unlock the SECRETS IN THE SOIL

Biological Indicators of Soil Health:

What they are, how they are measured, and what is on the horizon?

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USDA-NRCS, Portland, OR

USDA NRCS
United States Department of Agriculture
Natural Resources Conservation Service

Soil Health

The continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans

Healthy functioning soils:

- Produce food, fuel, fiber, and medicinal products
- Store, filter, and release water
- Provide resilience to environmental disturbances
- Resist diseases, pests, and pathogens
- Store and cycles carbon
- Store and cycle nutrients internally
- Maintain biodiversity and habitat

To Understand Soil Health We Must Understand Soil Biology

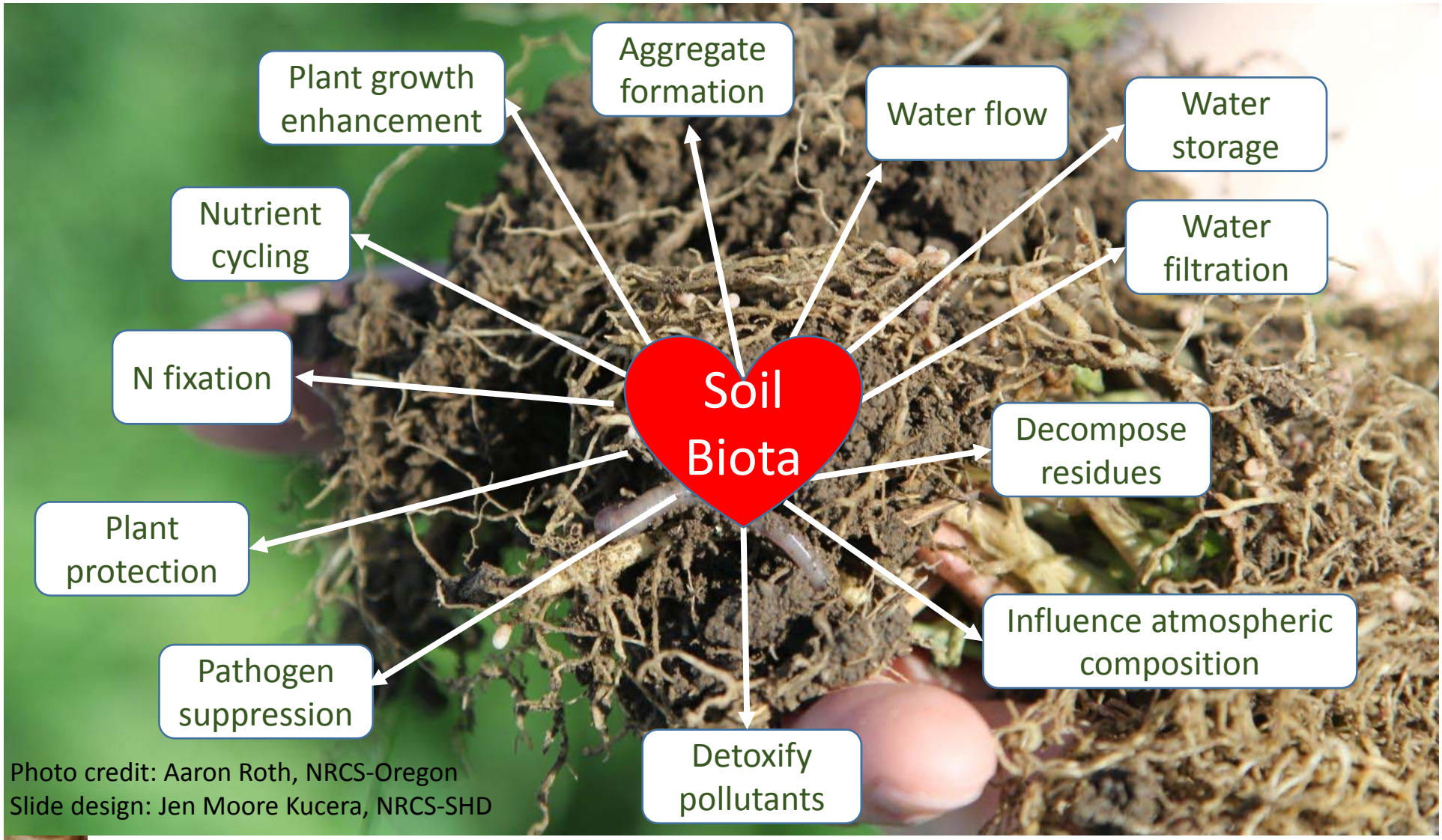

Photo credit: Aaron Roth, NRCS-Oregon
Slide design: Jen Moore Kucera, NRCS-SHD

Importance of Soil Biology: Decomposition → OM Formation

Soil Function

Decomposition

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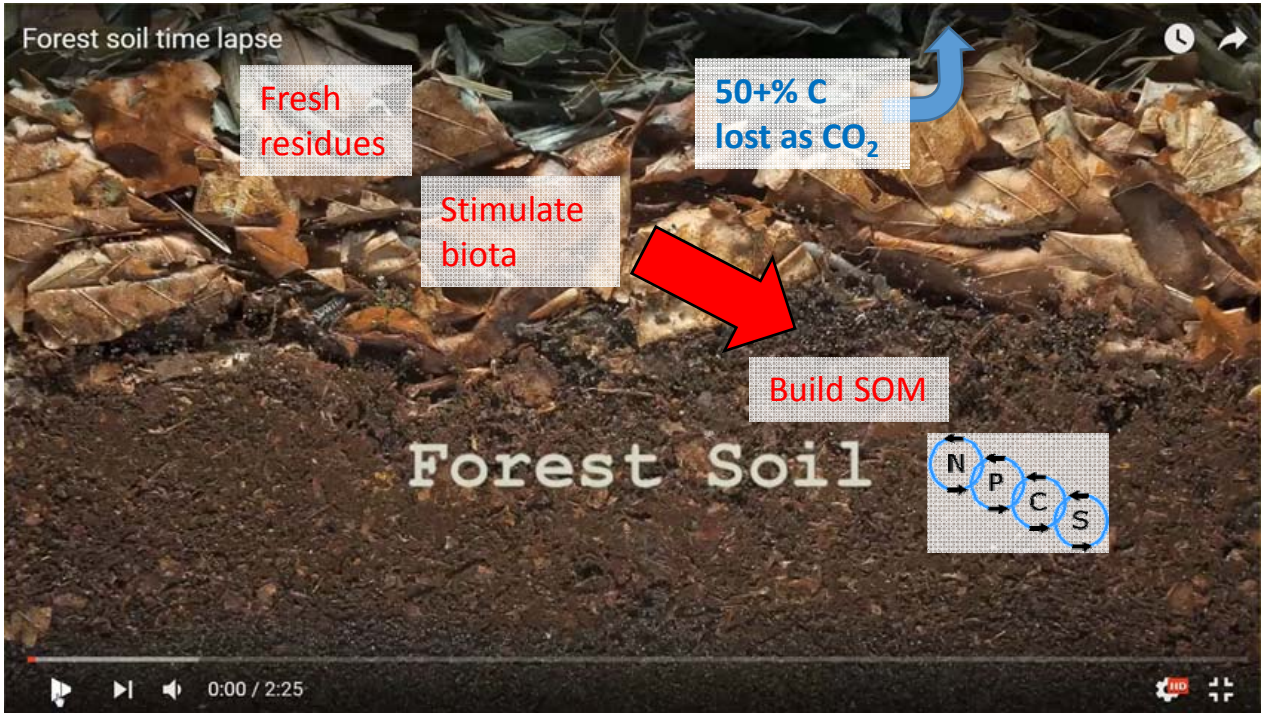
Decrease residue

