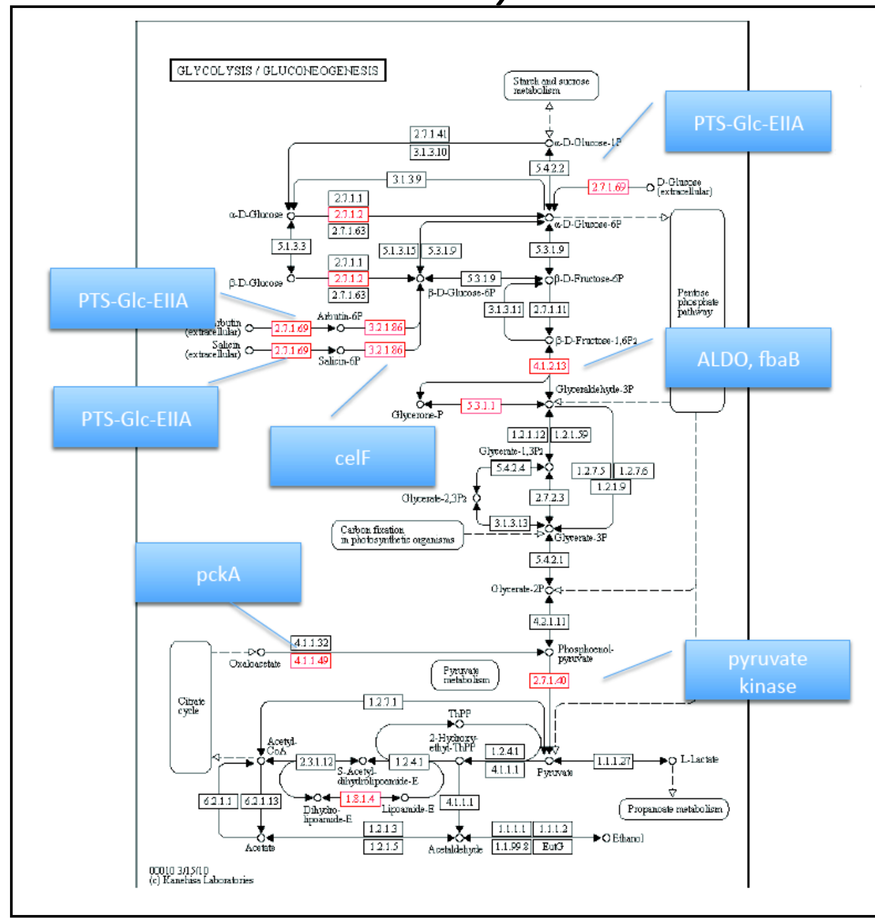
Although acetate and lactate production is low, the mutant grows slowly and ethanol yield is lower than expected.

In silico Comparison

C. thermocellum

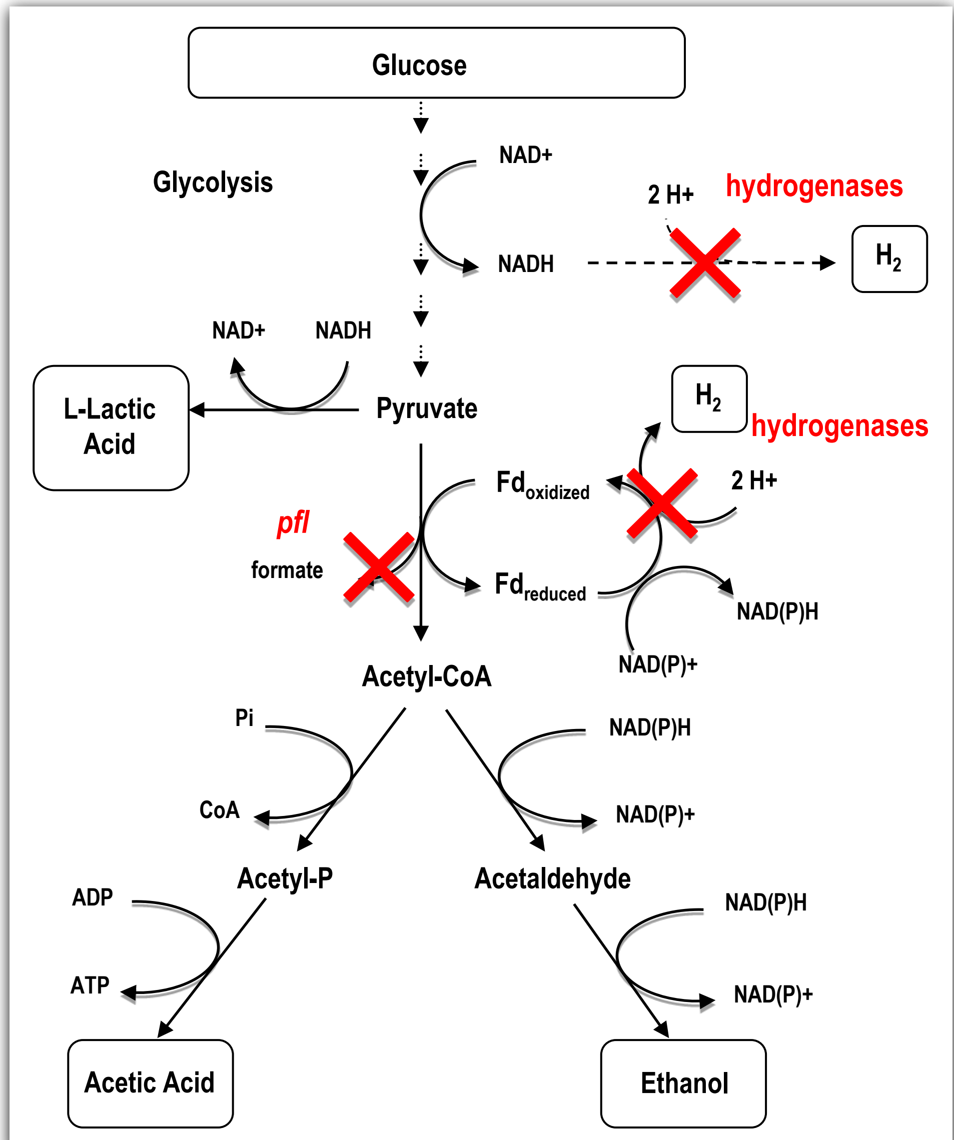


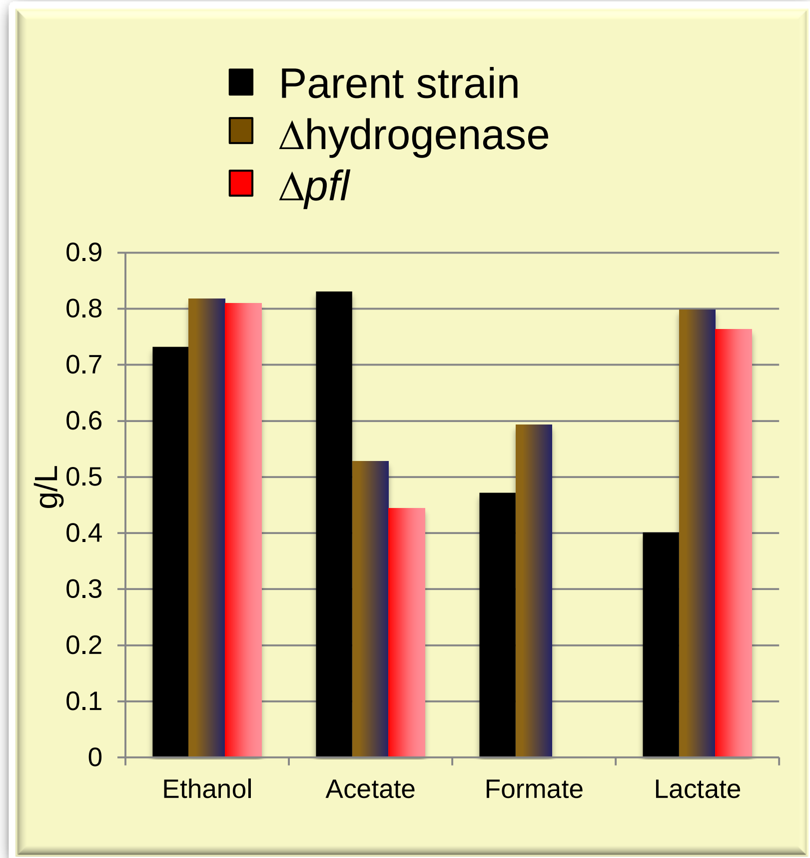
T. saccharolyticum



BESC/Xizeng Mao

Deletion of competing electron transport pathways in *C. thermocellum* for enhanced ethanol production





