Farming Carbon:

How Plant Roots, Microbial Ecophysiology, and Soil Minerals Shape the Fate and Persistence of Soil Carbon

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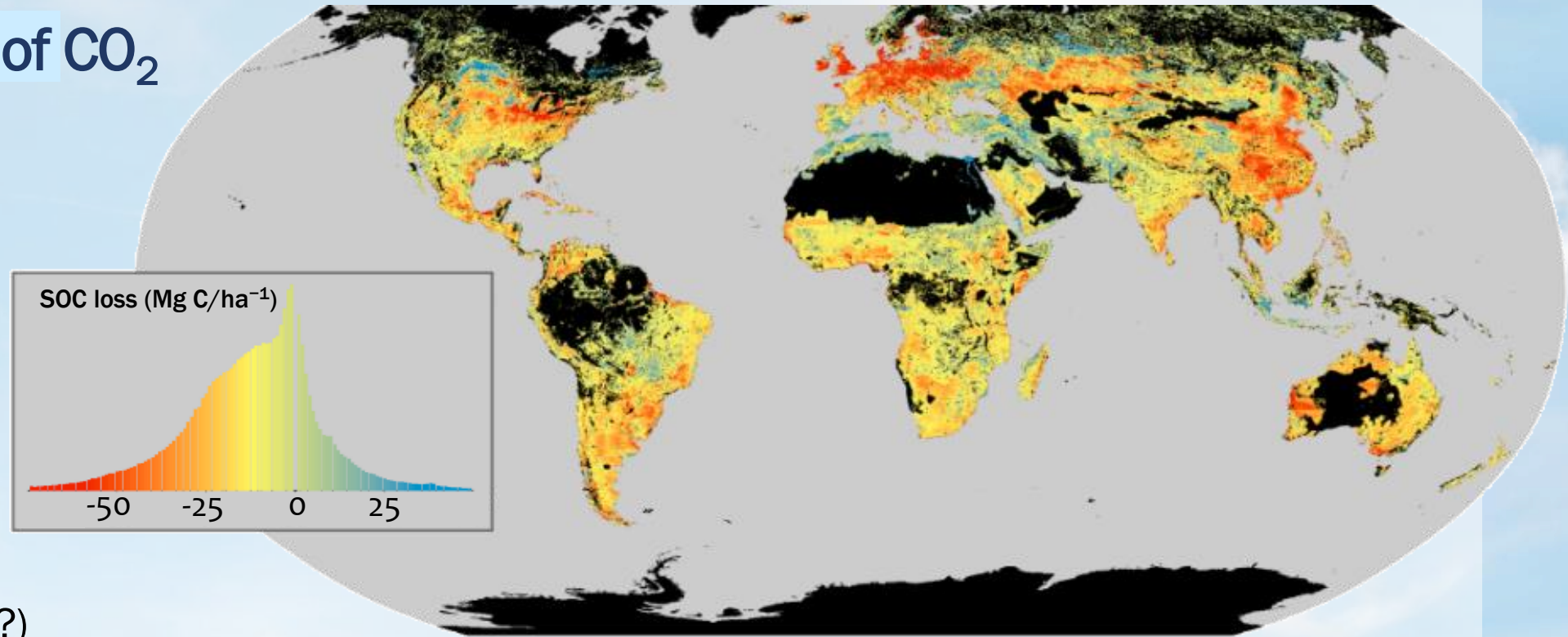Anne Kakouridis   Noah Sokol   Kate Zhalnina   Nameer Baker   Erin Nuccio   Eric Slessarev   Kat Georgiou   Craig See   Yoni Sher

The world's agricultural soils have lost at least 487 gigatons of CO_2 (equivalent)

SOC loss (Mg C/ha^{-1})

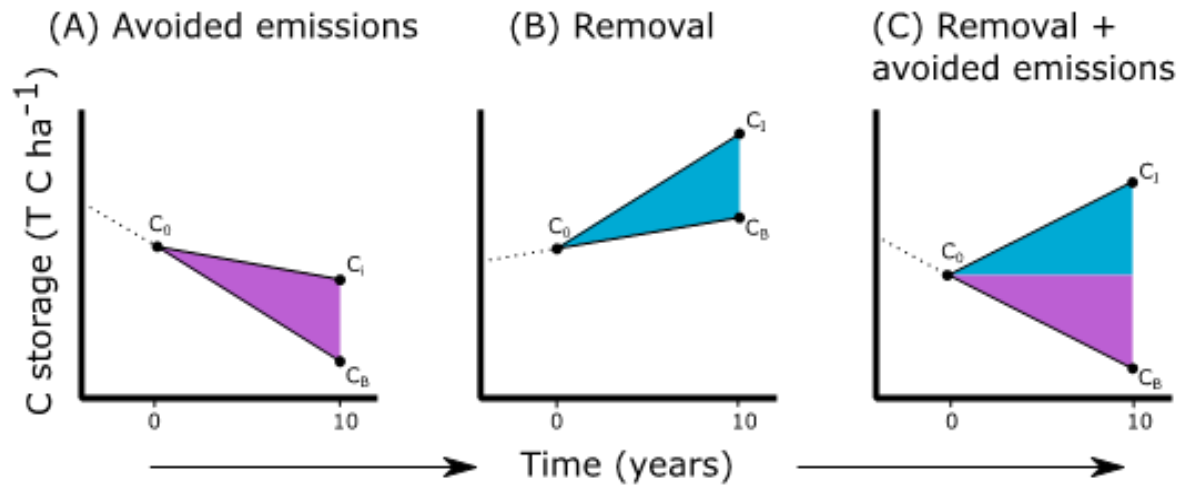-50   -25   0   25

(Can we put it back?)

In the USA, Paustian et al. estimate 0.5 - 1 Gt/yr could be sequestered (an overestimate?)

Thorny issues: MRV, durability, land tenure, producer economics, shallow/deep effects, additionality
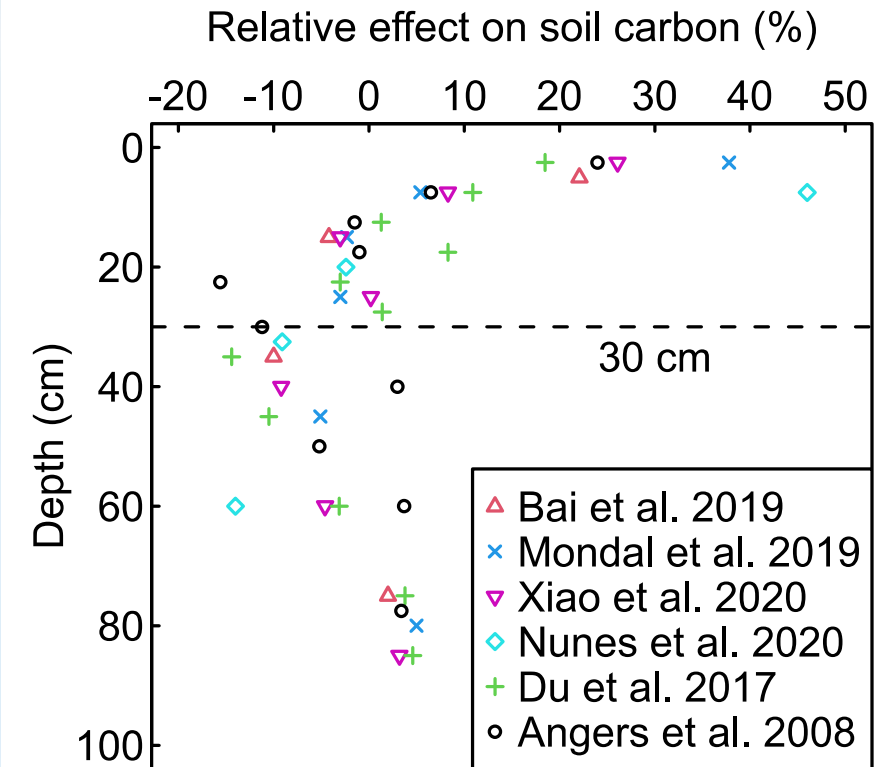
Sanderman et al. *PNAS* 2017

Thorny Issues: Additionality, Amendments, Unintended effects

No-till

Amendments

(A) Avoided emissions (B) Removal (C) Removal + avoided emissions

- Reduced / removed / avoided aren't well distinguished
- Amendments are mis-counted due to system boundaries

Relative effect on soil carbon (%)

- Bai et al. 2019
- Mondal et al. 2019
- Xiao et al. 2020
- Nunes et al. 2020
- Du et al. 2017
- Angers et al. 2008

- Soil C tends to increase at the surface, but can decrease at depth
- N_2O, CH_4 emission can increase

National analysis: Soil C solutions scorecard

| Removal Class	Subclass	Measurability of carbon storage	Removal vs. avoided emissions	Risk of unaccounted GHG emissions	Additionality	Leakage risk	Durability
Soil	Cover cropping	**	**	**	***	**	**
	Deep-rooted perennials	**	**	**	***	*	**
	Tillage reduction	*	**	**	*	**	**
	Organic amendments	*	**	*	*	*	**
	Grazing management	*	**	*	**	**	**
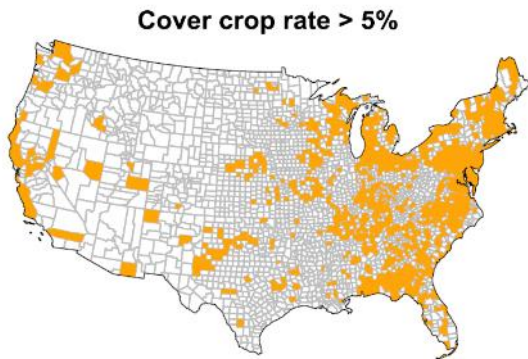
Cover crop rate > 5%

Figure 3-6. Counties where the rate of cover cropping exceeds 5%. These counties were excluded from our capacity estimates because they exceeded the 5% additionality threshold.