↓

Build organic matter → multiple benefits



Watch time-lapse video on YouTube
https://www.youtube.com/watch?v=ECh52Nt8WXc

http://soilquality.org/images/nutrient_cycling.gif

Importance of Soil Biology: Aggregate Formation and Stabilization

Soil Function

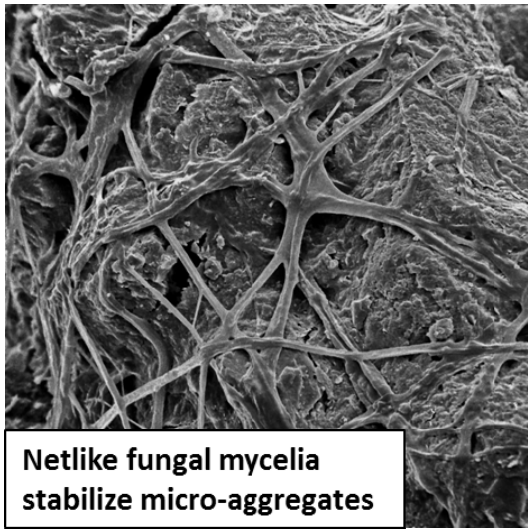
Aggregate formation

↓

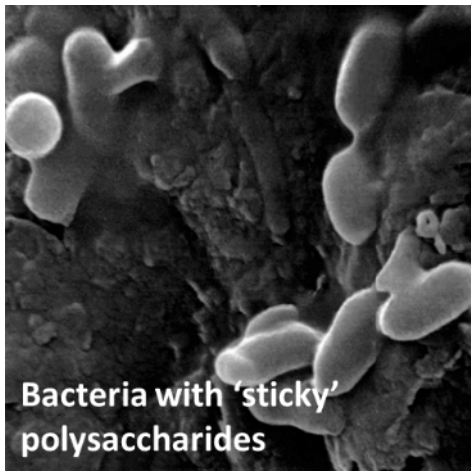
Increases water/O_2 flow

↓

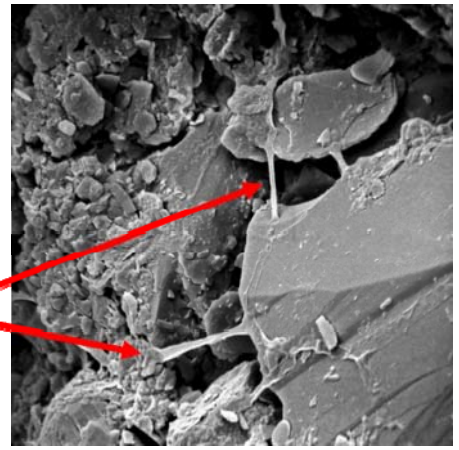
Resists erosion



Netlike fungal mycelia stabilize micro-aggregates



Bacteria with 'sticky' polysaccharides



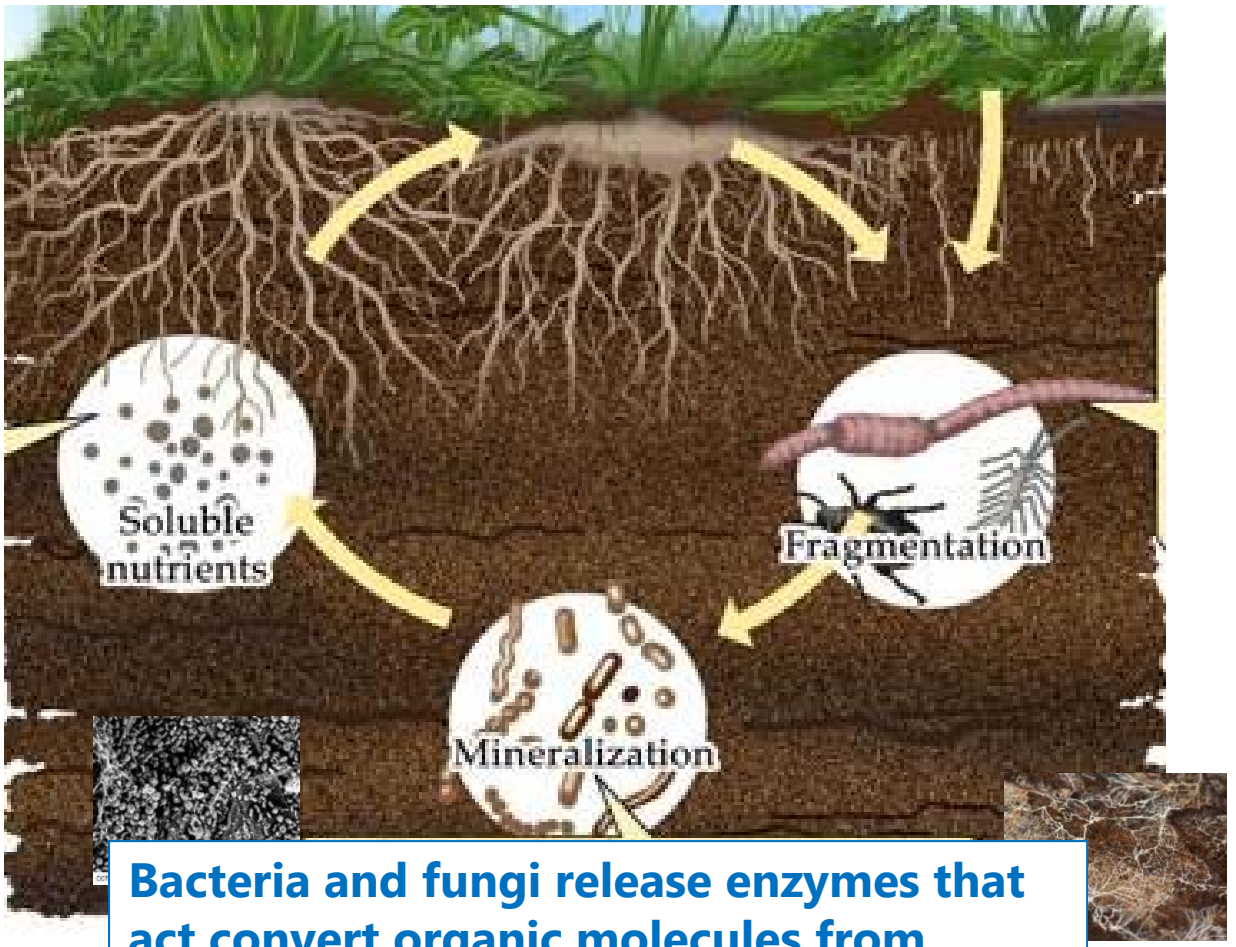
Stabilization of soil structure by actinomycete (bacterial) filaments

SEM photo source (accessed on 6/2/2016): Eickhorst, Thilo & Tippkoetter, Rolf. Micropedology – The hidden world of soils. University of Bremen, Germany. http://www.microped.uni-bremen.de

Importance of Soil Biology: Release Plant-Available Nutrients

Soil Function

- Nitrogen mineralization
- Plant-available N release
- Reduction in fertilizer needs



Bacteria and fungi release enzymes that act convert organic molecules from residues into soluble nutrients (N, P, S)