On one key road block for cellulosic ethanol production

U.S. DEPARTMENT OF ENERGY

- End-product titer is an important contributor to capital and downstream processing costs.
- “This makes the engineering of ethanol-tolerant strains, which can tolerate the adverse environment in which the process takes place, of the utmost importance”.
- “Not much progress has been made on this front, ***perhaps because of the preconception that a complex phenotype such as ethanol tolerance could be modulated by a single gene***, or at most a handful of genes.
- “There is now accumulating evidence that no single gene can endow microbes with tolerance to ethanol and other toxic compounds.”

G. Stephanopoulos. Challenges in Engineering Microbes for Biofuels Production. *Science* **315**, 801 (2007).

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Resequencing an ethanol tolerant *C. thermocellum* mutant

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Williams et al. Appl Microbiol Biotechnol (2007)

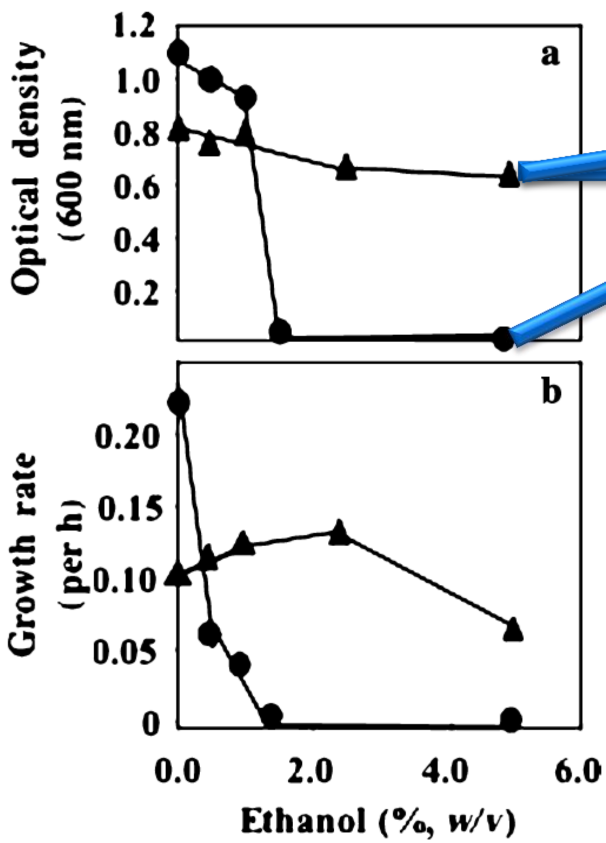
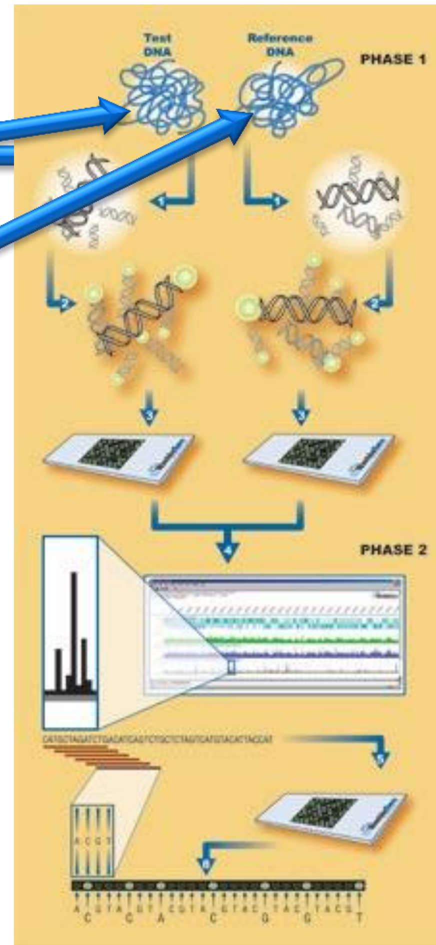


Fig. 1 Effect of ethanol on growth of wild-type (circles) and ethanol-adapted (triangles) cultures. Ethanol-sensitive cells (wild-type) and cells adapted to 5% ethanol were incubated in media containing varying concentrations of ethanol. Optical density (**a**) and growth rate (**b**) were measured

gDNA resequenced via microarray



Resequencing via 454

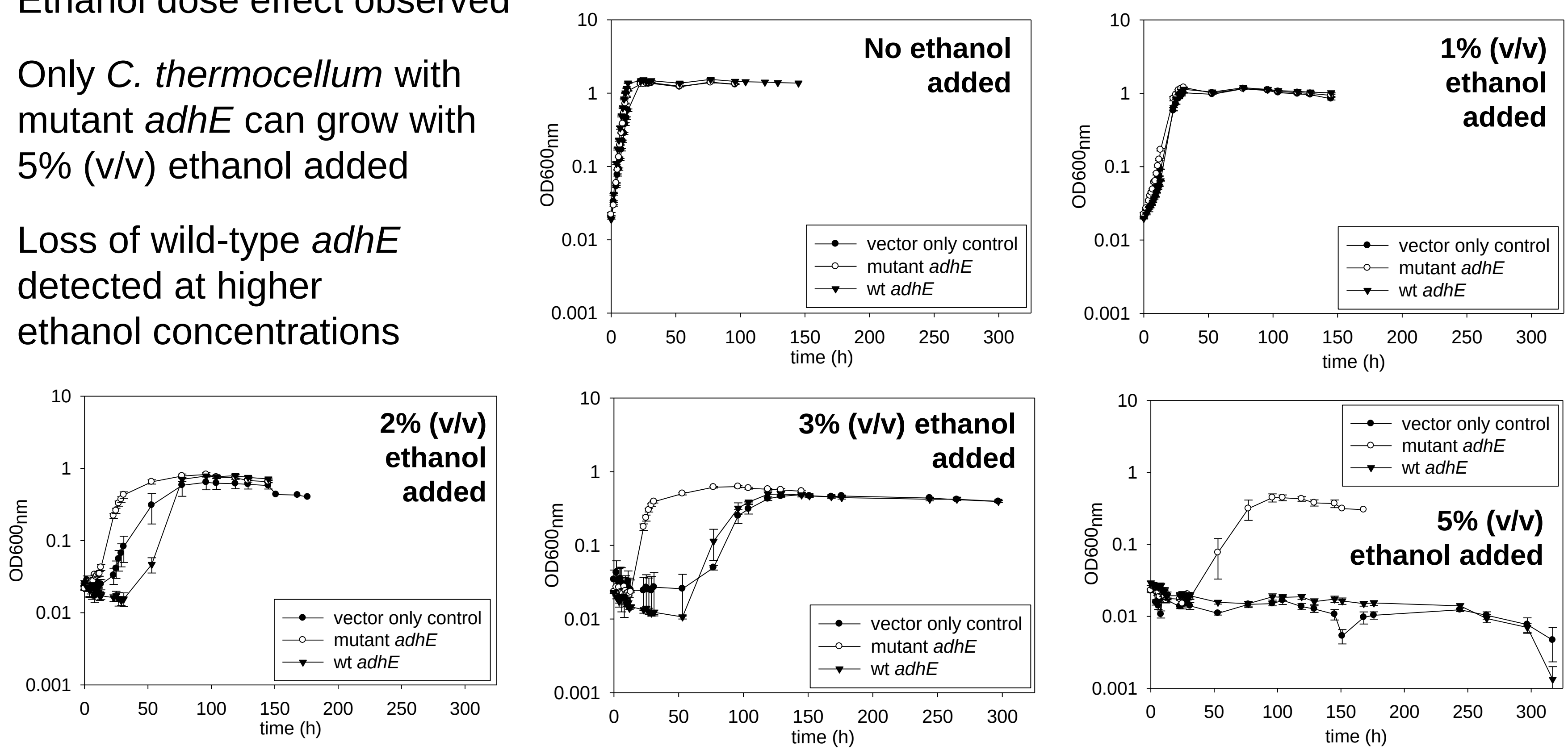
Brown S. D., et al. submitted. U.S. 61/346,660.