* low	* avoided only	* high risk	* relatively low	* high risk	* Less than 10 years
** intermediate	** mixed	** medium risk	** intermediate	** medium risk	** 10 - 100 years
*** high	*** removal only	*** low risk	*** relatively high	*** low risk	*** 1000's of years

na = not applicable

Panicum virgatum
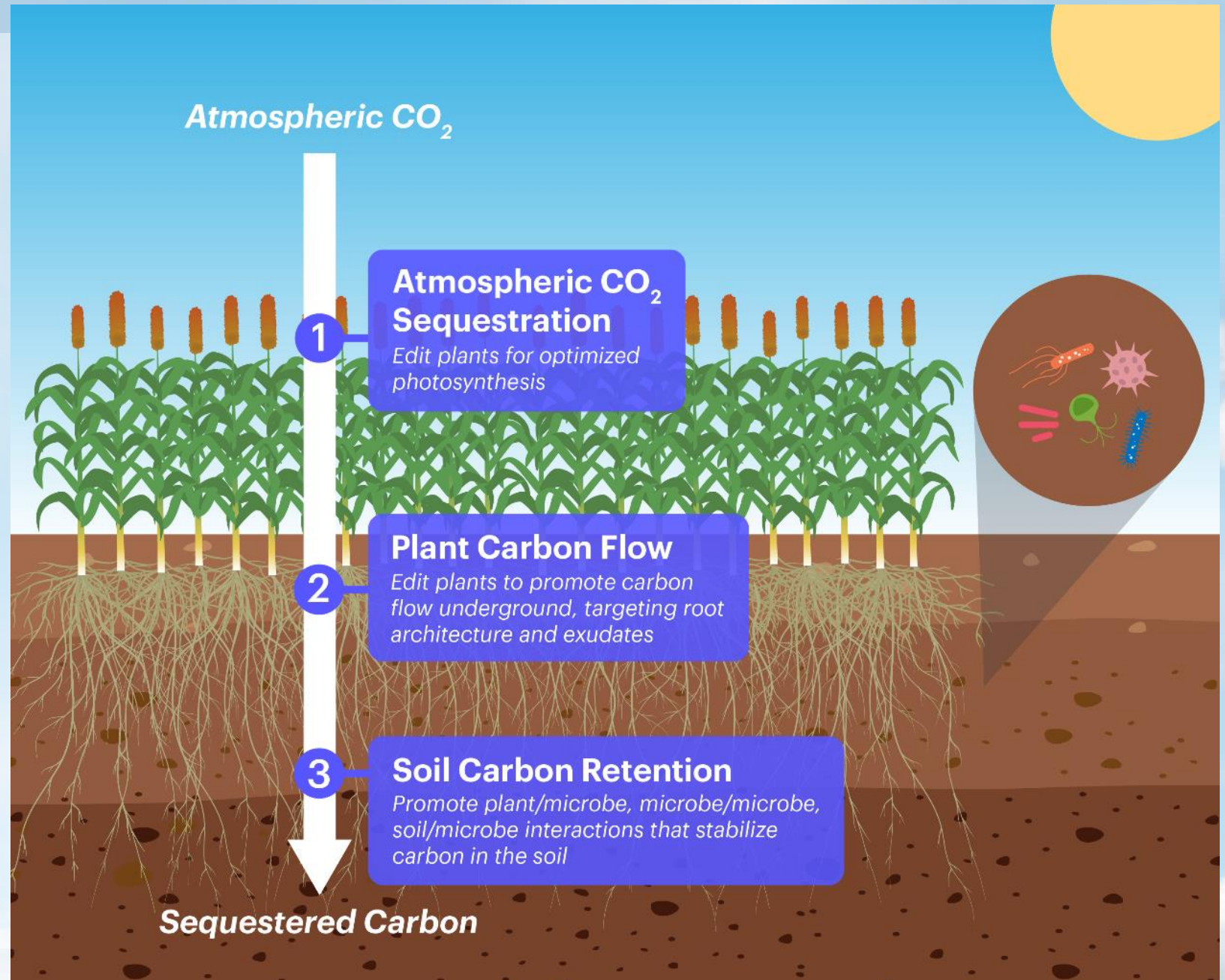
Schmidt et al., *Carbon Negative by 2030: CO$_2$ removal options for an early corporate buyer*, 2022
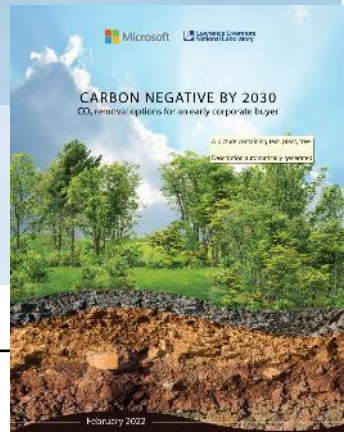
To achieve Gt-scale carbon removal, we must harness <u>both</u> plants and soil microbes

..and maybe minerals too!

Atmospheric CO$_2$

Atmospheric CO$_2$ Sequestration
1 *Edit plants for optimized photosynthesis*

Plant Carbon Flow
2 *Edit plants to promote carbon flow underground, targeting root architecture and exudates*

Soil Carbon Retention
3 *Promote plant/microbe, microbe/microbe, soil/microbe interactions that stabilize carbon in the soil*

Sequestered Carbon

~50% of soil organic carbon is formed from dead soil microorganisms: 'microbial necromass'
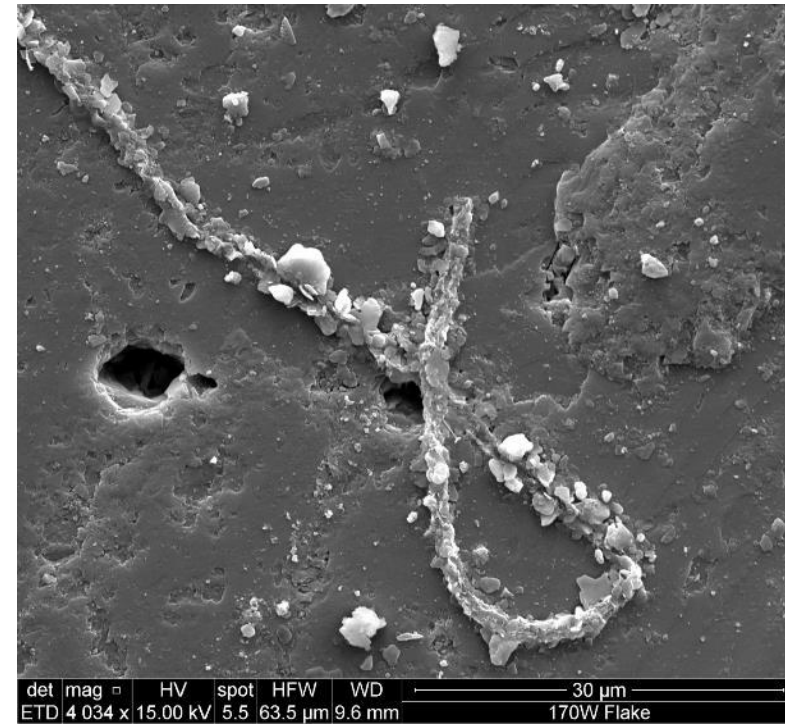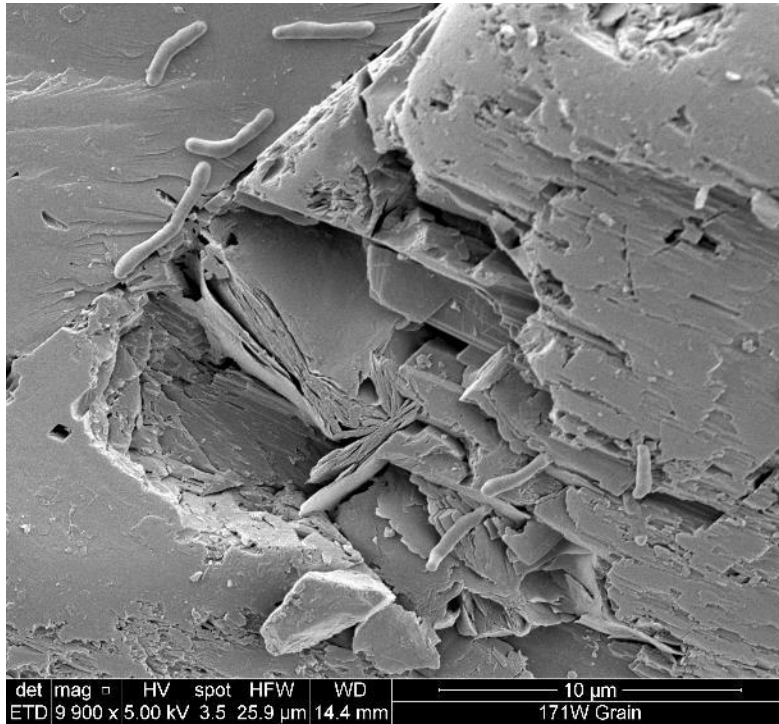
SEM Images: Noah Sokol/Christina Ramon LLNL

pu·tre·fac·tion (aka 'decomposition)