Importance of Soil Biology: Release Plant-Available Nutrients

Soil Function

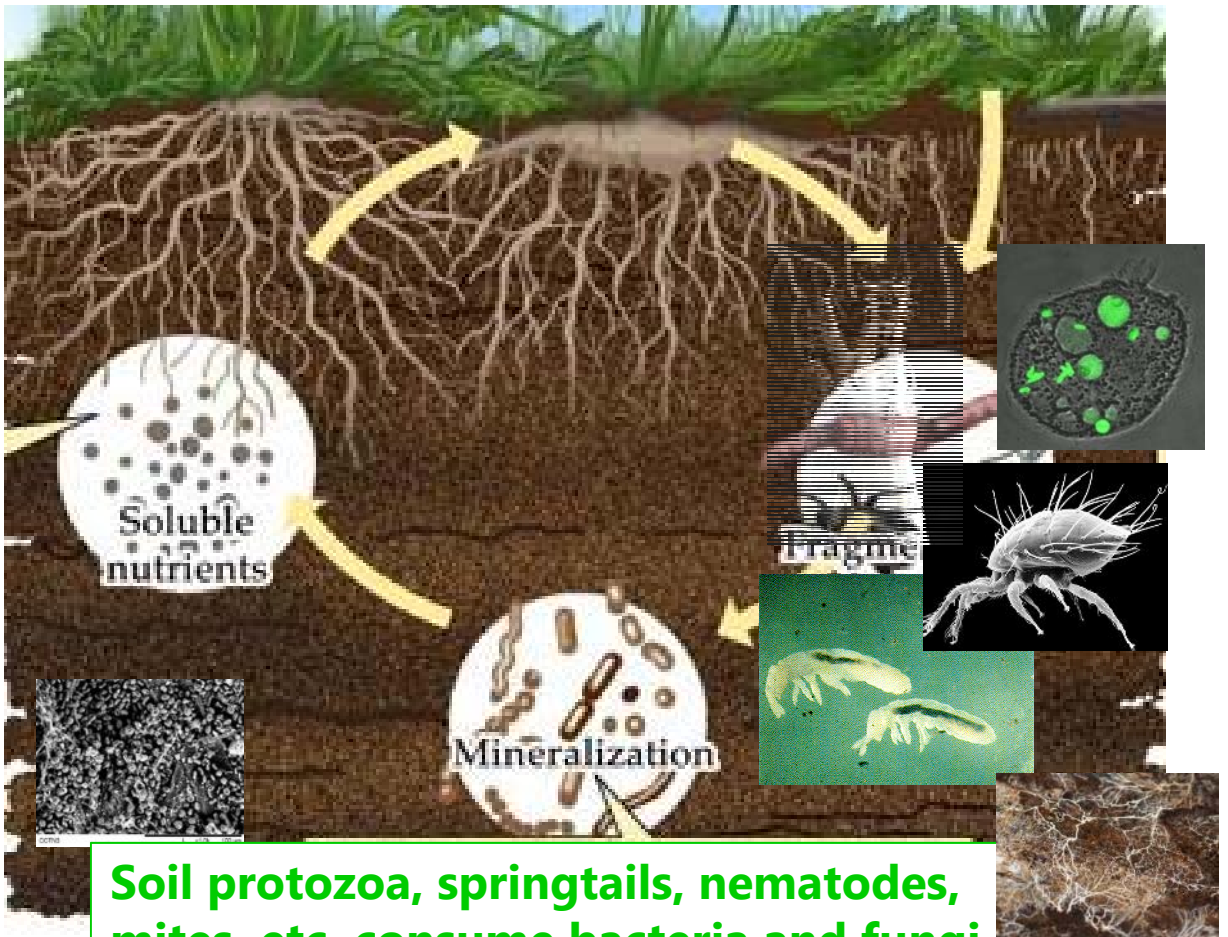
Nitrogen mineralization

↓

Plant-available N release

↓

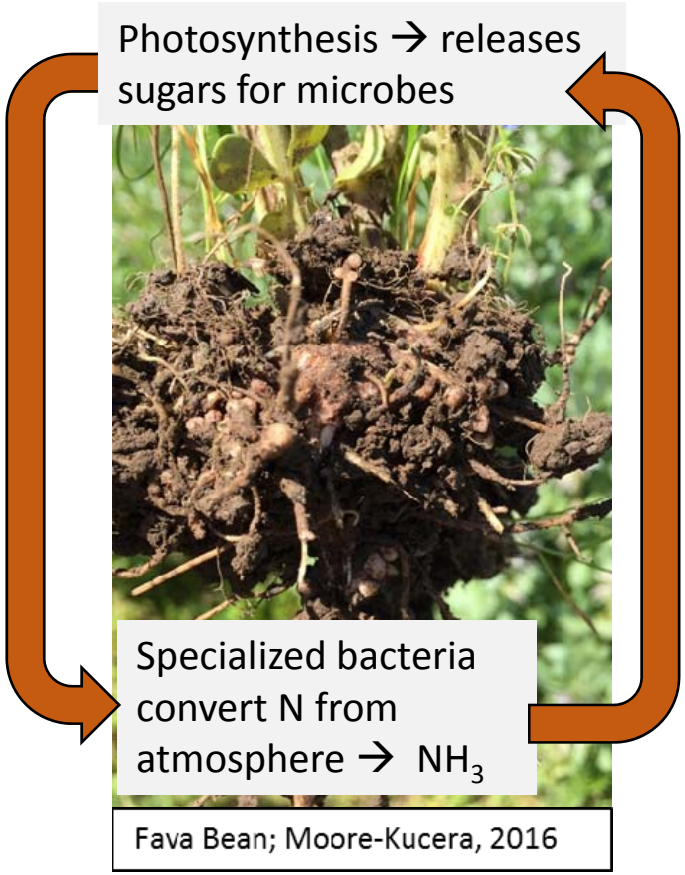
Reduction in fertilizer needs



Soil protozoa, springtails, nematodes, mites, etc. consume bacteria and fungi and mineralize more nutrients (N, P, S)

Importance of Soil Biology: Release Plant-Available Nutrients

Soil Function



Symbiosis between soil bacteria associated with some plant roots supply:

25-75 lb/ac in natural systems

100-200 lb/ac in cropland

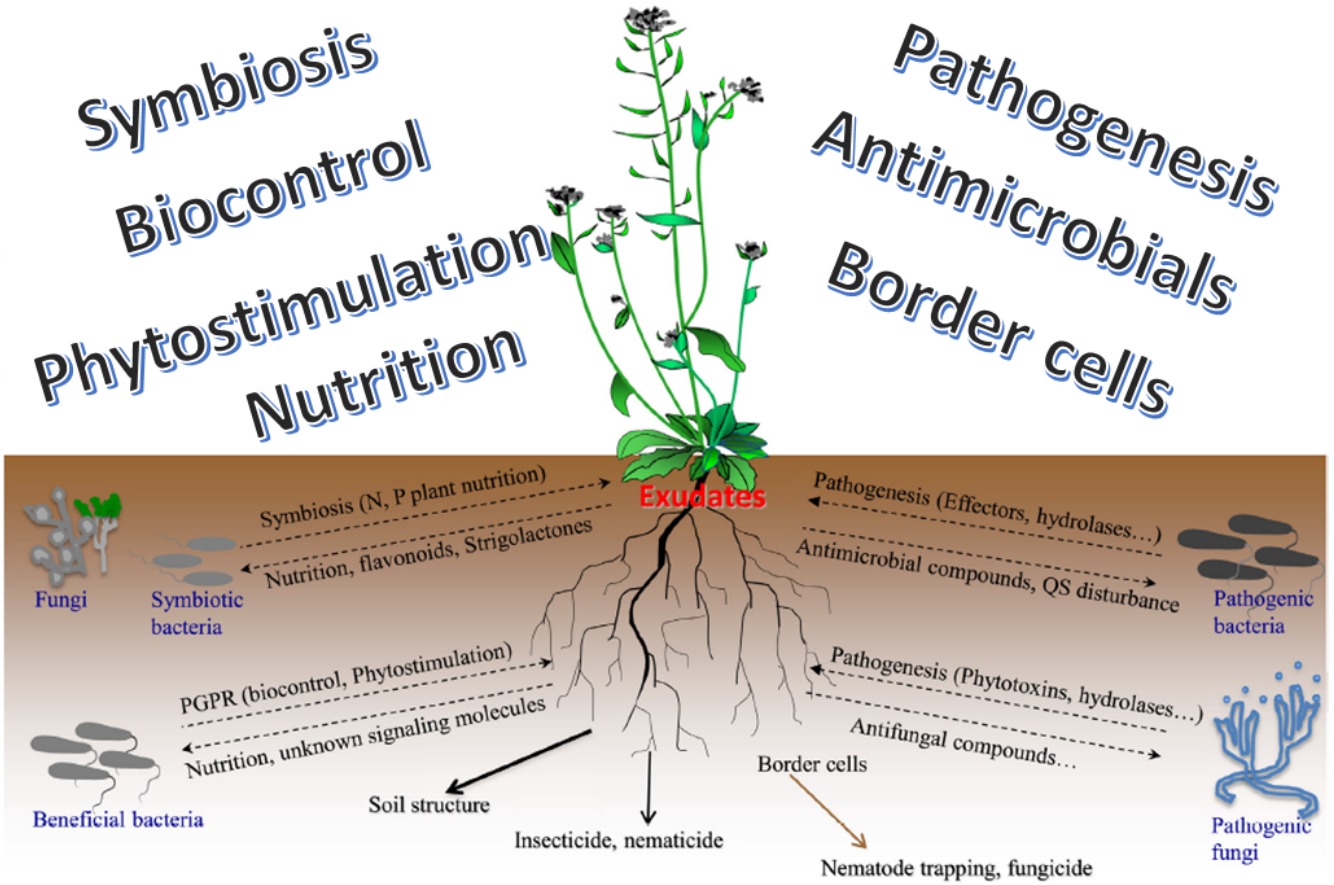
Importance of Soil Biology: Plant Stimulant/Defense