BESC BioEnergy Science Center

Mutant *C. thermocellum* alcohol dehydrogenase leads to enhanced ethanol tolerance

U.S. DEPARTMENT OF ENERGY

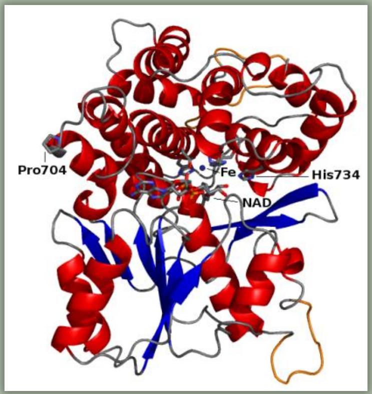
- Three strains tested in *C. thermocellum* DSM 1313 wild-type background (i.e. *adhE*+), vector only control, additional wt *adhE* via plasmid, mutant *adhE* via plasmid
- Ethanol dose effect observed
- Only *C. thermocellum* with mutant *adhE* can grow with 5% (v/v) ethanol added
- Loss of wild-type *adhE* detected at higher ethanol concentrations



Brown S. D., et al. submitted. U.S. 61/346,660.

BESC BioEnergy Science Center

Carbon and electron flow partition differently in AdhE mutant strain



Mutation in NADH binding domain of ADH

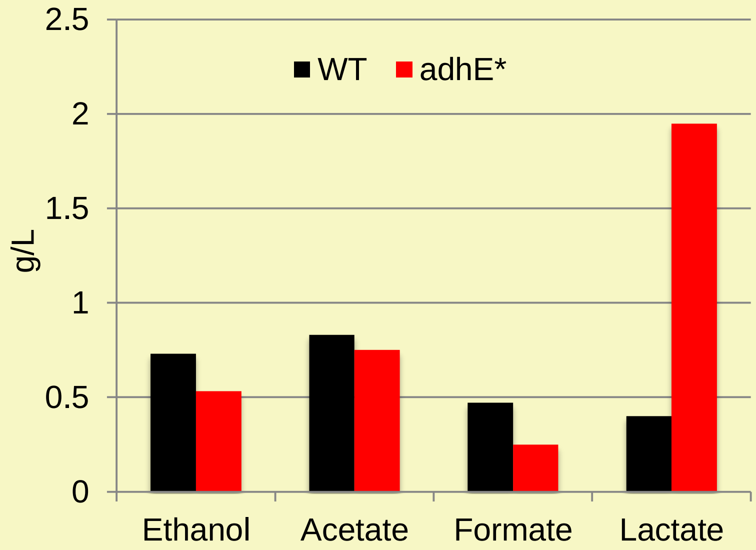
Mutant ADH co-factor specificity changes to NADPH dependence

	Specific Activity[a] (Std dev)	
	NADH	NADPH
WT	2.7 (0.18)	0.025 (0.005)
EA	<0.005[b]	0.052 (0.007)
adhE*(EA)	<0.005	0.12 (0.03)

[a] µg NAD(P)H oxidized.mg crude extract protein-1.min-1

[b] Below assay detection limit

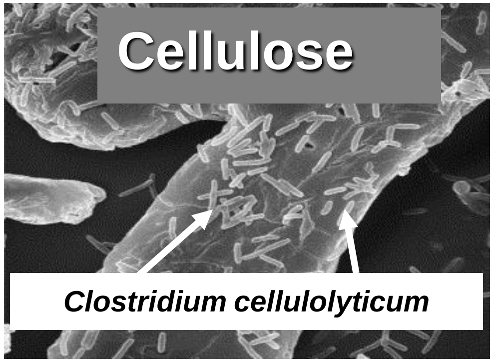
Carbon flow also effected in *C. thermocellum* containing mutant ADH



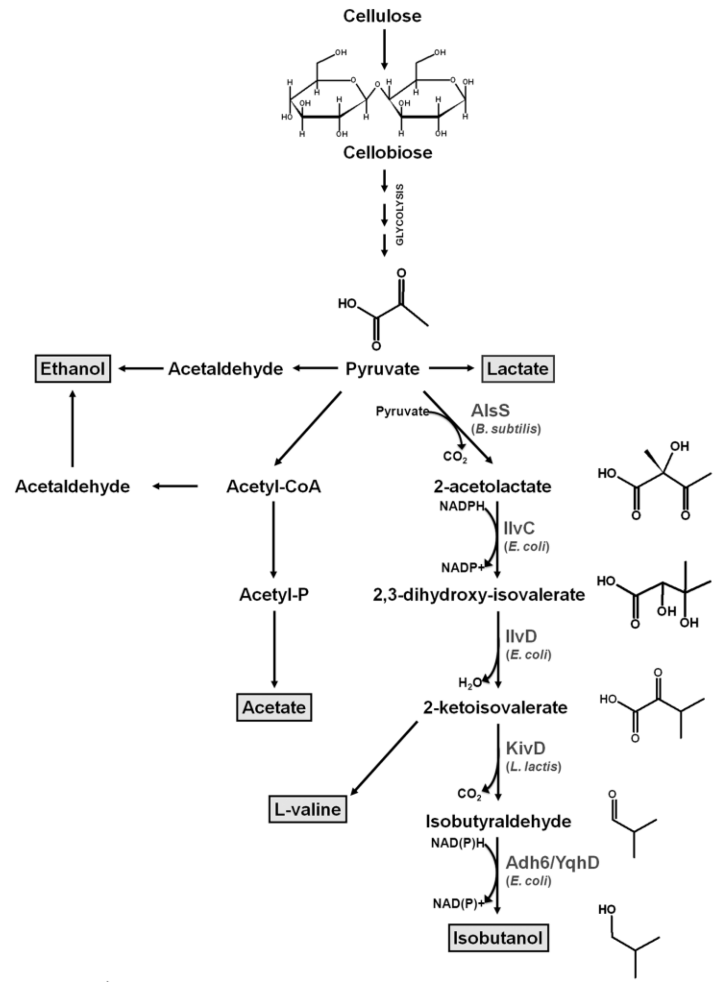
Studies underway to further optimize carbon and electron flow for productivity advances

Brown S. D., et al. submitted. U.S. 61/346,660.

Demonstration of the direct production of an advanced biofuel from cellulose



- Conferred the ability to make isobutanol into a native cellulose-degrading microbe, *Clostridium cellulolyticum*.