CO$_2$

- **Much of soil carbon starts as dead root carbon**
  Belowground inputs 5X more likely to be retained (Jackson et al. 2017; Sokol & Bradford 2019)

- **Microbes colonize decaying roots, encasing them in protein- and polysaccharide-rich 'goo' (EPS)**

- **This EPS, and lipid-rich globs of microbial necromass, become precursors for stabilized C**
  Root EPS promotes soil aggregation (Sher, Baker et al. 2020)
  Oldest SOM resembles microbial cells, metabolites (Kleber 2011)

- **Microbial necromass is a primary ingredient in soil organic matter**
  (Darwin 1881; Waksman 1936; Kögel-Knabner 2002; Liang and Balser 2008; Keiluweit et al. 2012; Kallenbach 2016 …& many others)

Desorption

Microbial Turnover
(Lysis, Predation, Stress)

Carbon Inputs

Sorption

Microbial Colonization

C Transformations
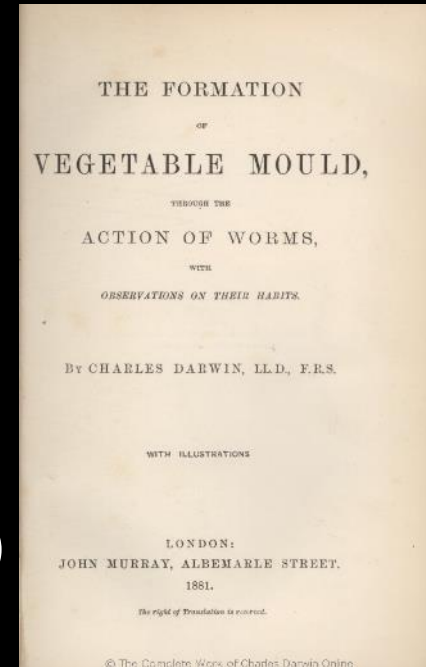
Darwin, 1881
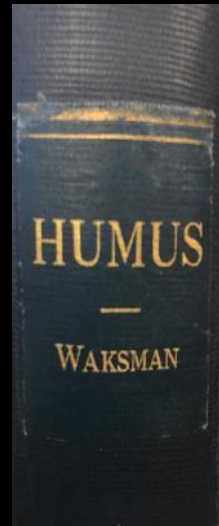
Waksman, Humus, 1936

Microbial traits important for soil carbon accrual

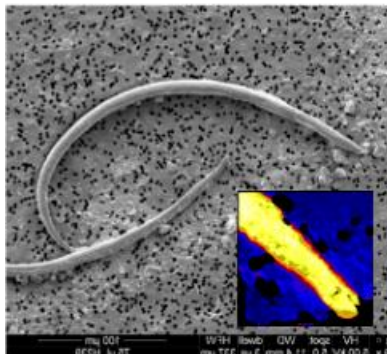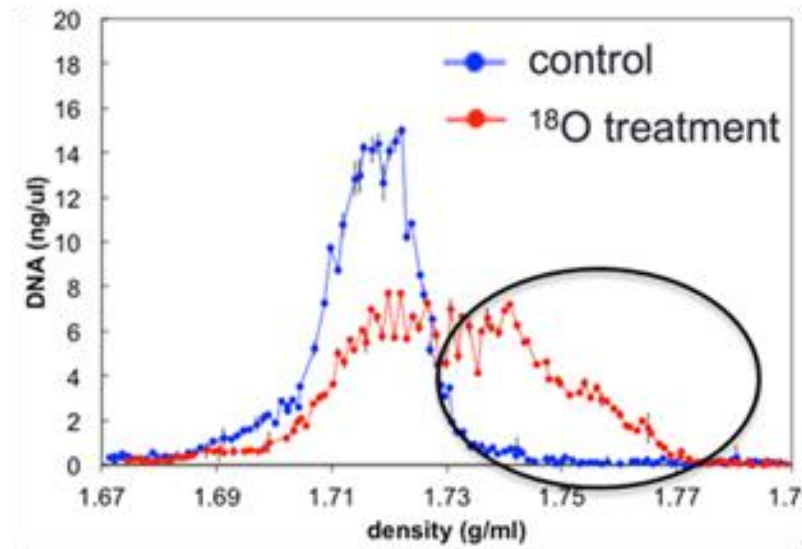|	Traits	Prediction and validation[a]
Life history traits	Minimum generation time	Codon usage, rRNA copy number, microscopy, optical density
	Optimum growth temperature	Amino acid frequencies, microscopy, optical density
Biophysical	Genome	Assembled genome length, DNA yield per cell, GC content
	Cell size and shape	Genome size to cell size, SEM, light microscopy, FACS (isolates or Nycodenz)
	Adhesion and motility	Adhesins, holdfast genes; Pilli, flagella genes, microscopy, capillary assays
Cellular composition	Cell wall or envelope composition	Polysaccharide, lipid, glycoprotein, pigment or Gram-type genes, lipidomics, FTIR, NMR, HPLC, mass spectrometry
	EPS or other residues	EPSac genes, bulk EPS quantification, FTIR, mass spectrometry
Resource acquisition	Exoenzymes	Secreted enzyme genes, activity essays, protein-SIP
	Transport systems	Transporter genes
	Secretion systems	Secretion genes, SEM or TEM
	Metallophores	NRPS siderophore genes, siderophore assays, mass spectrometry
	Storage materials	Phosphoester, phospholipid, polyhydroxybutarate, microscopy, FTIR
Stress tolerance	Stress regulation	Regulatory genes (sigma factors, anti-sigmas, two-component)
	Spore formation	Sporulation genes, spore stains, bulk quantification, DNA-SIP–dormancy
	Osmotolerance	Osmotic response genes (osmolytes, efflux pumps), viral integrity experiments, mass spectrometry, protein-SIP
Antagonism or defence	Antibiotics, toxin–antitoxin systems	Biosynthetic clusters, toxin or antitoxin genes, mass spectrometry
Emergent traits	Realized growth rate	Genome inferred (iREP[46]), heavy water DNA-SIP[18]
	CUE	Genome predicted ranges, quantitative SIP[173], isotope tracing, bulk CUE
	Stoichiometric range	Genome predictions and allometric scaling, nanoSIMS, bulk measurements

Sokol et al. 2022 *Nature Reviews Microbiology*
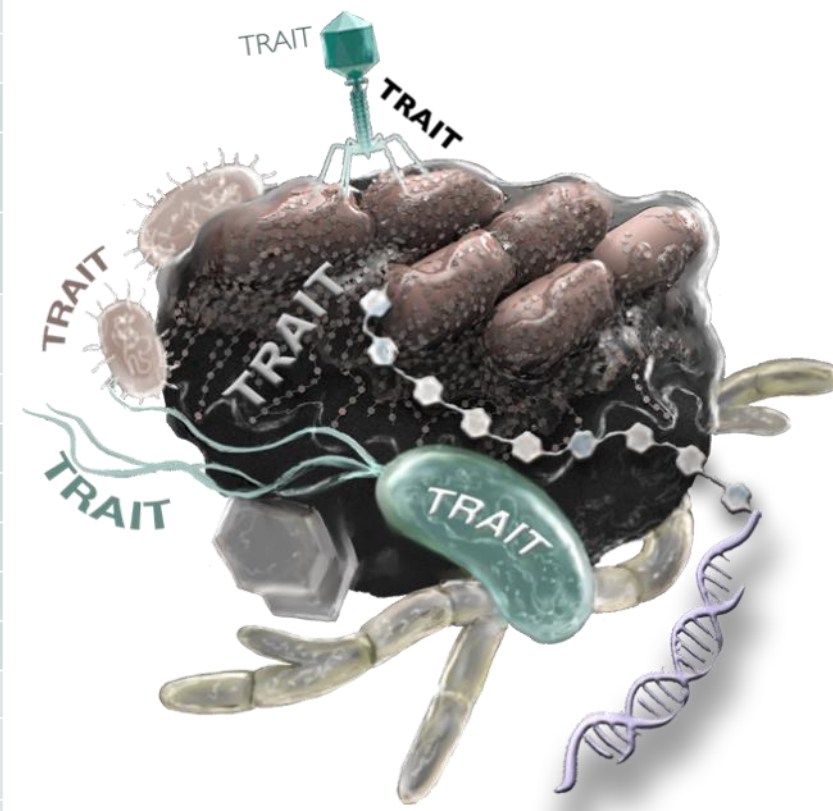
Its essential we study the 'right' microorganisms...