Soil Function

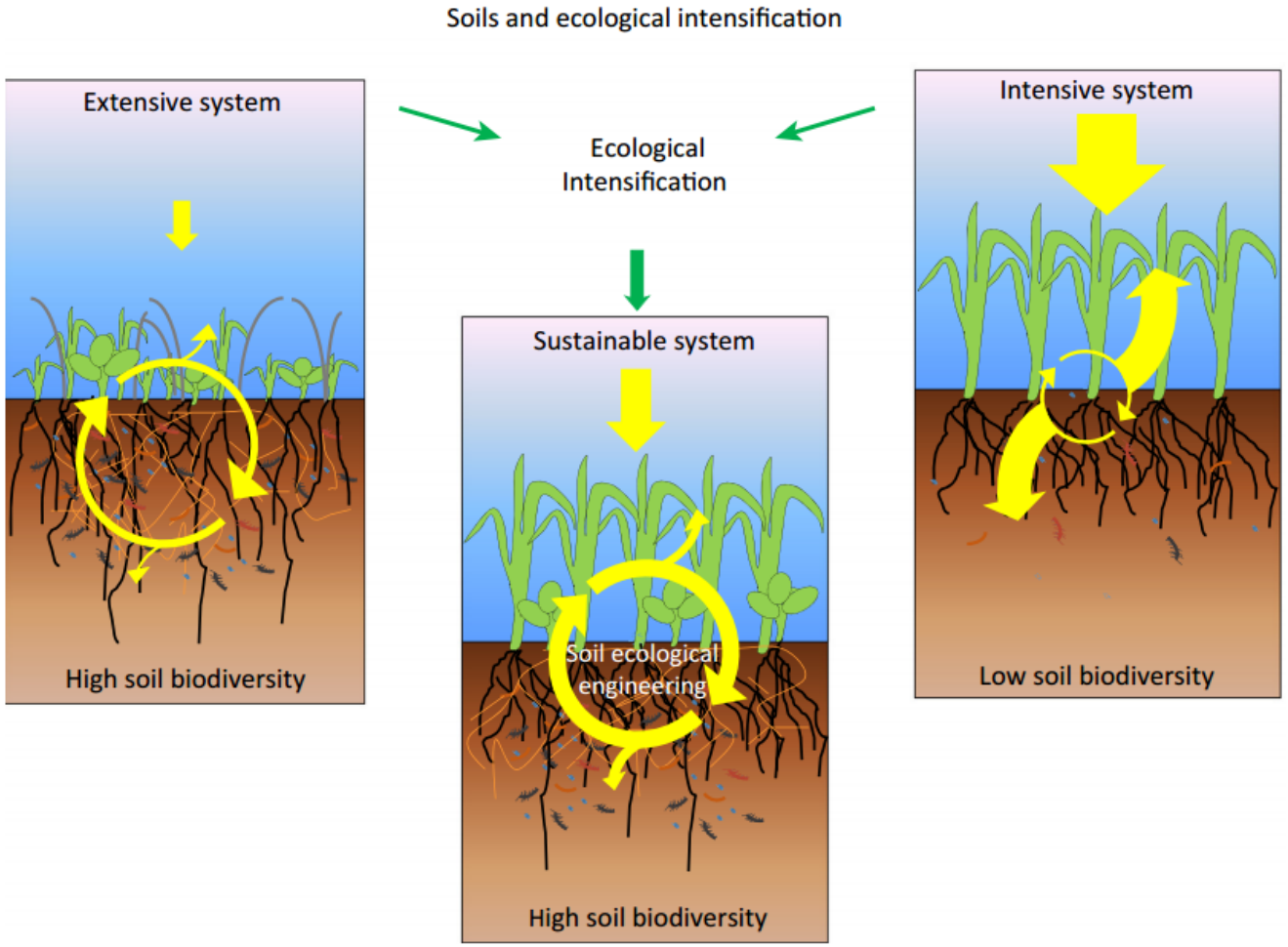
Plant enhancement/ protection

Fights disease/ pathogens

Reduces stress (H_2O, salinity)



Importance of Soil Biology: Plant Productivity, Ecosystem Resiliency and Self Sufficiency



Bender et al. 2016. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol.

Criteria of 'Good' Soil Health Indicators

- Cover a diverse set of soil functions or processes relevant to agricultural or ecological systems
- Sensitive to changes in soil/crop management systems
- Show changes within 1 to 3 years
- Ability to be adopted by commercial laboratories:
  - Relatively easy to sample for and measure
  - Cost effective
  - Repeatable
  - Interpretation is easily accessible by public stakeholders
  - Interpretation must help guide management recommendations

Soil (Micro-)Biological Indicators for Soil Health

Advantages

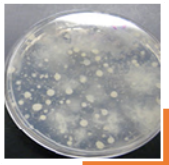
- Tend to be more sensitive to management shifts - can reflect changes within 1-2 y
- Microbes can be indicators of multiple soil functions
- Costs are decreasing as technological advances are made
- Highly expanding knowledge base of unknown potential

Disadvantages

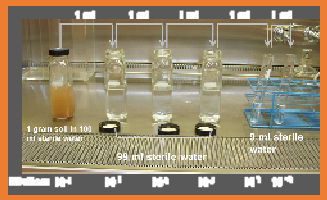
- High seasonal and temporal variability
- Can be too sensitive and respond to environmental variables (e.g., soil moisture and temperature)
- Many methods require advanced skillset for commercial adoption
- Interpretative power is lacking due to delay in microbial ecology research

Microbial Indicators of Soil Health: Abundance-Composition-Activity

Abundance



- Microscopic counts
- Microbial biomass



Life in the Soil is Abundant, Massive, and Complex

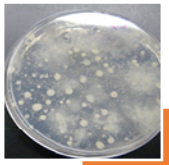
In 10 ft^2, the soil has…

- 10 trillion (10^{13}) bacteria
- 100 billion (10^{11}) fungi
- 10 billion protozoa
- 5 million nematodes
- 100,000 mites
- 50,000 springtails
- 5000 insects and spiders
- 3000 earthworms & potworms
- 100 snails and slugs
- 1 vertebrate

Soil organism	Biomass (lb/ac)
Fungi	9760
Bacteria	5840
Actinomycetes	5840
Algae	2720
Earthworms	1840
Protozoa	400
Nematodes	240
Springtails	80
Total:	**26,800 lb/ac**

Biota numbers adapted from Lindbo et al. 2012. Know Soil, Know Life.

Microbial Indicators of Soil Health: Abundance-Composition-Activity



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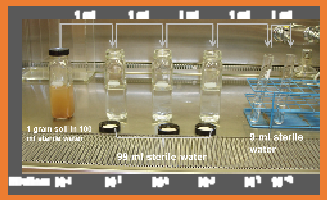
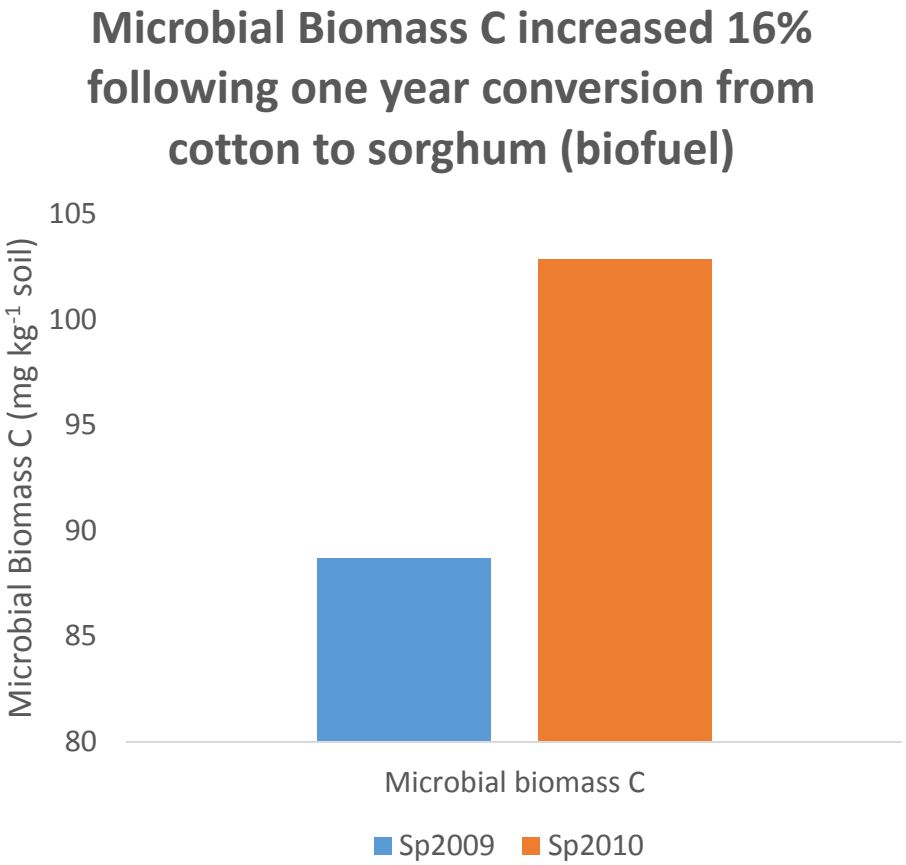


Soil microbial biomass measurements provide an estimate of the mass of soil bacteria and fungi.