Contacts: James Liao, liaoj@seas.ucla.edu

Citation: Higashide (UCLA), Li (ORNL), Yang (ORNL), & Liao (UCLA) Applied Environmental Microbiology (2011) *Accepted.*

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Demonstration of the direct production of an advanced biofuel from cellulose

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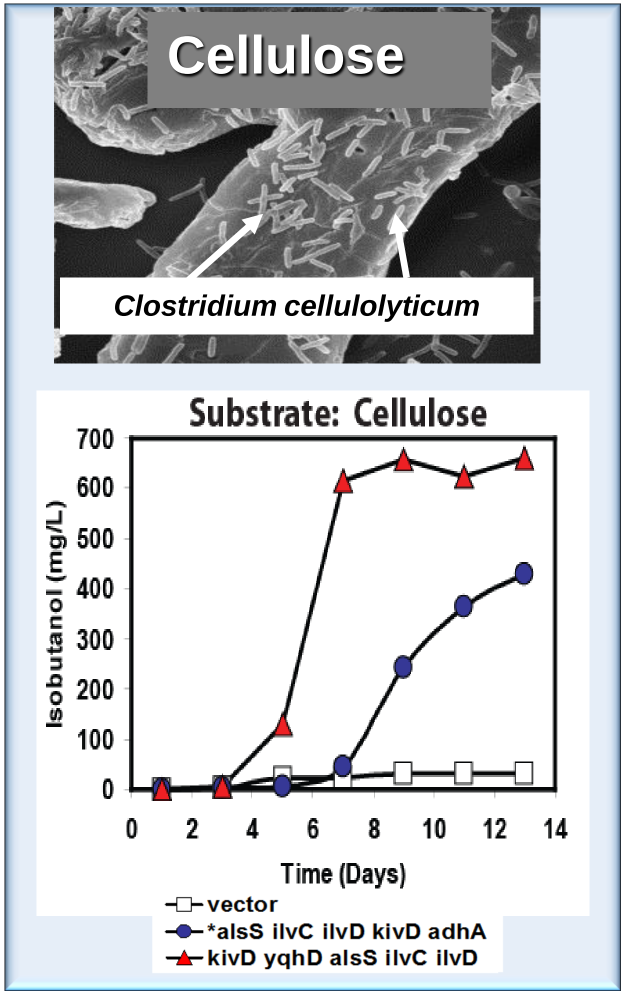
Demonstrating the ability to combine CBP (consolidated bioprocessing) with production of next generation biofuels.

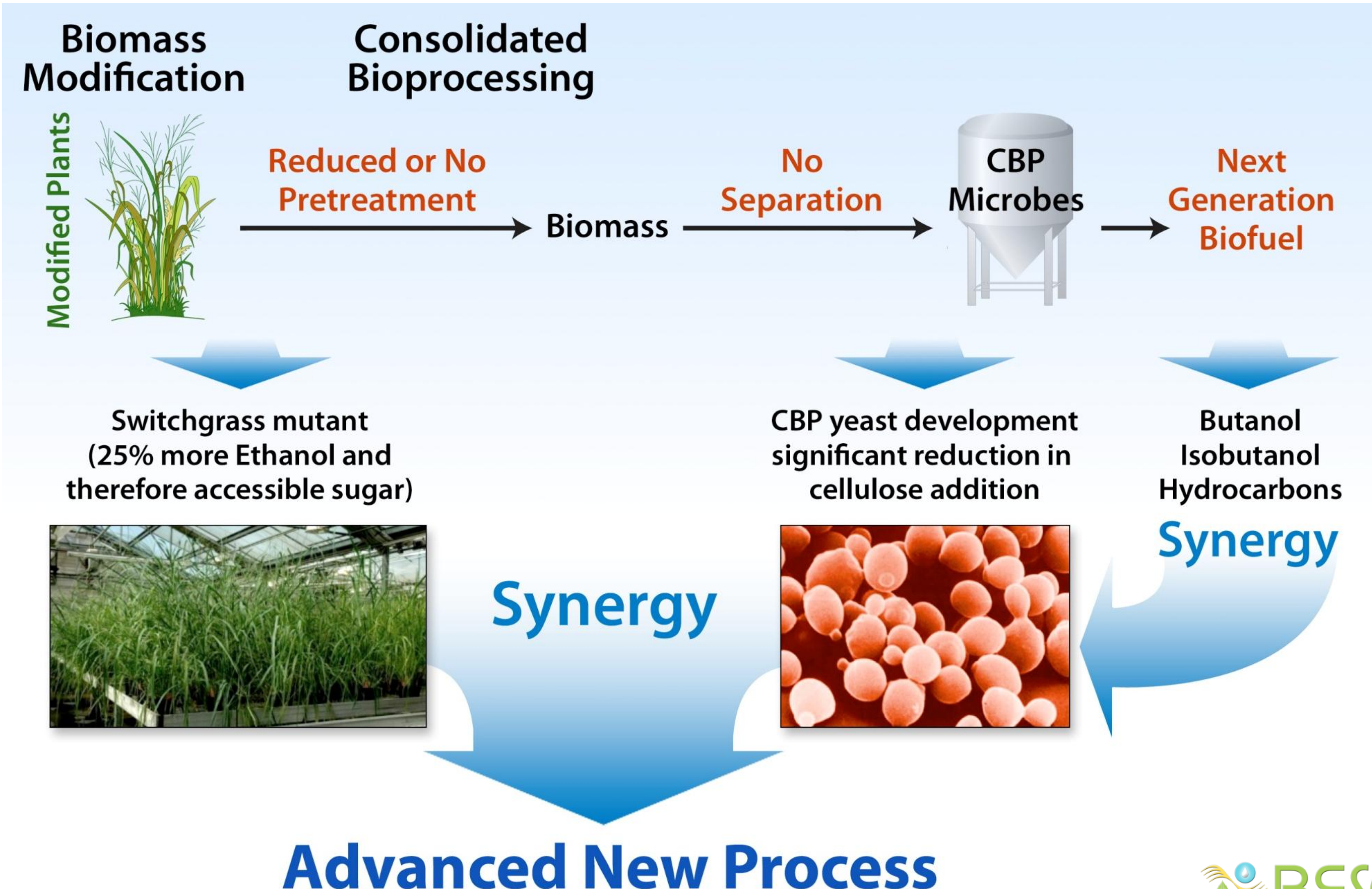


Contacts: James Liao, liaoj@seas.ucla.edu

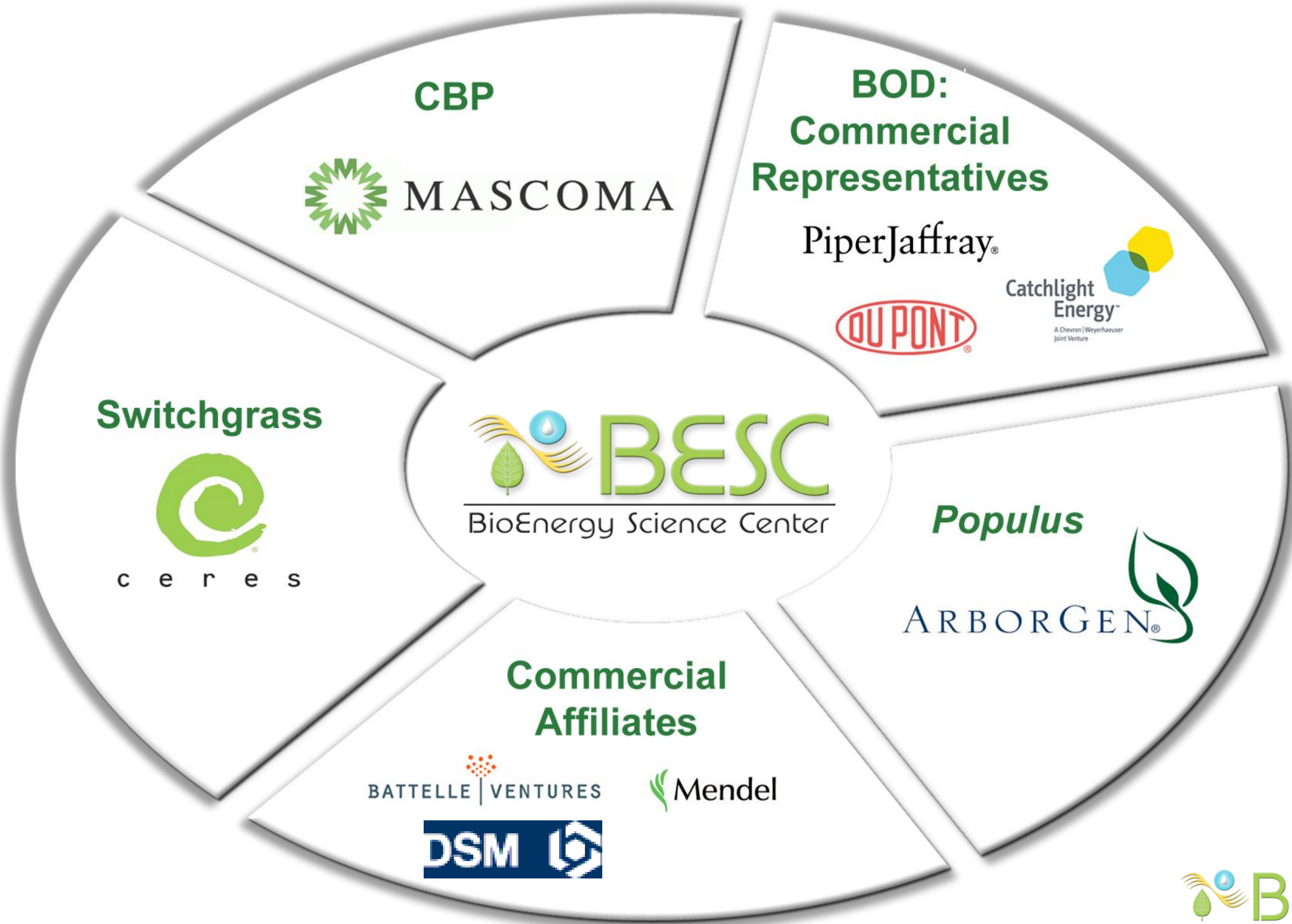
Citation: Higashide (UCLA), Li (ORNL), Yang (ORNL), & Liao (UCLA) Applied Environmental Microbiology (2011) *Accepted.*