Quantitative stable isotope tracing helps ID the ACTIVE taxa
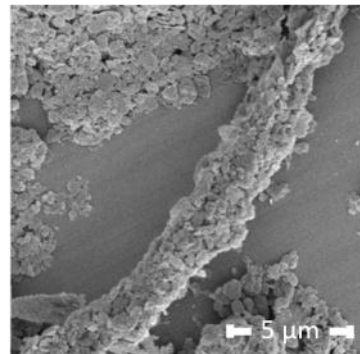

Greenhouse $^{13}CO_2$ labeling
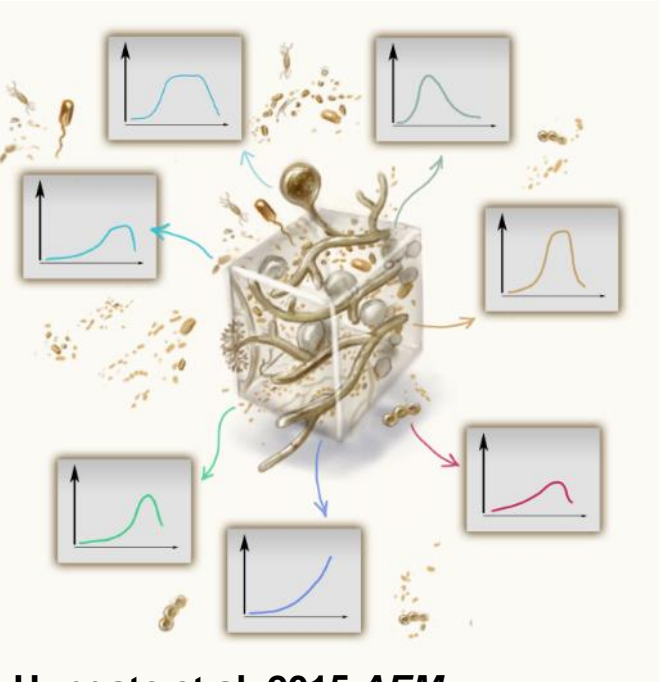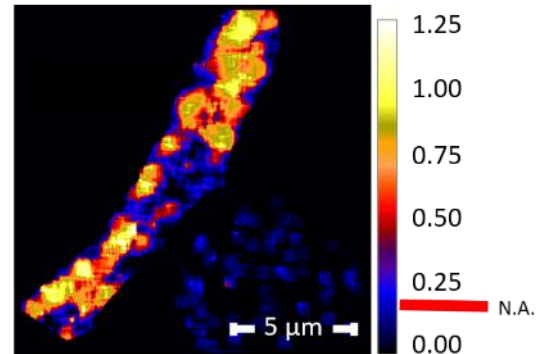

Hungate et al. 2015 *AEM*
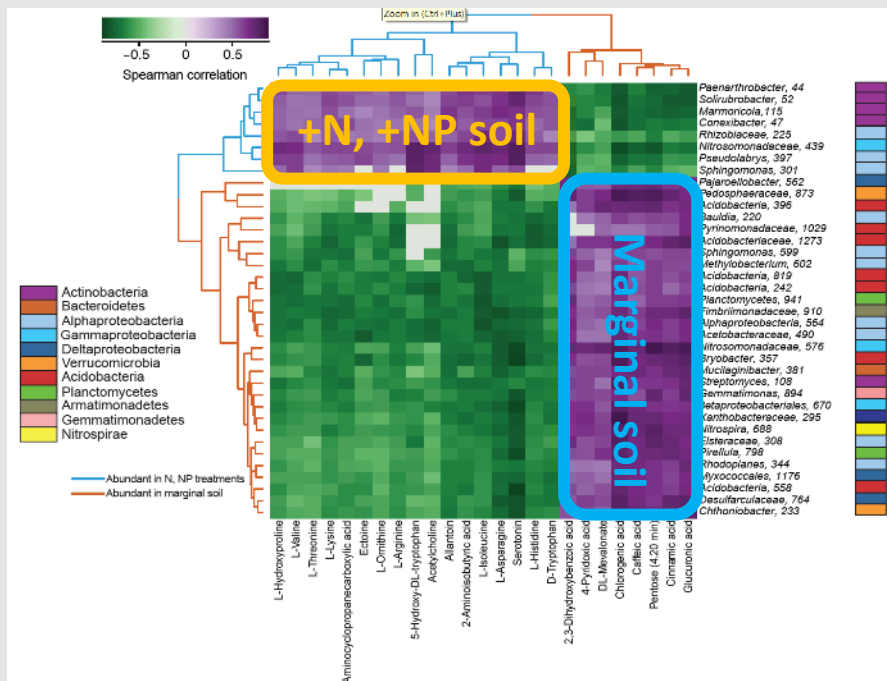**Koch et al. 2018** *Ecosphere*

SEM   NanoSIMS $^{13}C/^{12}C$
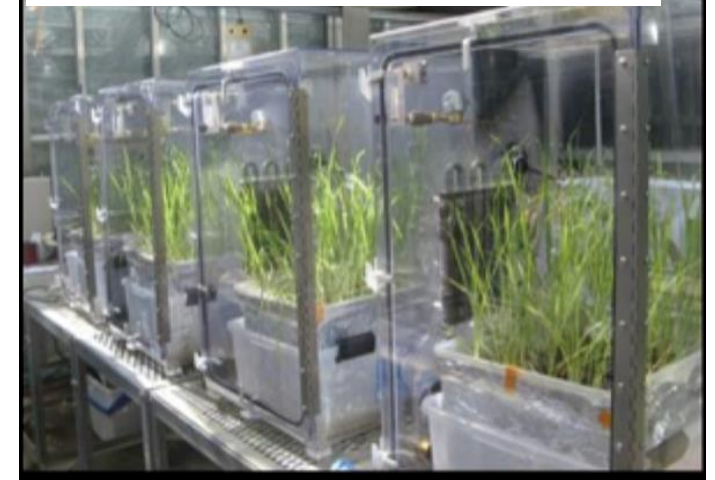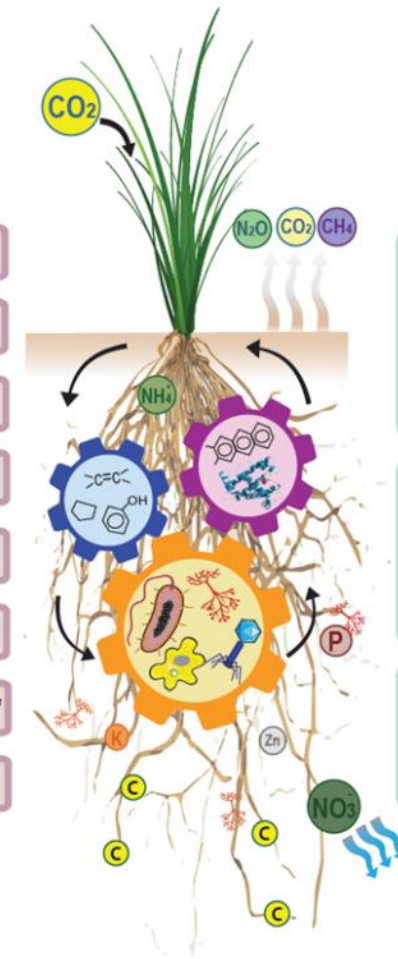

Field $^{13}CO_2$ labeling

I. Engineering the Microbiome

- Focus on obligate biotrophs

- By managing plant exudates, we manage the root microbiome



Baker, Zhalnina et al. 2022 *bioRxiv*

Zhalnina et al. 2021 *Phytobiomes*

Arbuscular mycorrhizal fungi

--provide significant amount of plant N, P & water

--can 'rescue' rhizo-biome during water stress

--transport substantial plant-fixed C outside the root zone

--key mechanism leading to organic matter-mineral interactions

Density in soil	Global Mean
Total hyphae (cm cm^{-3})	102,000 (100-1,255,400)
AMF only (cm cm^{-3})	2,000 (100-15,000)
Fine roots (cm cm^{-3})*	6.8

See et al. *Global Change Biology* 2022

100 μm

AMF transported C becomes rapidly mineral-associated

SEM | NanoSIMS $^{13}C/^{12}C$
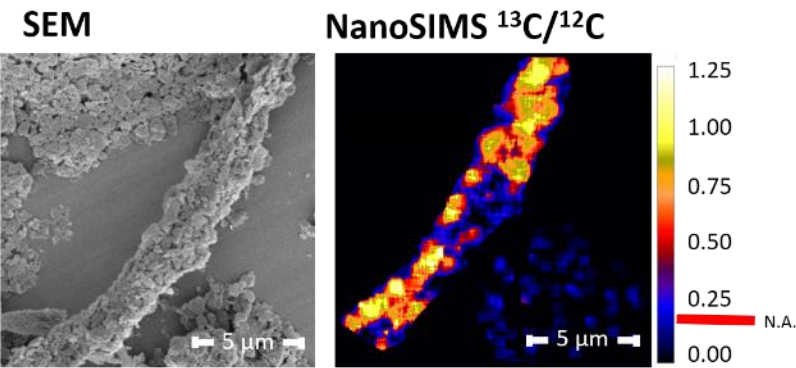
^{13}C-enriched AM fungal hyphae covered with kaolinite minerals