- Up to 5% of total soil organic carbon (SOC) is in the microbial biomass
- Microbial biomass can hold up to 50 lb N/ac
- Changes in microbial biomass occur much sooner (1-2 years) than changes in total SOC (years to decadal)

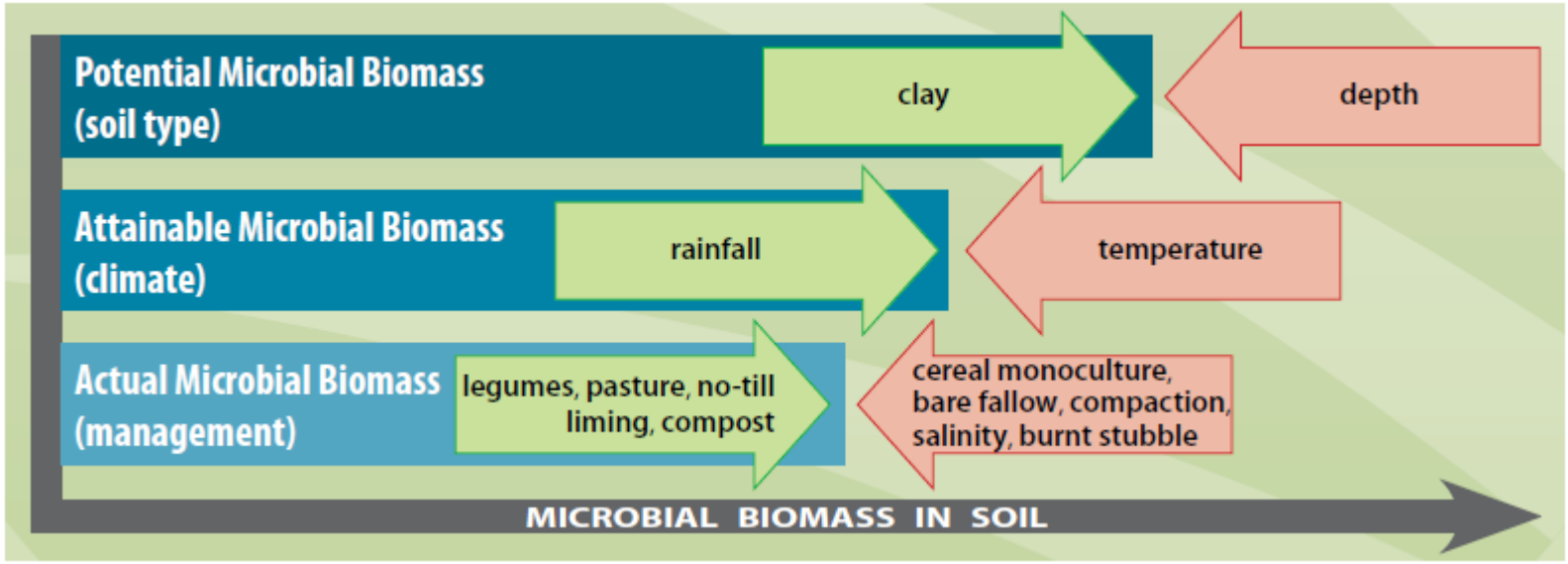
Sensitivity of Microbial Biomass C to Management Shifts

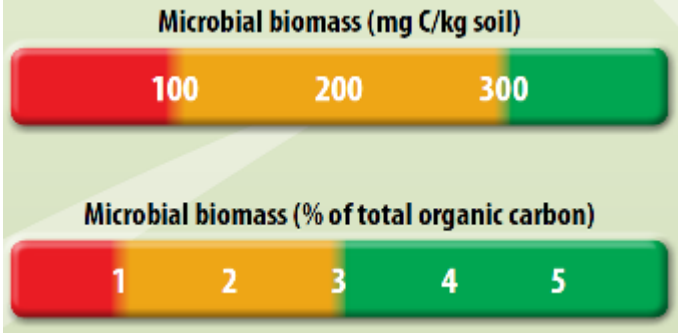


- No changes in soil organic C (average 0.4%)
- No observable changes in soil physical attributes
- MBC:SOC ratio increased from 2.2% to 2.6%

Cotton, J., et al. (2013). "Early changes due to sorghum biofuel cropping systems in soil microbial communities and metabolic functioning." Biology and Fertility of Soils 49(4): 403-413.

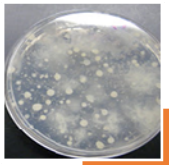
Microbial Biomass: Interpretation (Based on Australian Data Set)





http://soilquality.org.au/factsheets/interpreting-microbial-biomass-carbon;
http://soilquality.org.au/factsheets/making-sense-of-biological-indicators (accessed 7/2016)

Microbial Indicators of Soil Health: Abundance-Composition-Activity



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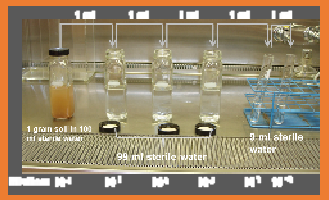
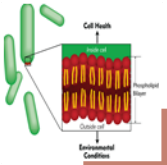
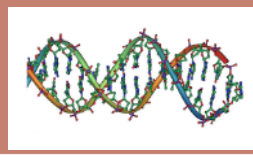




Composition/Diversity

- Biochemical markers (lipid profiles)
- DNA/RNA Sequencing



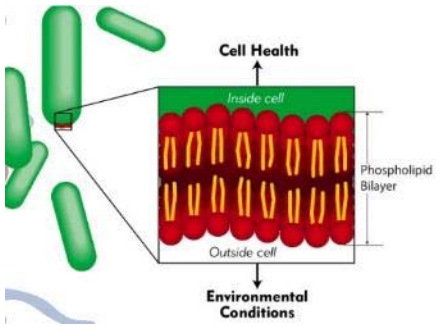
Biochemical Markers: Phospholipid Fatty Acid Profiles (PLFAs)

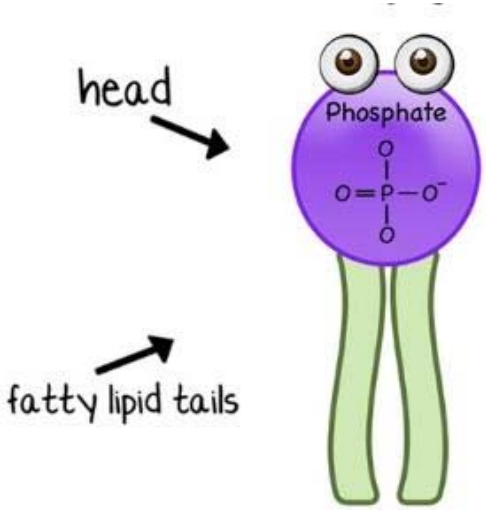
What are they?

Essential cellular components that help form a protective layer around cells.

Composed of a phosphate group at one end (head) and two fatty acids (tails)

1. Phosphate group is quickly degraded upon cell death; thus PLFA targets the 'viable' microbial community
2. Different types of 'tails' are biomarkers of different microbial groups





Bacterial PLFA Biomarkers and Interpretation

Group	Generalizations for interpretation
Gram negative (GM-) Copiotrophs; r-strategists	Fast growers (tourists); Respond rapidly to fresh inputs; increase with increased SOM; higher in rhizosphere relative to GM+; Members of Proteobacteria and Bacteriodetes
Gram positive (GM+) Oligotrophs; K-strategists	Slow growers (residents); more resistance to environmental stress; Acidobacteria;
Actinomycetes	Degrade complex SOM; aggregation via filaments; tolerant of salt, high pH
GM+:GM- ratio	Higher ratios associated with low C content and organic inputs; high ratios common in cultivated soils compared to grasslands