BESC BioEnergy Science Center

BESC will revolutionize how biomass is processed and converted

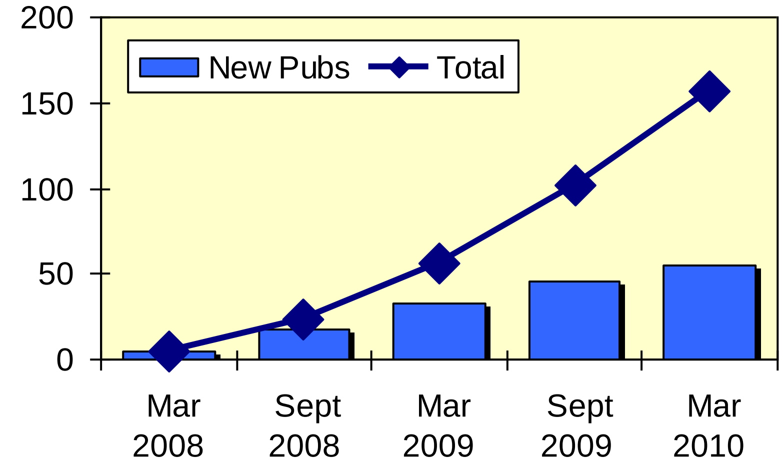


Industrial partners facilitate strategic commercialization



Translating discoveries to the scientific community

- 210 scientific publications
  - 33% of publications include external collaborators at non-BESC Institutions
- BESC publications have already been cited 395 times in peer-reviewed journals



- Several publications in top-tier journals
  - *Nature Biotechnology*, 2008, Lynd *et al*., How biotech can transform biofuels
  - *PNAS*, 2008, Shaw *et al*., Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield
  - *Nature Nanotechnology*, 2010, Tetard *et al*., New modes of subsurface atomic force microscopy through nanomechanical coupling
- 22 inventions disclosed (under evaluation by BESC Commercialization Council)

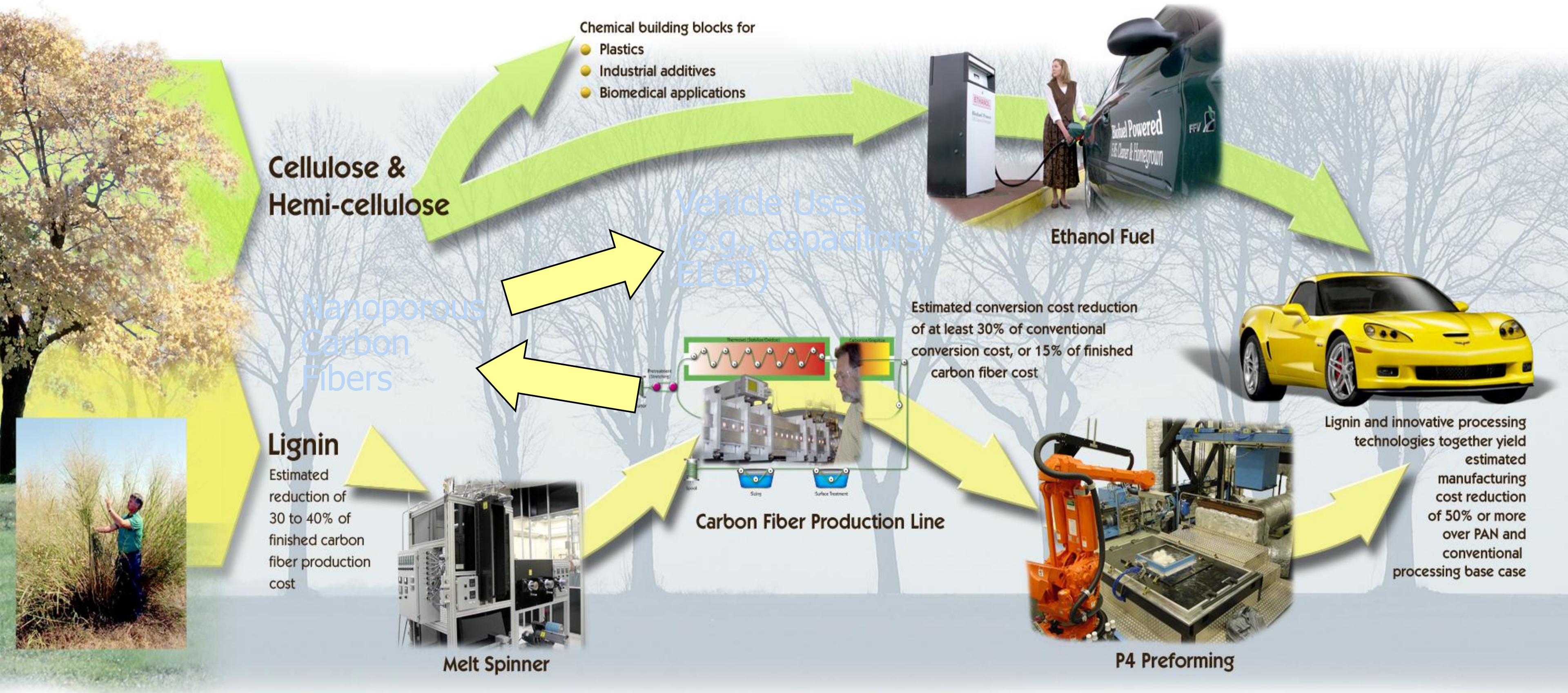
Influencing next generation of scientists

U.S. DEPARTMENT OF ENERGY

- National Geographic, The Jason Project, filmed and generated an educational module on bioenergy with BESC researchers
  - This module is available from www.jason.org
- Created an interactive biofuels outreach lesson for students in Grades 3-8
  - Piloted more than 220 lessons which reached over 6,000 students
  - Partnered with the Creative Discovery Museum
  - Available on www.bioenergycenter.org
- Piloted ten Biofuels Family Science Nights with an average attendance of 250 people each