Neurath et al. 2021 *ES&T*
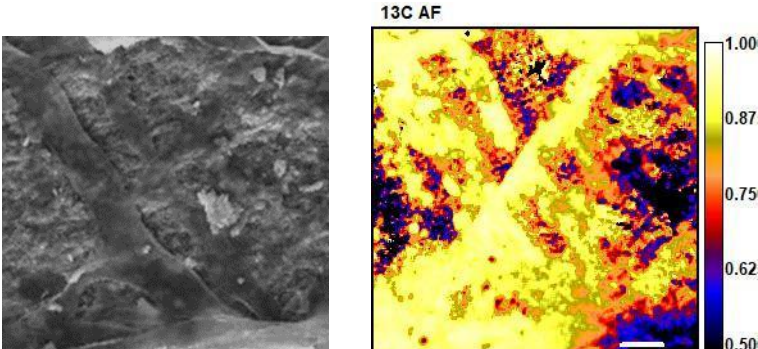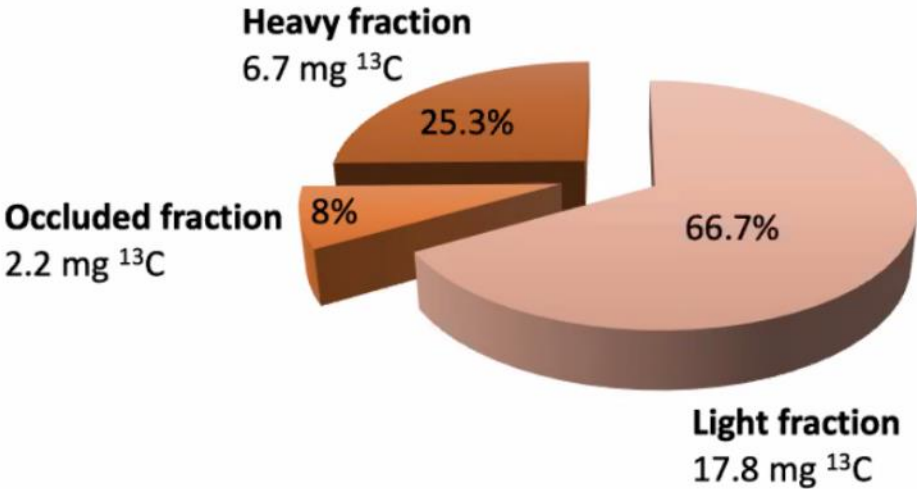
^{13}C-enriched decaying fungal necromass
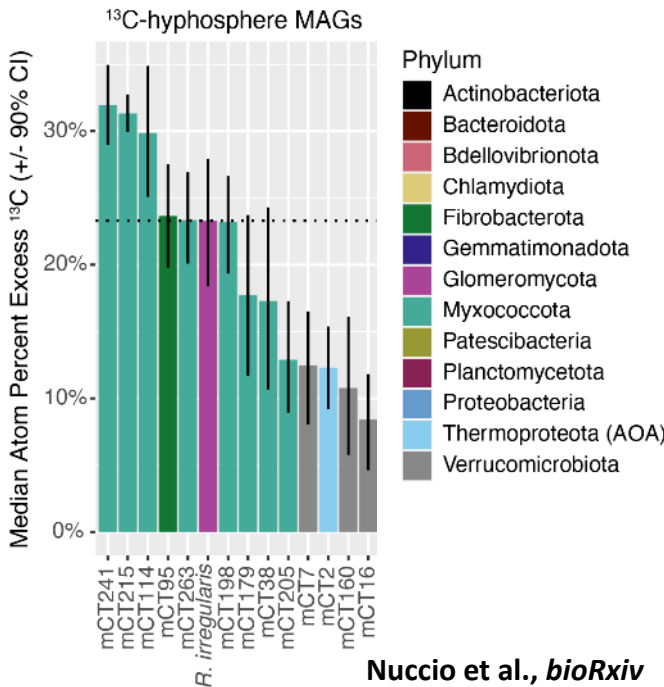
See et al. 2021 *Global Change Biology*

Hyphae mediate aggregate formation

Heavy fraction
6.7 mg ^{13}C
25.3%

Occluded fraction
2.2 mg ^{13}C
8%

66.7%

Light fraction
17.8 mg ^{13}C

In 6 weeks of plant growth, AMF hyphae moved 27mg of ^{13}C

Kakouridis et al., *in prep*

^{13}C-hyphosphere MAGs

Phylum
- Actinobacteriota
- Bacteroidota
- Bdellovibrionota
- Chlamydiota
- Fibrobacterota
- Gemmatimonadota
- Glomeromycota
- Myxococcota
- Patescibacteria
- Planctomycetota
- Proteobacteria
- Thermoproteota (AOA)
- Verrucomicrobiota

Nuccio et al., *bioRxiv*

Carbon moved by AMF hyphae is preferentially accumulated in predatory bacteria

II. Improving the effects of roots

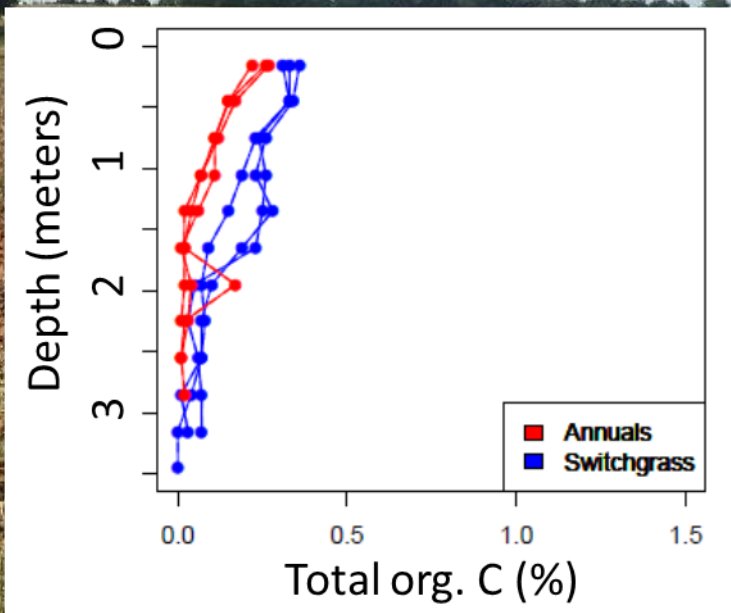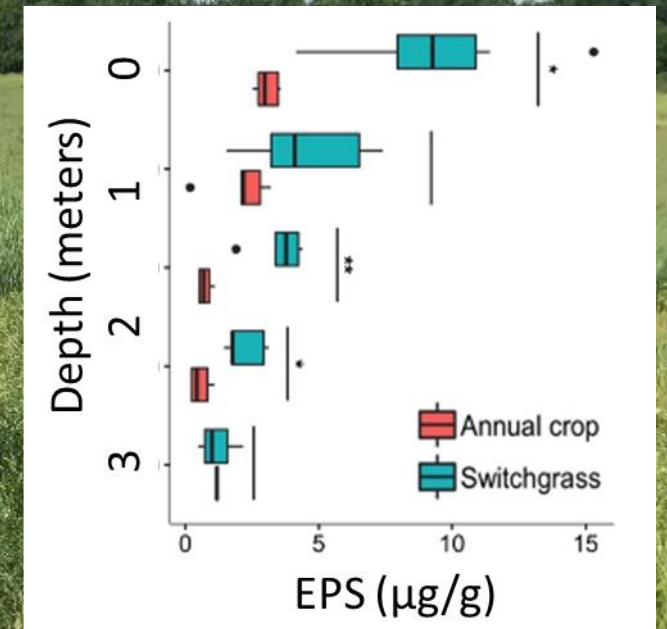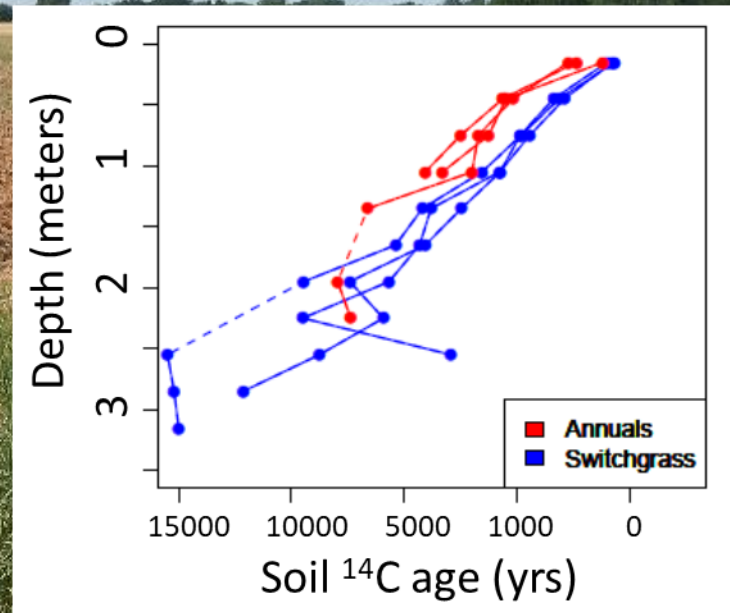*Panicum virgatum*

Shallow-Rooted Annuals

vs.