Fungal & Protozoan PLFA Biomarkers and Interpretation

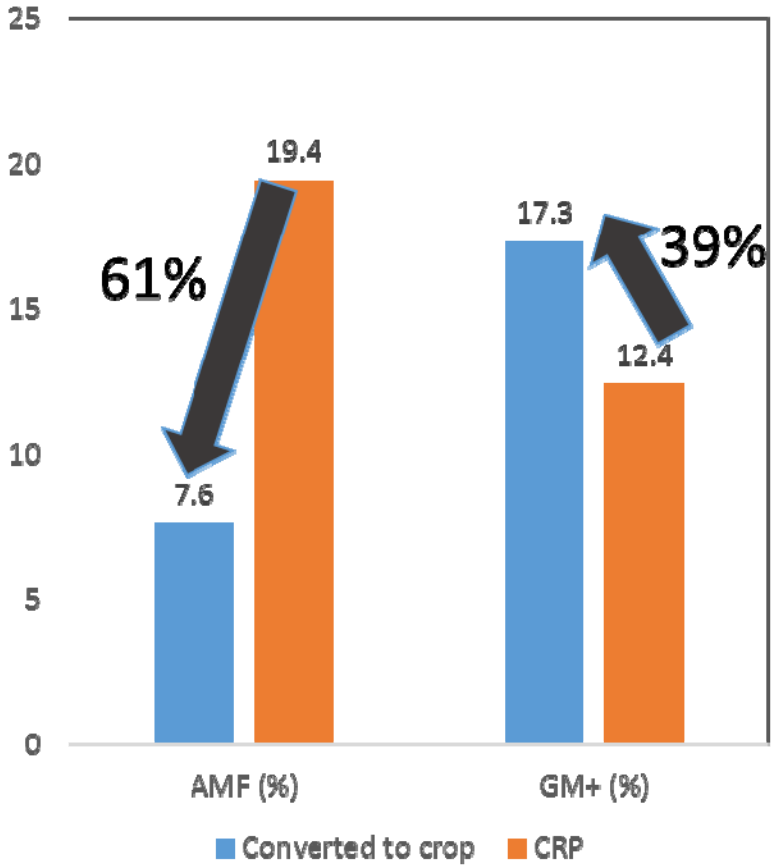
Group	Generalizations for interpretation
Saprophytic fungi	Associated with high organic matter; complex SOM decomp; low pH;
Ectomycorrhizae	Associated with woody species; Same biomarkers as saprophytic fungi so ecosystem type important for interpretation
Arbuscular mycorrhizae	Higher in less (physically) disturbed lands; important for aggregation, P, H_2O uptake, plant protection
F:B ratio	Higher values generally associated with greater functional benefits and less soil disturbance
Protozoa (possibly also nematodes)	N mineralization; population control

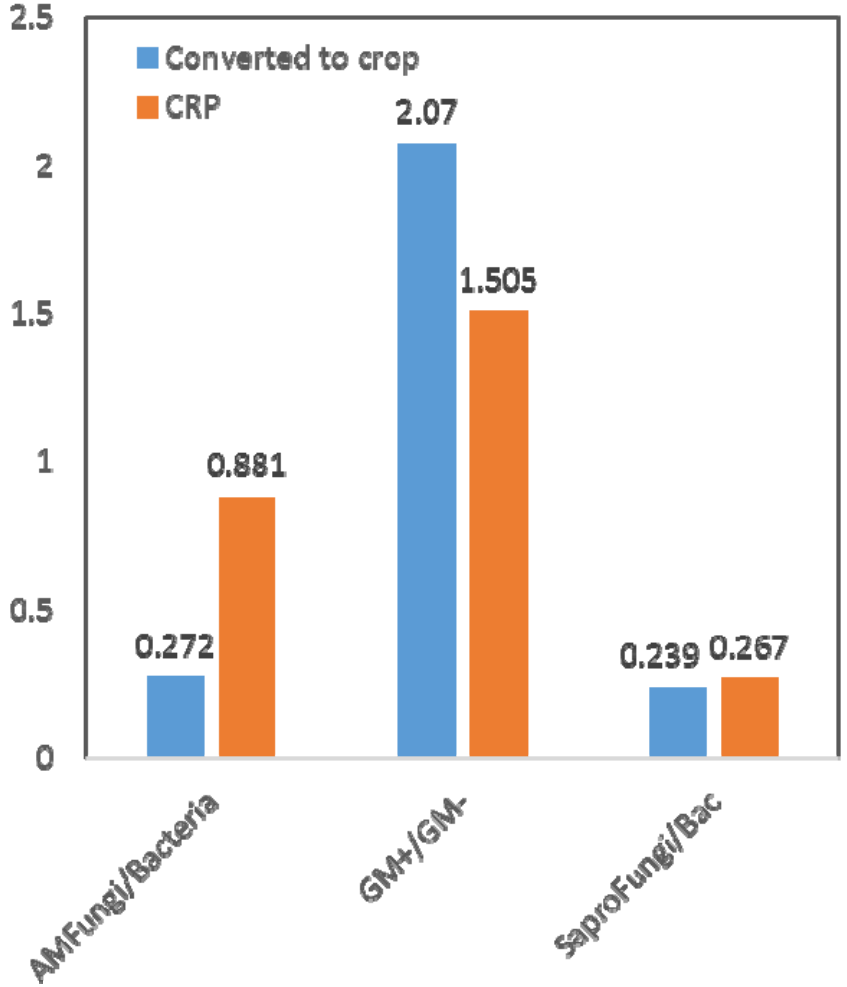
*18:1w9 less reliable as fungal marker in agricultural fields (use and misuse article)
**16:1w5 less reliable as AM marker when bacterial biomass is high unless neutral lipid is used

PLFA: Interpretation Cautions

- Values are dependent upon method and instrumentation configuration
- Biomarkers are guides; not absolute indicators
- Biomarkers of the same group don't always track in same direction
- Biomarkers identified based on culture studies
- Currently, best used for comparisons
- Databases are needed under standard procedures to expand interpretation

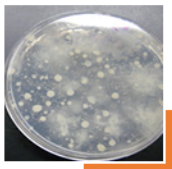
Within 5 years following conversion of CRP to cropland, AMF markers decreased, GM+ bacteria increased





Li, C. et al. (unpublished data)

Microbial Indicators of Soil Health: Abundance-Composition-Activity



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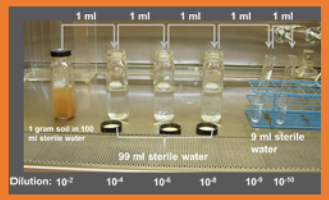

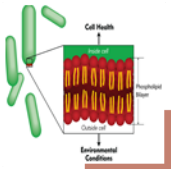

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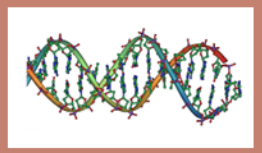
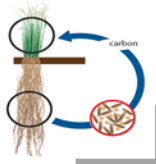
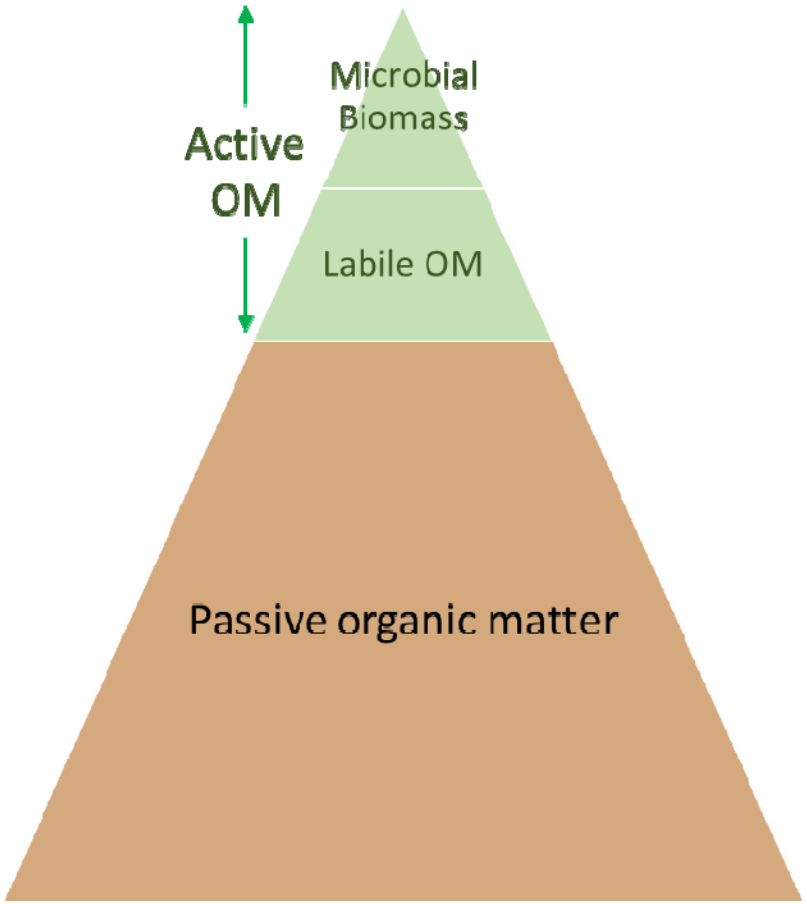




Function/ Activity

- Active carbon
- C and N Mineralization
- Enzyme activity
- Gene abundance (qPCR)
- Metabolomics

Active Organic Matter



- Active Fraction (5-25%):
  - Highly degradable OM (lasts days to years);
  - Serves as food source of microbes; partially decomposed plant and animal tissue, microbial cells, biological compounds
- Passive Fraction (75-95%):
  - Resistant to decomposition (lasts 100s to 1000s of years)
  - Important for WHC and CEC

Adapted from: Weil, R. 2005. Laboratory Manual for Introductory Soils, 7th Ed.