BESC BioEnergy Science Center

ORNL Research Directed Toward Production of Multiple Value-added Streams from Biomass Feedstock

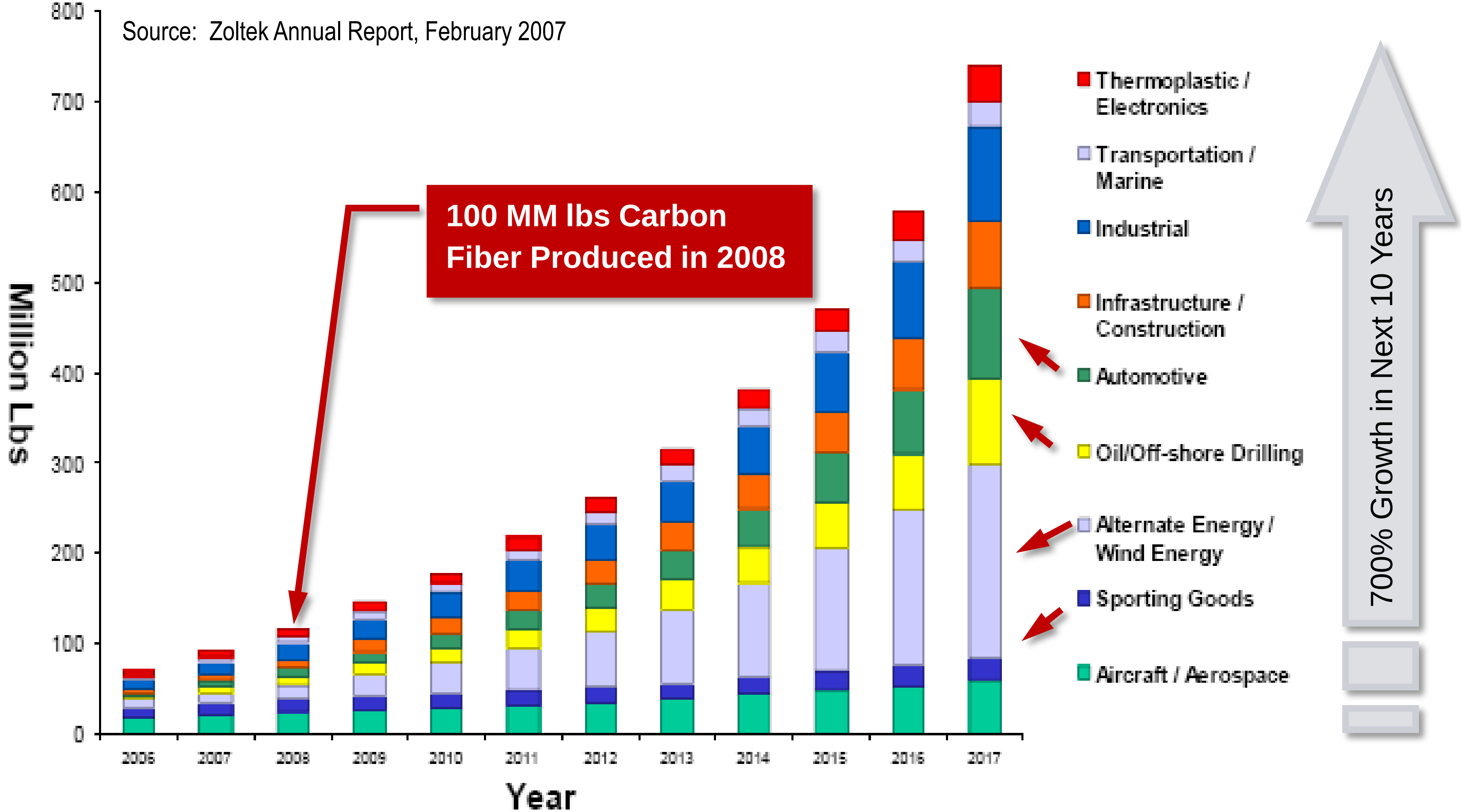


DOE Office of Vehicle Technologies
Lightweight Materials Program

Reason for Project:

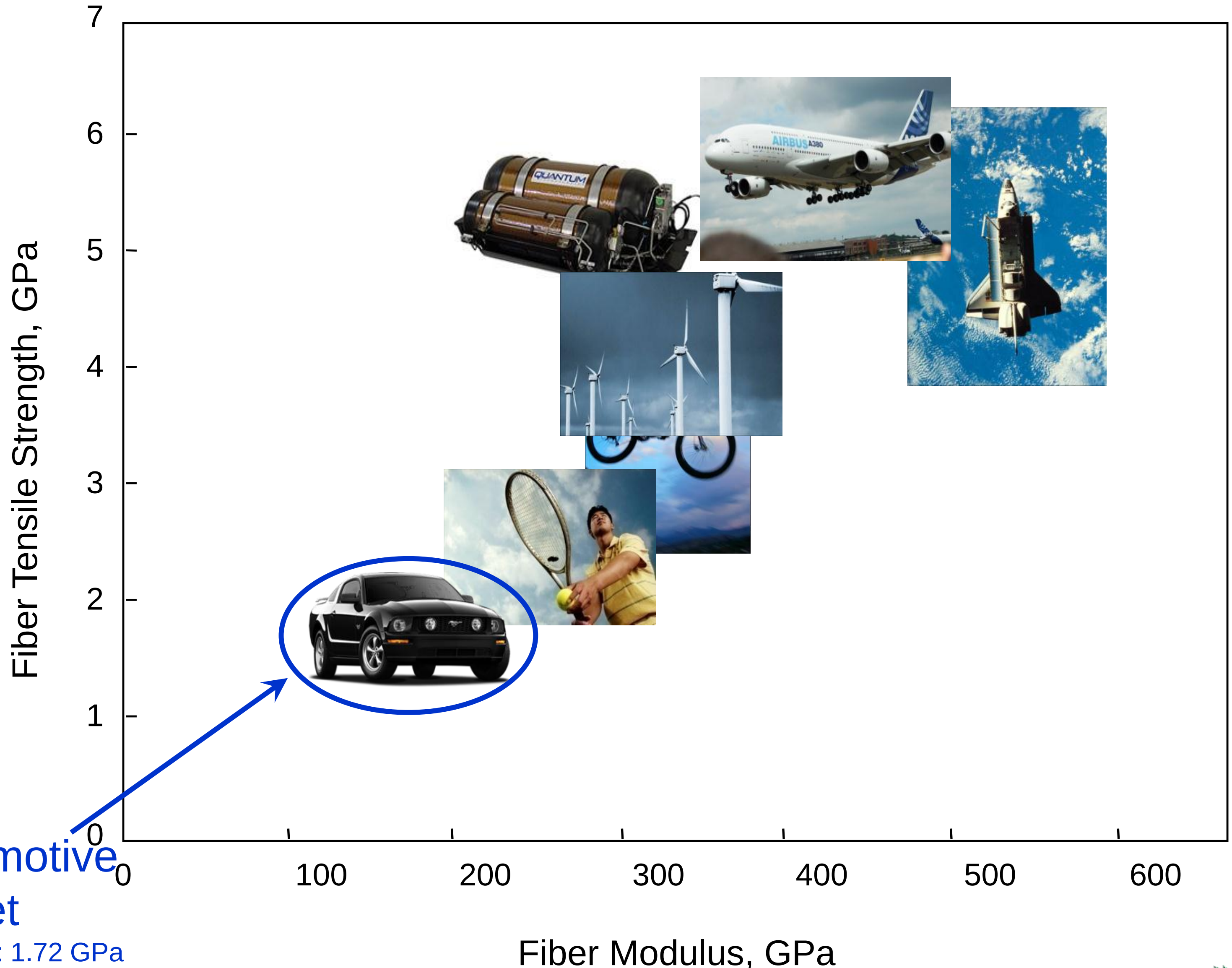
- Weight reduction is one of the most practical ways to increase the fuel economy of vehicles
- 10% Vehicle Weight = + 6-8% Vehicle Mileage/gallon
- An important additional benefit of increasing fuel economy is a reduction in greenhouse gas emissions, notably CO_2
- 1.85 billion metric tons (1,850,000,000 tons) of CO_2 emitted by vehicles on U.S. roads in 2002