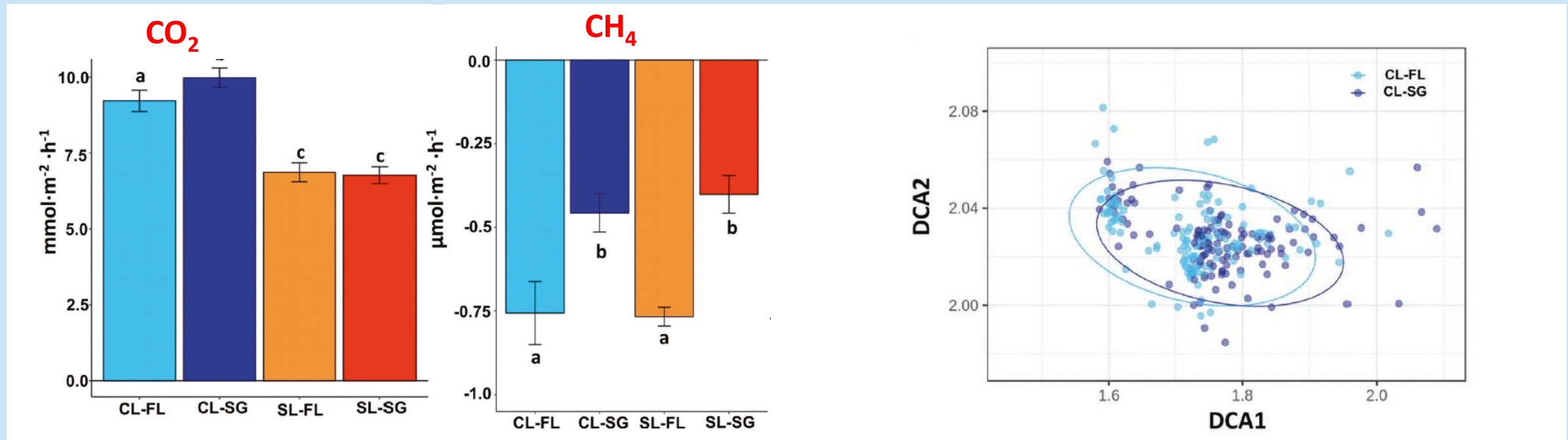# Deep-Rooted Perennials

10 years, twice as much soil carbon

Slessarev et al. *GCB Bioenergy*, 2020

Sher et al. *Soil Bio & Biochem, 2020*

Burneyville, OK

Roots & microbial biofilms 'EPS' lead to increased soil aggregation

GHG fluxes with conversion to a deep rooted perennial

CO$_2$

CH$_4$

- GHG fluxes for 1.5 yrs after conversion to switchgrass, two soil types, compared to fallow
- Minor CO$_2$ effect, no N$_2$O effect (not fertilized)
- Significant reduction in CH$_4$ consumption in both soil types

- Correlated with shift in microbiome composition
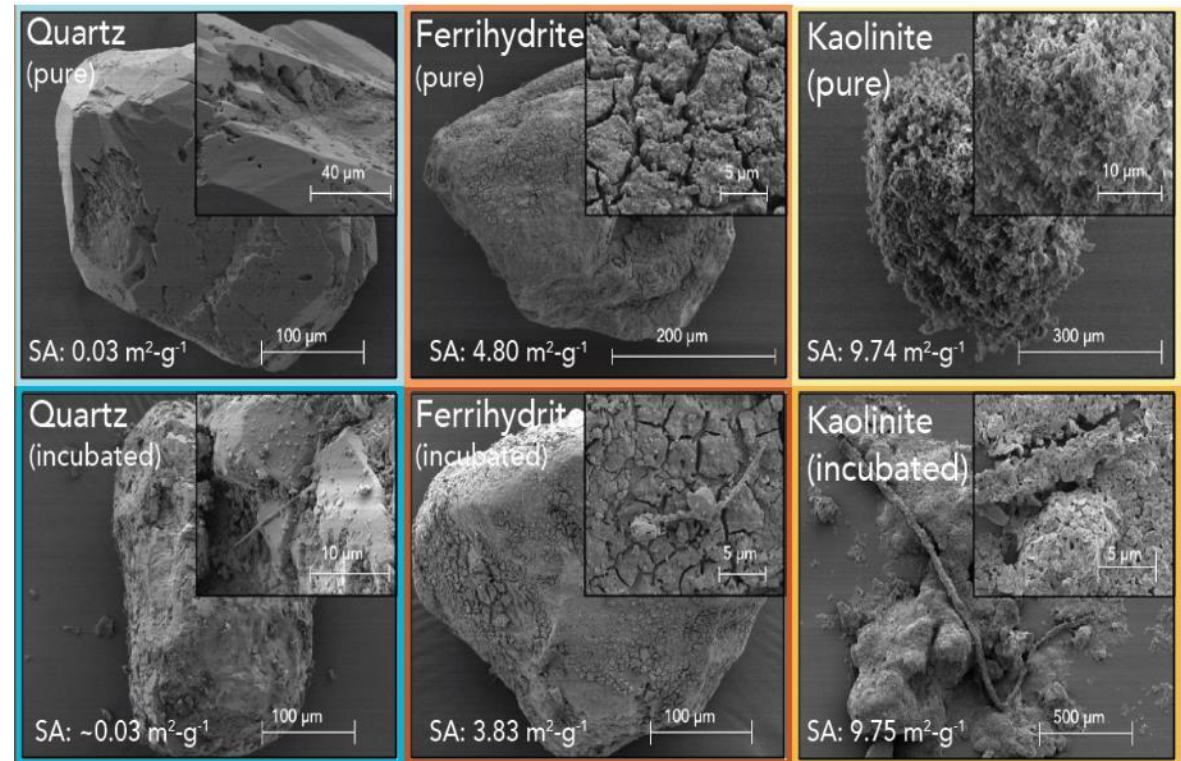- Need SIP approach to determine which specific taxa are responding

Ability to add deep C depends on soil type and initial C stocks

- Switchgrass vs. shallow-rooted annuals
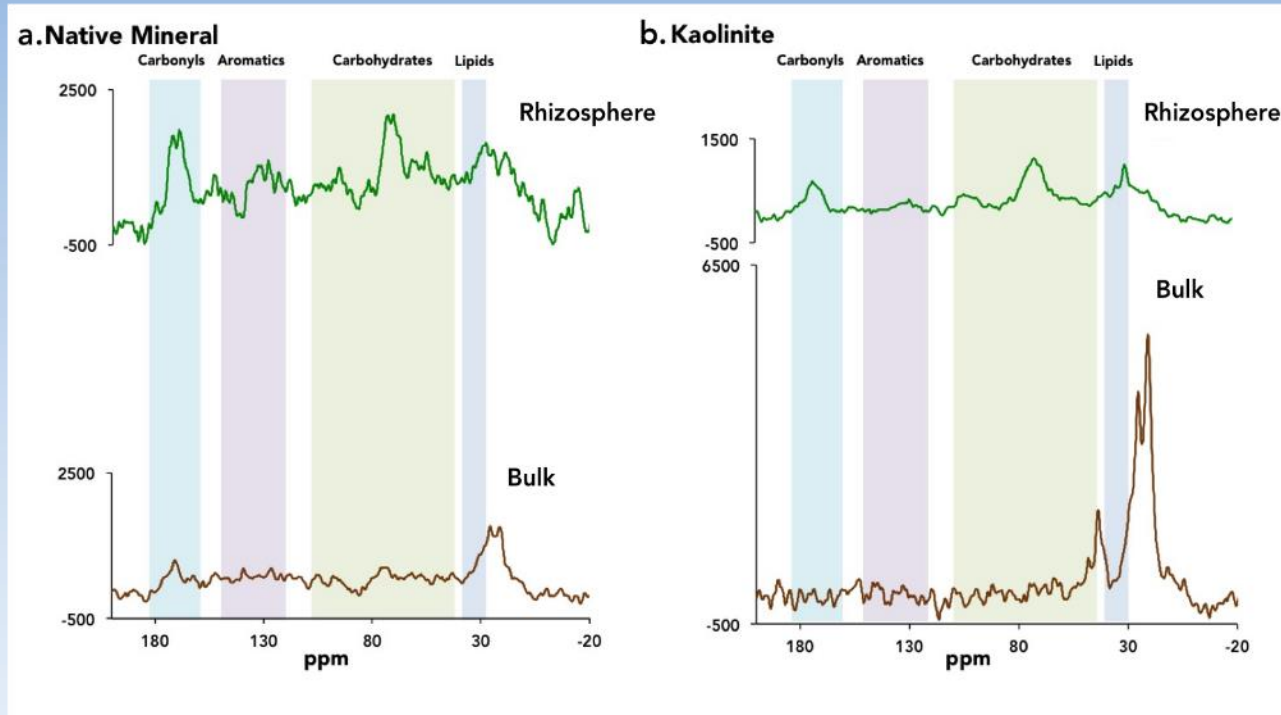- 2.5m cores x 9 depths (470 samples)