Active Organic Carbon-

Potassium permanganate ($KMnO_4$) oxidation

- Provides an indication of the portion of SOM that provides food and energy to soil microbes.
- $KMnO_4$ has a deep purple color and is a strong oxidizing agent.
- Reacts with labile portions of organic matter (eg. simple carbohydrates, amino acids, amine sugars and various 'simple' C-compounds) and becomes lighter in color.
- The color change is measured and amount of C oxidized is calculated.

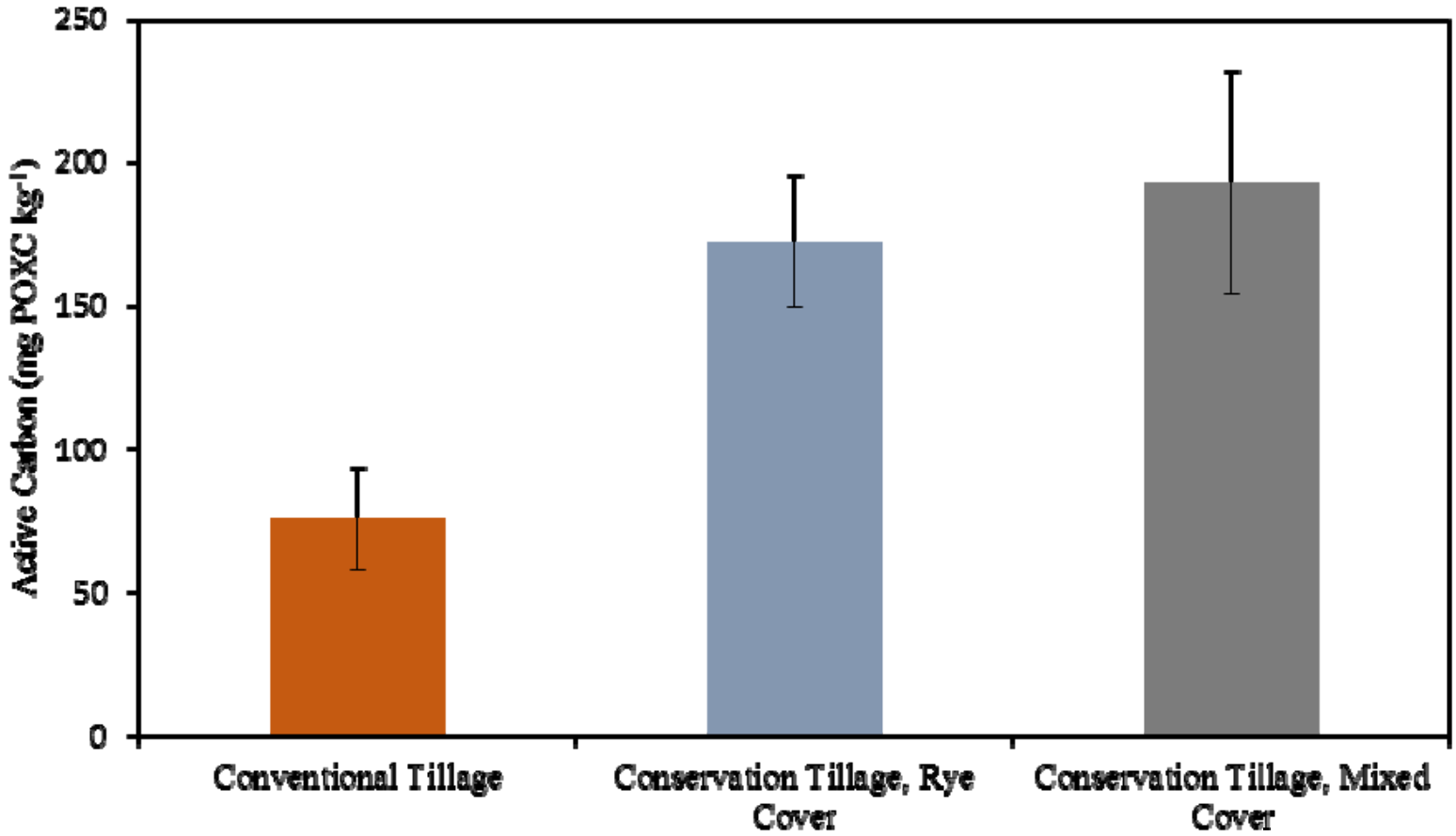
Adapted from: Weil, R. 2005. Laboratory Manual for Introductory Soils, 7th Ed.

Active C: Interpretation Cautions

- Active C does not always correlate with microbial biomass and other labile pools of soil C
- Unclear which fractions permanganate oxidizable C most closely reflects
- Geographic, climatic, and edaphic factors have begun to be tested but further studies are needed for broader interpretation

Tirol-Padre, A. and J. Ladha (2004). Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. Soil Sci Soc Am J 68(3): 969-978.
Culman, S. W., et al. (2012). Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Sci Soc Am J 76(2): 494-504.

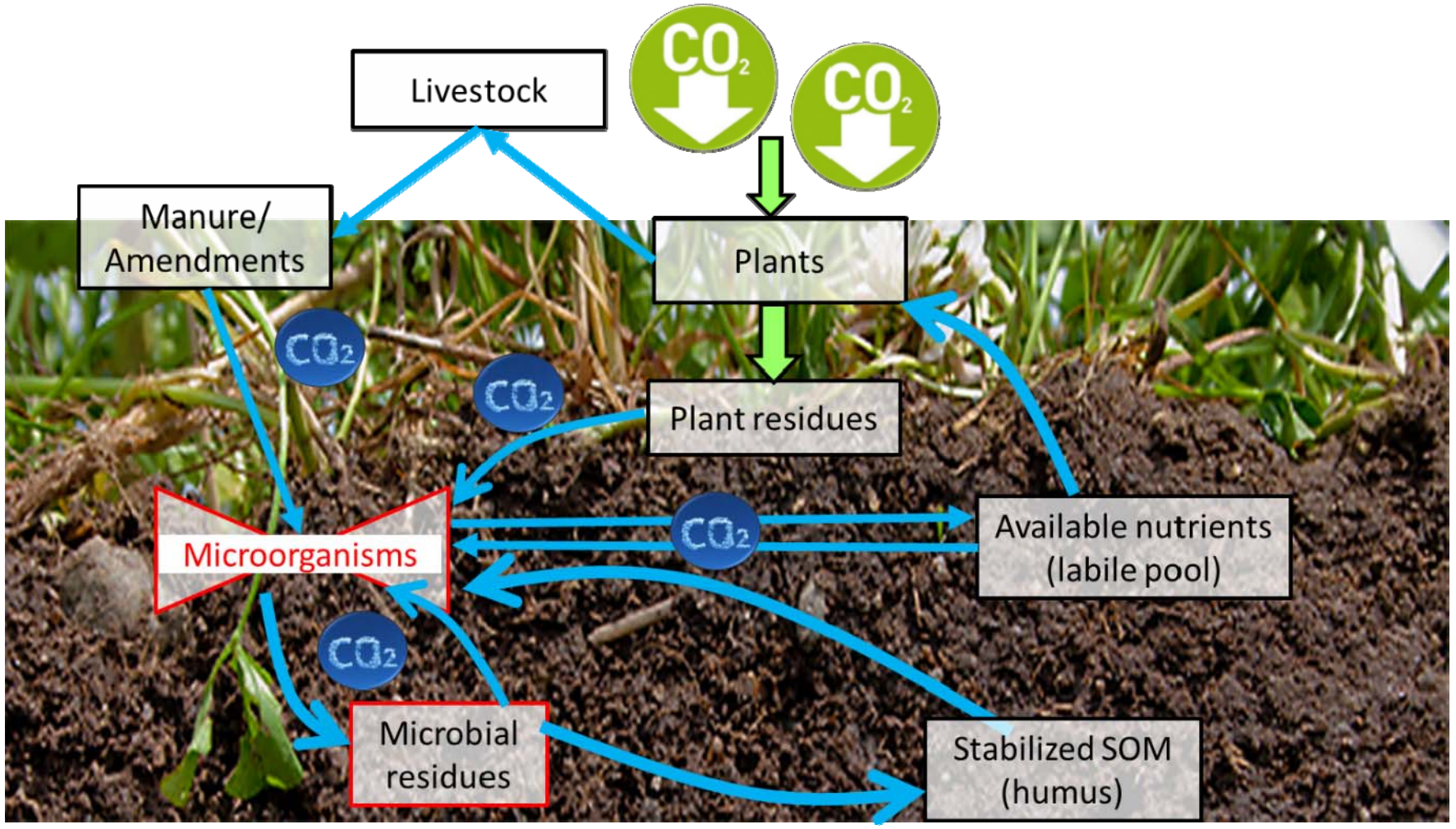
Reduced till treatments with cover had >2x higher Active C levels than conventional till cotton systems



Mixed Cover includes: hairy vetch (*Vicia villosa* Roth), radish (*Raphanus sativus* L.), Winter pea (*Pisum sativum* L.), and rye (*Secale cereal* L.)