Projected carbon fiber market demand



The Growth and Challenges are
<u>Multi-Industry, not just Automotive</u>

Structural Applications of Carbon Fibers



Automotive Target

Strength: 1.72 GPa

Modulus: 172 GPA

Melt Spinning and Thermal Processing of Lignin into Carbon Fibers

Mechanical Properties (to date):

Tensile Strength: 155 Ksi (62% of Target 250 Ksi)

Modulus: 10-12 Msi (40-48% of Target 25 Msi)

- Properties as measured. No adjustment for porosity (density) of fiber; e.g., adjusted for 20% porosity, highest mechanical properties "increase" to:
  - Tensile strength of 194 Ksi (78% of target)
  - Modulus of 15 Msi (60% of target)
- Highest values obtained with softwood lignin

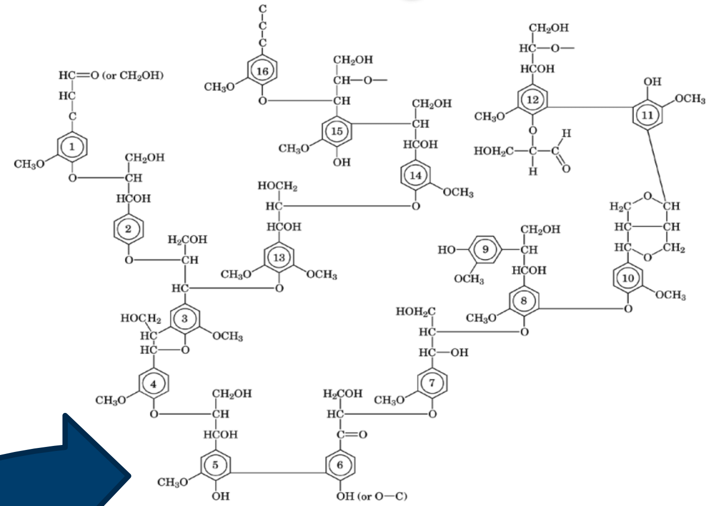
Melt Spinning and Thermal Processing of Lignin into Carbon Fibers

Does all this mean that the targeted carbon fiber properties cannot be obtained with a lignin-based system ??

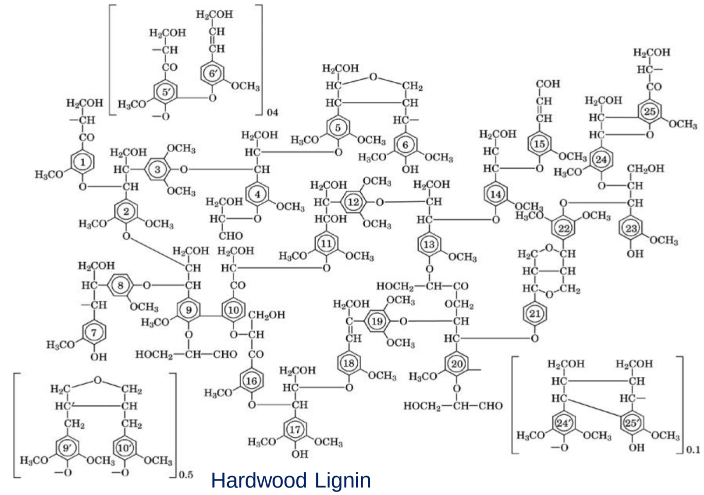
It means that although we have come a long way in developing an understanding of lignin chemistry as it relates to carbon fiber production, we still have more to learn about what is a complex system, and especially the role of lignin chemistry

Note: During the development of PAN-based carbon fibers, it took roughly ten years to reach the engineering properties achieved for lignin-based carbon fiber!

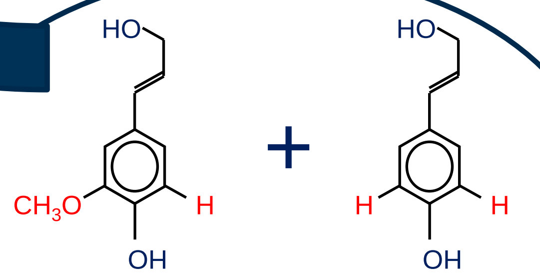
Influence of Lignin Chemistry



Softwood Lignin
E. Adler, *Wood Science & Technology*, 11, 169 (1977)

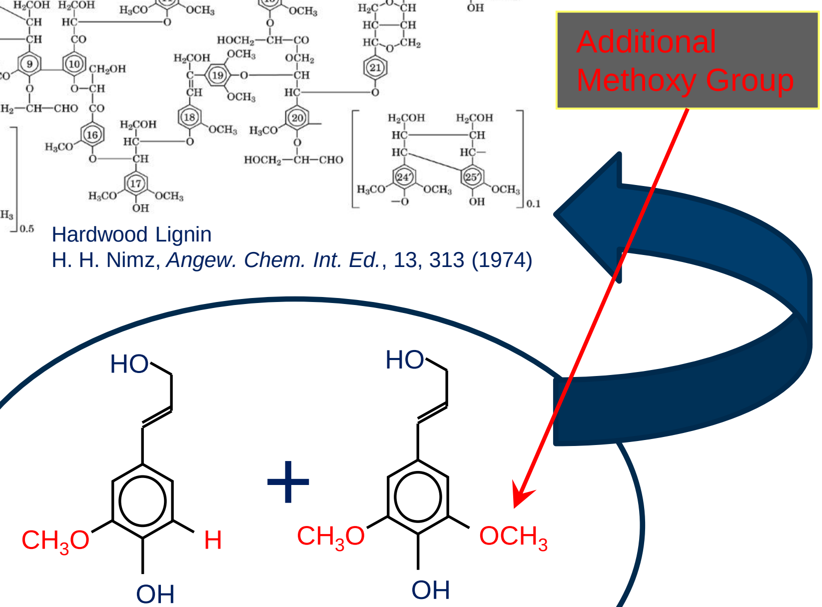


Hardwood Lignin
H. H. Nimz, *Angew. Chem. Int. Ed.*, 13, 313 (1974)



coniferyl alcohol
(> 90%)

p-coumaryl alcohol



coniferyl alcohol

synapyl alcohol

(various ratios)