Less -> More Weathered Soil
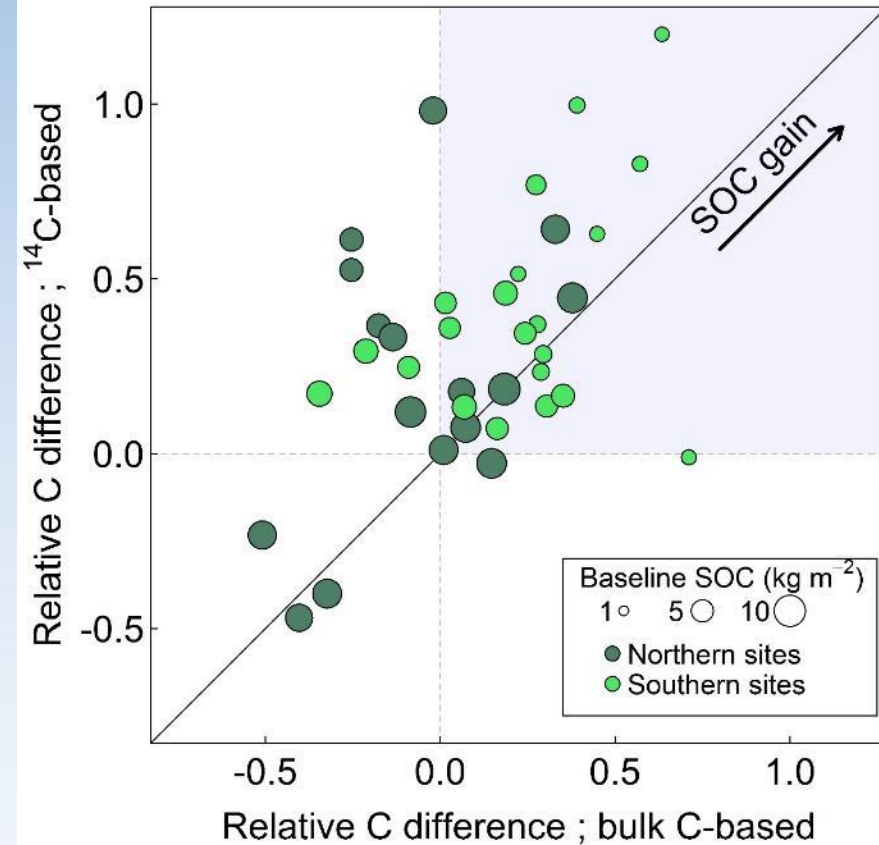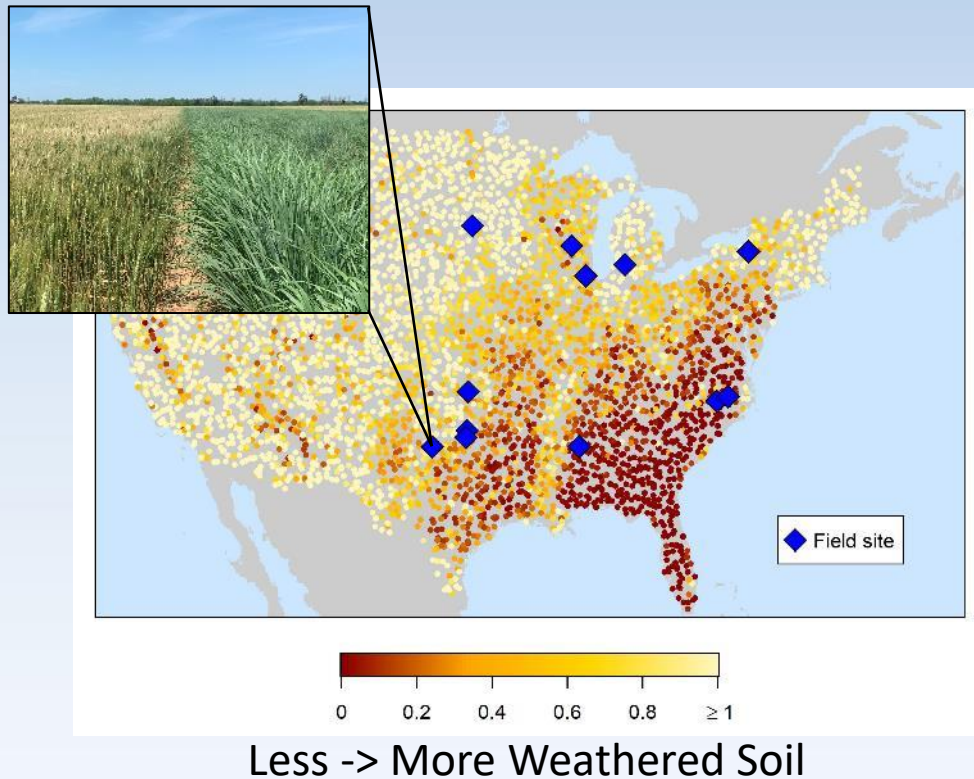
- Accrual of new C us most apparent in <u>marginal soils</u> with low initial C

III. Considering Soil Minerals

Neurath et al. *ES&T*, 2021; Whitman et al. *Env. Microbiology,* 2018

Different microbes prefer different minerals, leave distinct residues behind

- diverse rhizosphere-derived compounds are a *transient fraction* of mineral SOM
- rapid exchange with mineral surfaces

- Distinct microbial communities found on different mineral types
- Rhizosphere minerals had more diverse compounds (different functional groups—carbonyl, aromatics, carbohydrates, lipids) than minerals in bulk soil

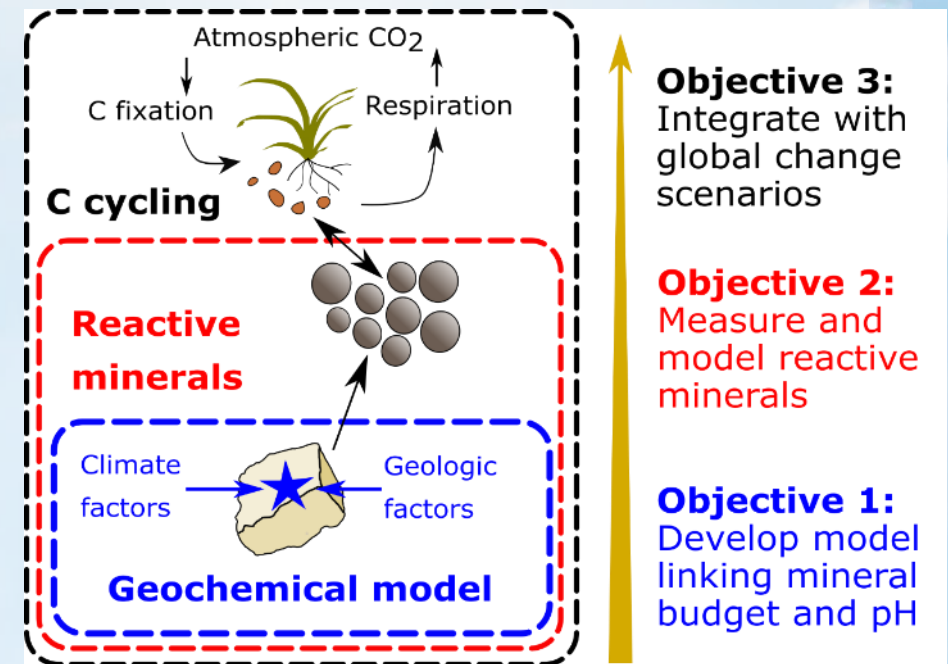Whitman et al Env. Micro. 2018; Neurath et al. *ES&T*, 2021
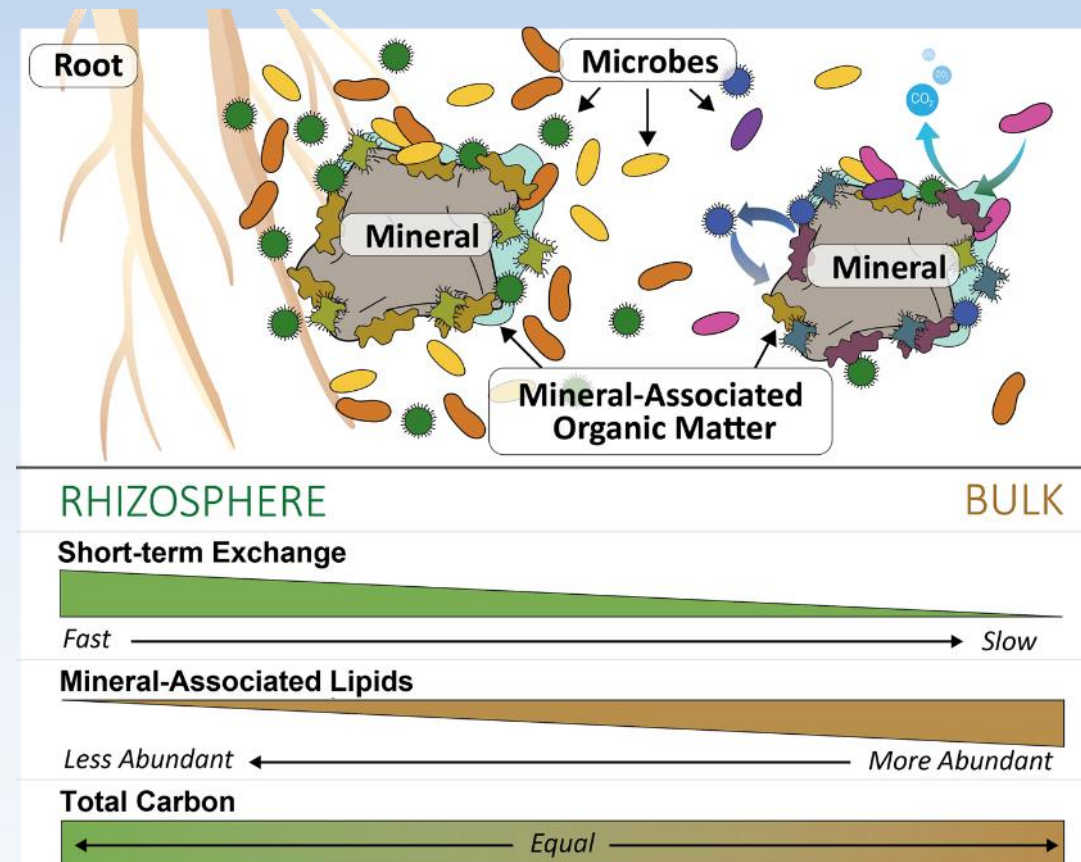
The opportunities for carbon farming are not equal across US agricultural lands
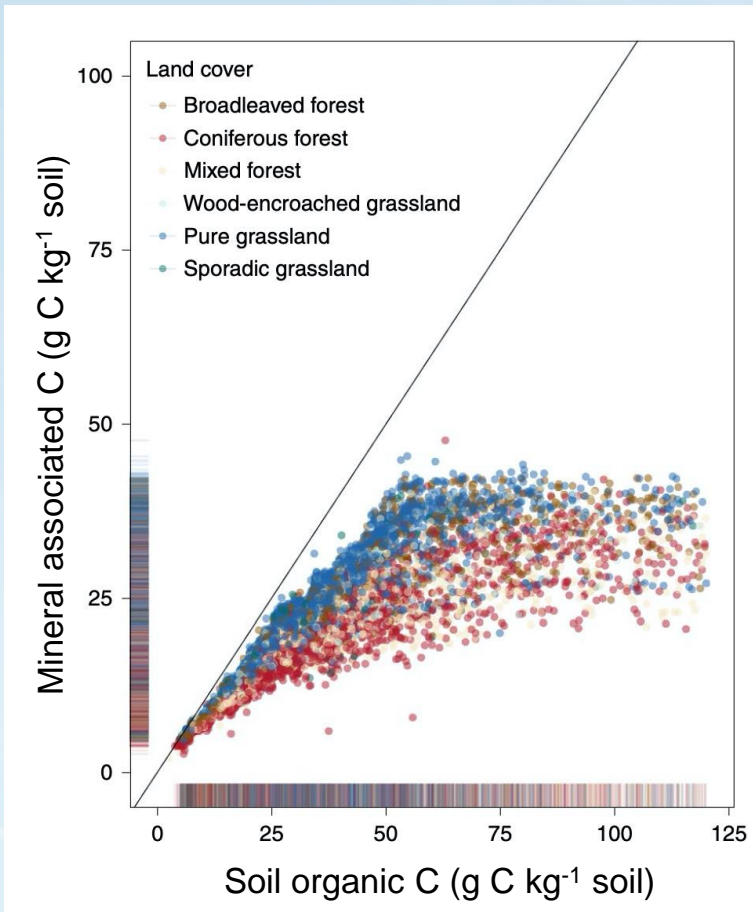

Where to park the carbon?
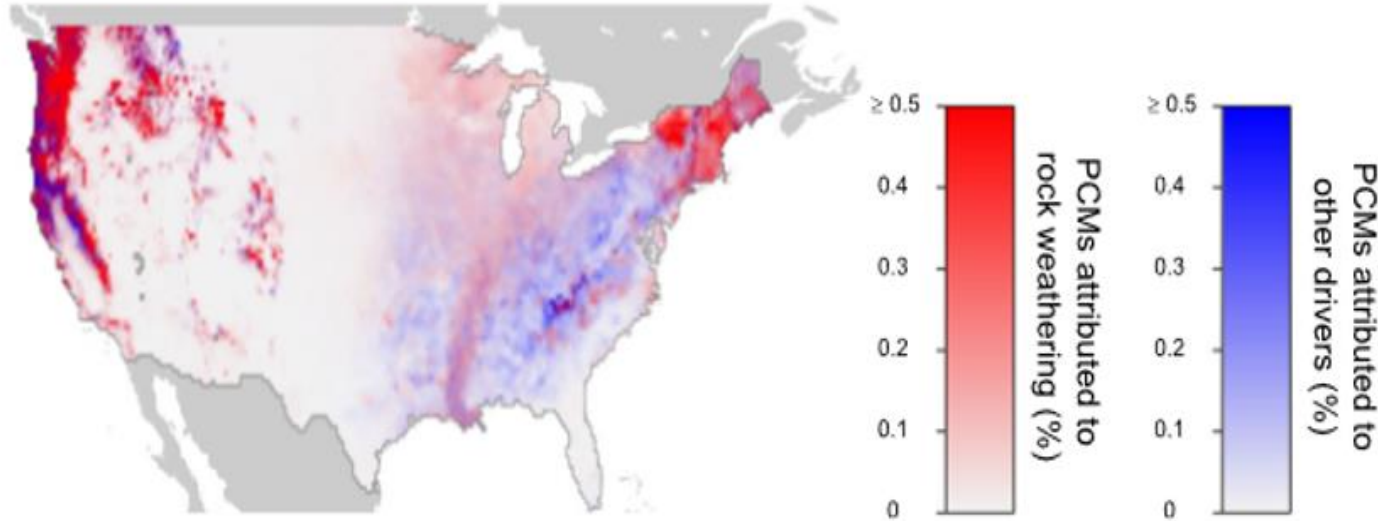
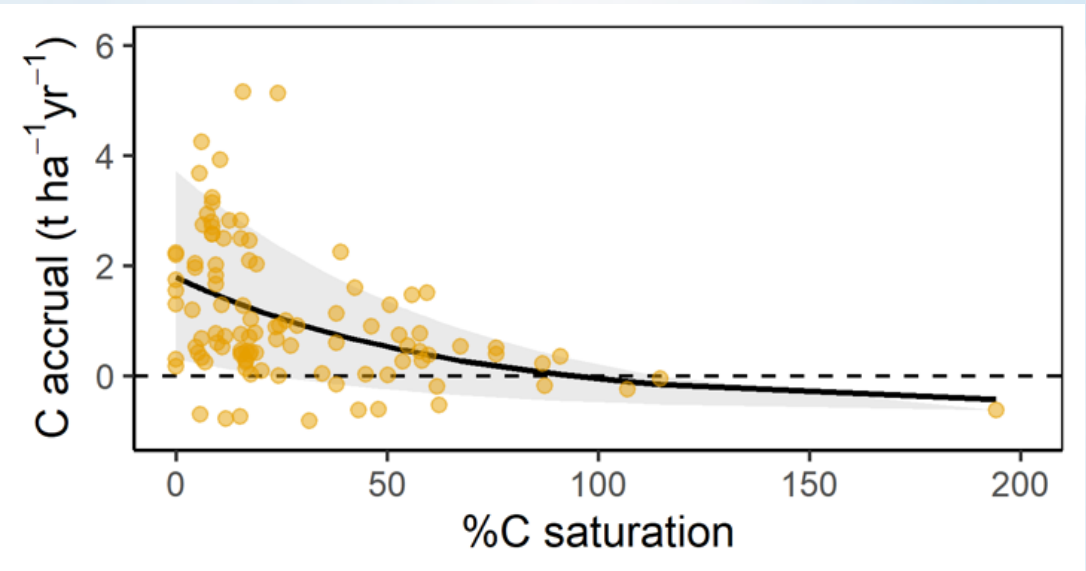Slessarev et al. *Biogeochemistry Letters,* 2021

Slessarev et al. *in prep.*

Mineralogical C capacity in practice

186 soil profiles and model-predicted
values across Europe

Global synthesis of C accrual studies from 103 soil profiles

Cotrufo et al. *Nat. Geosci,* 2019

Accrual rates were ~3x higher in soils at
10% saturation than at 50% saturation.

Georgiou et al., *Nat. Comms.* 2022

Opportunities:

- Biotrophs/beneficial fungi transport N, P and water to the plant host, and fungal hyphae transport C to mineral surfaces → **select for enhanced mycorrhizal symbioses**

- Deep rooted plants, particularly perennials, can have a net positive impact on SOC → **engineer for deep, robust root systems**

- Rhizodeposits (extracellular polysaccharides, "EPS") play an important role in promoting soil aggregation/carbon persistence → **engineer for EPS production**

- We need to measure the geographic patterns of biophysical constraints and mineral capacity → **include dynamic minerology in our carbon farming strategies**

Thanks to…

BER Sustainable Switchgrass Project
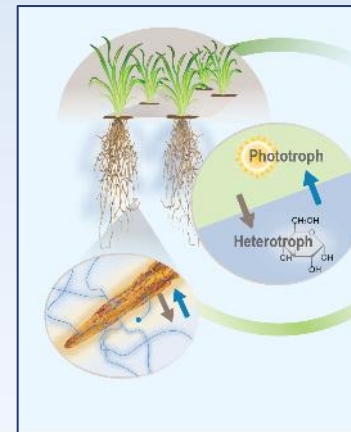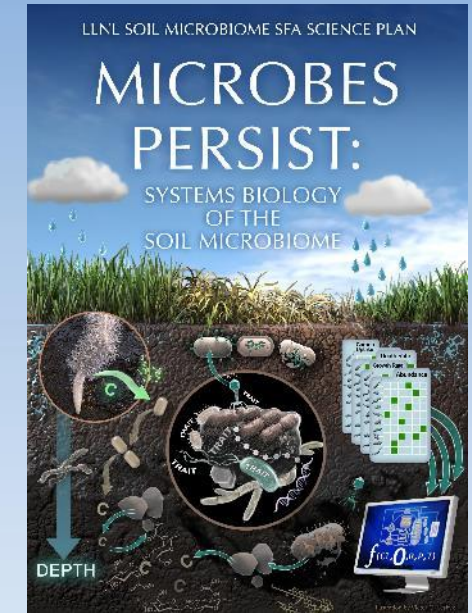
LLNL Soil Microbiome SFA

LLNL µBiospheres SFA