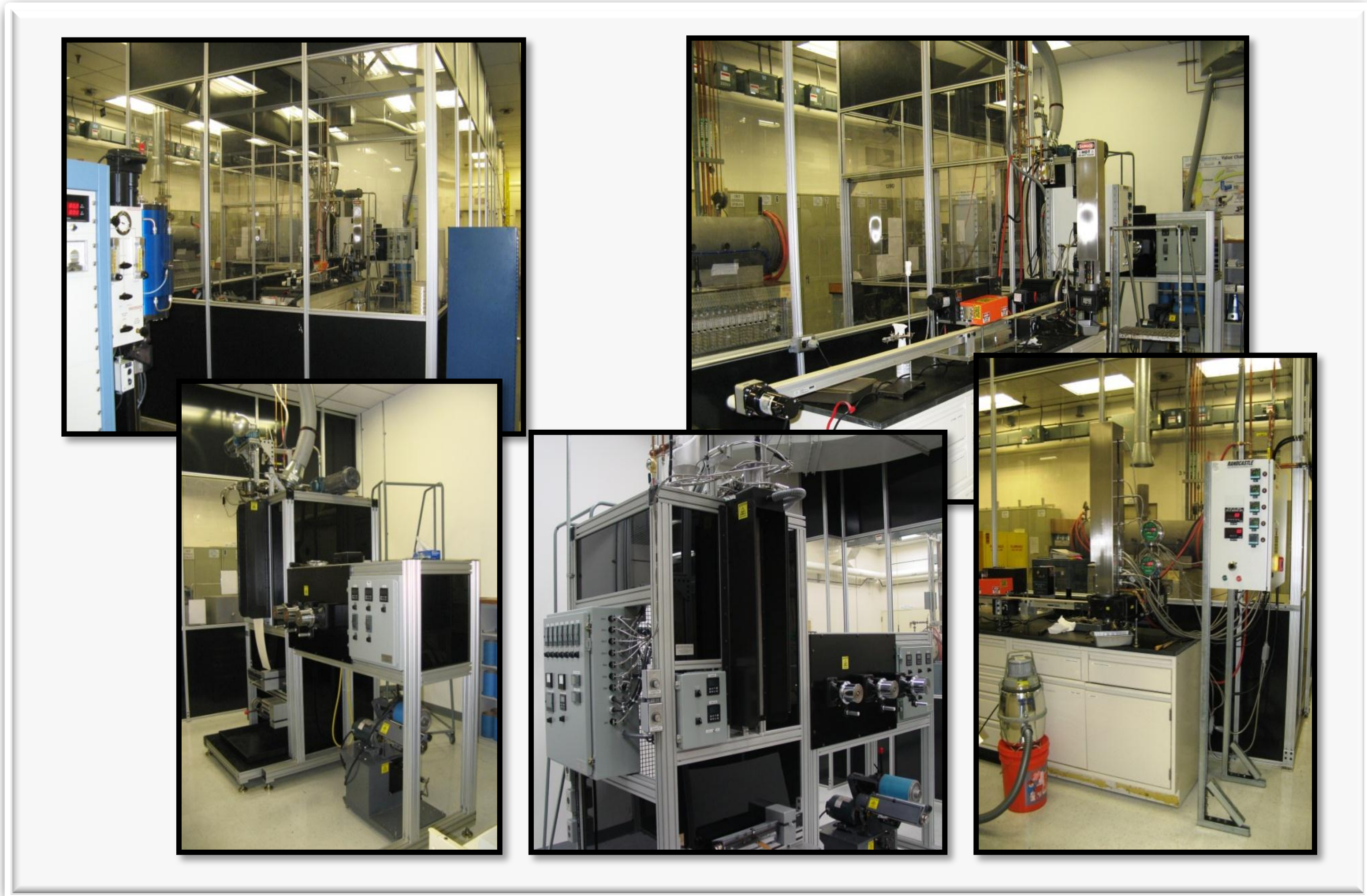
More difficult to melt spin
More amenable to X-linking

More amenable to melt spinning
More difficult to X-link

Conversely

Oak Ridge National Laboratory

Melt Spinning Facilities Installed at ORNL

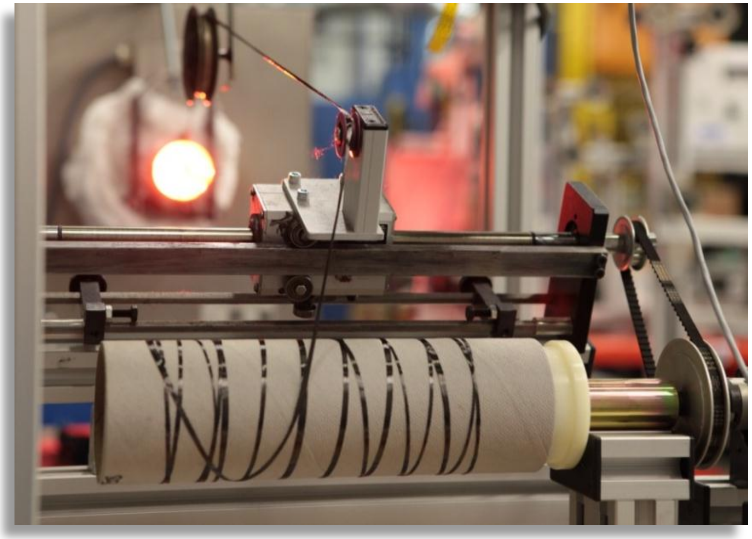


Compounding/Pelletization Line and Multifilament Melt Spinning Line

ORNL Carbon Fiber Processing Equipment)

Multi-pass oxidation oven

Oxidized fiber entering carbonization furnace



Finished carbon fiber being spooled

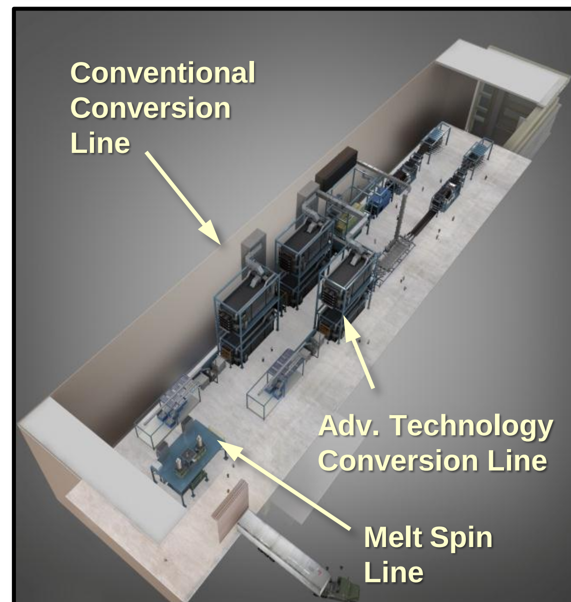
Pilot-scale, multiple-tow carbon fiber conversion line (≈ 20 lb/day)

Oak Ridge National Laboratory

Carbon Fiber Technology Center (≈ $50 million)

- North America's most comprehensive carbon fiber material and process development capabilities
- Development and demonstration of carbon fiber technology for energy and national security applications
- Low-cost and high-performance fibers
- Fast, energy efficient processing
- Capability to evaluate micrograms of candidate materials and produce up to 25 tonnes/year of carbon fibers
- Produce fibers for large-scale material and process evaluations by composite manufacturers
- Train and educate workers

Facility and equipment perspective



ORNL's vision for a sustainable community

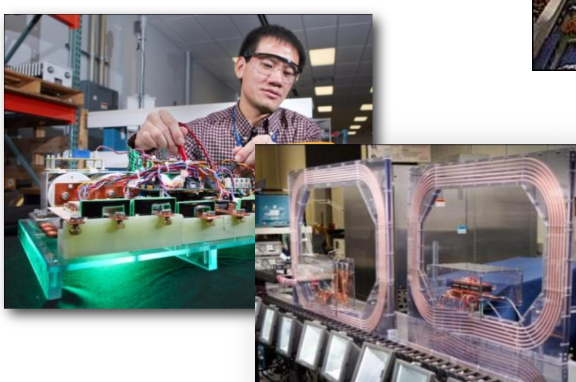
ORNL's vision for a sustainable community

Climate Impact

An integrated approach is needed!

Energy Technologies

Transportation



Building Technologies

ORNL's vision for a sustainable community

Green Intelligent Buildings

- Commercial and residential integration
- Envelopes
- Appliances
- Cool roofs

Smart Grid

- Situational awareness
- Advanced communications and controls
- Energy storage

Industrial

- High efficient processes

Renewables

- Bioenergy
- Solar
- Geothermal systems
- Wind

Sustainability

- Waste reduction
- Water management

Intelligent Transportation Systems

- Integrated land use planning
- Public transit friendly
- Alternate mobility choices (incl. freight)
- Clean fuels
- Intelligent vehicles and infrastructure

Thank you

SCIENCE RETREAT DECEMBER 2008

SCIENCE RETREAT JUNE 2009

DOE Bioenergy Research Centers

DOE GENOMICS:GTL
SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

Office of Science
U.S. DEPARTMENT OF ENERGY

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OAK RIDGE National Laboratory