Ongoing Texas research, unpublished data - J. Burke and K. Lewis

Microbial Activity Method: C Mineralization

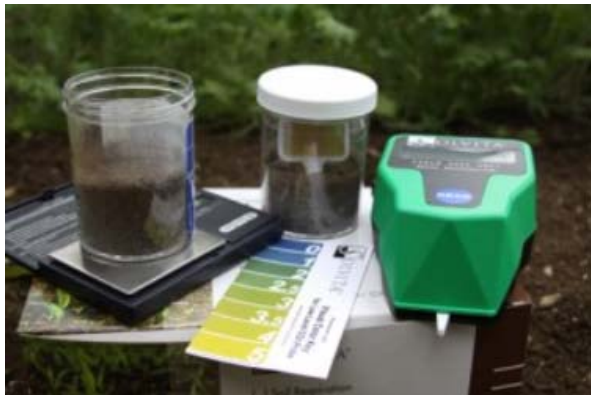


C Mineralization: CO_2 release

- What does it tell us?
  - Measure of metabolically active soil microbes
- Interpretation
  - Greater amounts reflect a larger, more active population
  - Increased decomposition and breakdown of organic matter
  - May be associated with release of nutrients

How Do We Measure C Mineralization?

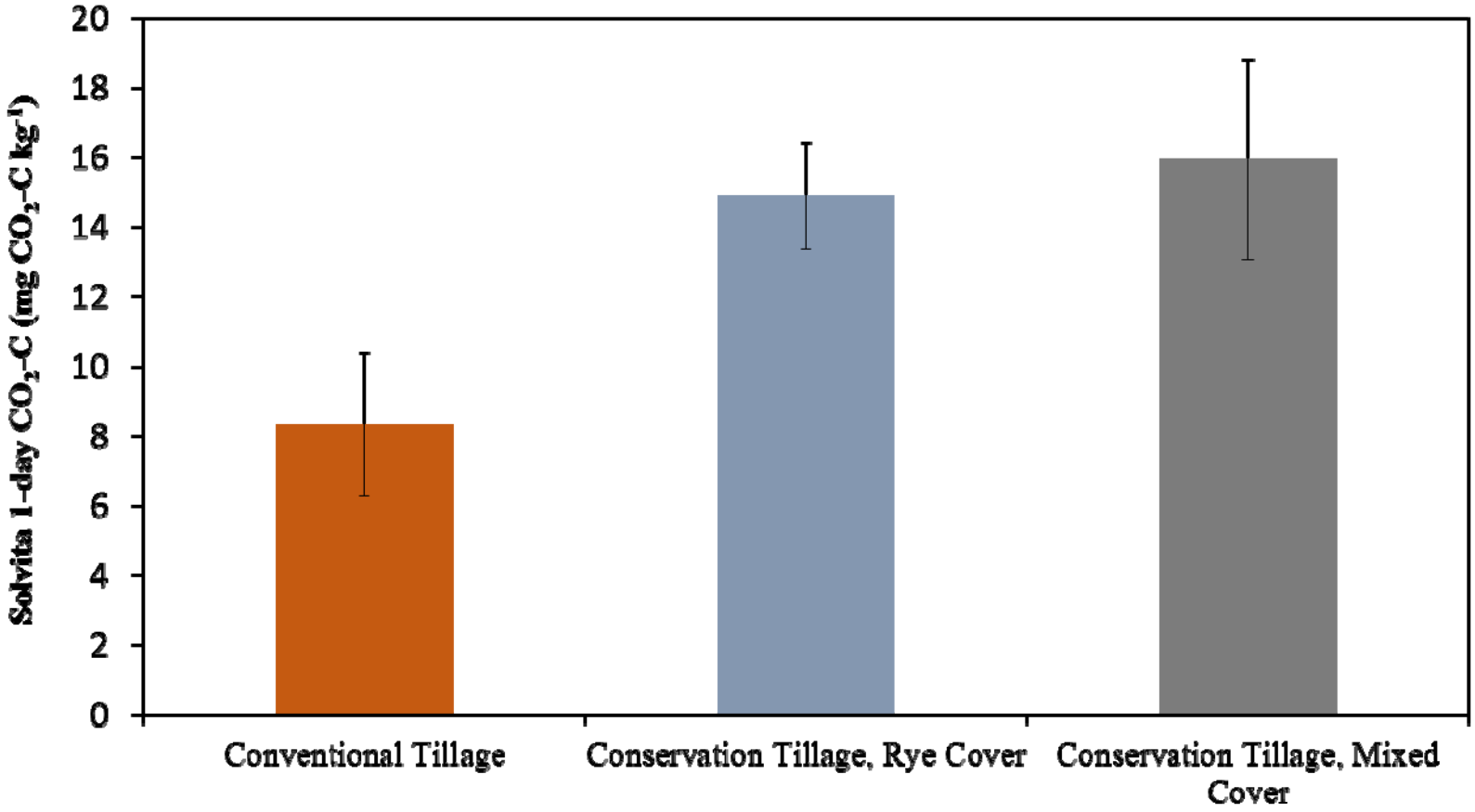
- Air-dried soils are rewetted and incubated 24h-28d
- CO_2 is determined (multiple tools possible)
- Commercially available approaches:
  - Solvita Soil Burst: 24h incubation & proprietary gel paddle
  - Cornell: 4-day incubation & electrical conductivity of base trap.



C Mineralization Cautions

- Soil respiration tests do not account for microbial or ecological stress
- Soil CO_2 may be very high following disturbance
  - Tillage, harvest, fire, clear-cutting, etc.
- By coupling this measure with microbial biomass (CO_2/MBC) may provide an indication of microbial stress
- High CO_2 without an increase in microbial biomass C suggests community is stressed and SOC loss >>> SOC formation

Reduced till treatments with cover had ~2x higher CO_2 burst levels than conventional till cotton systems



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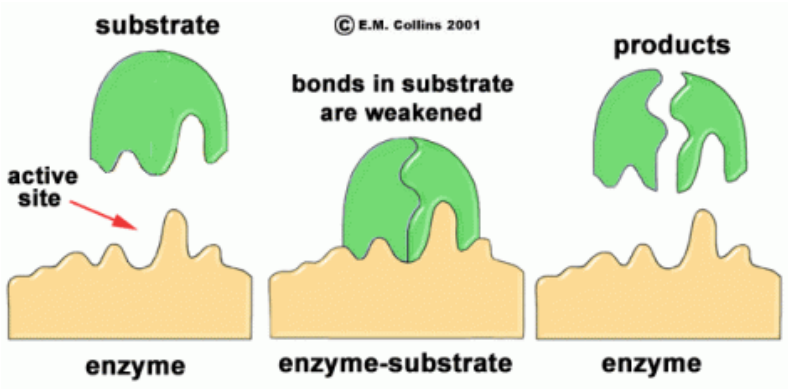
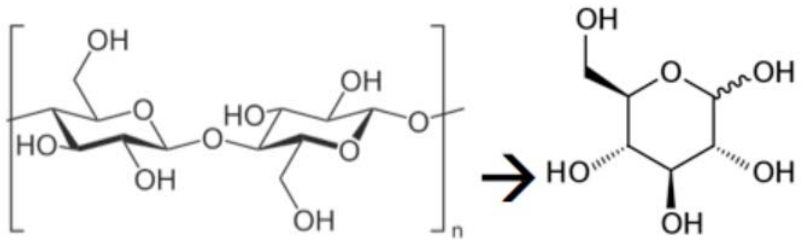
Ongoing Texas research, unpublished data - J. Burke and K. Lewis

Microbial Activity: Enzyme Activities

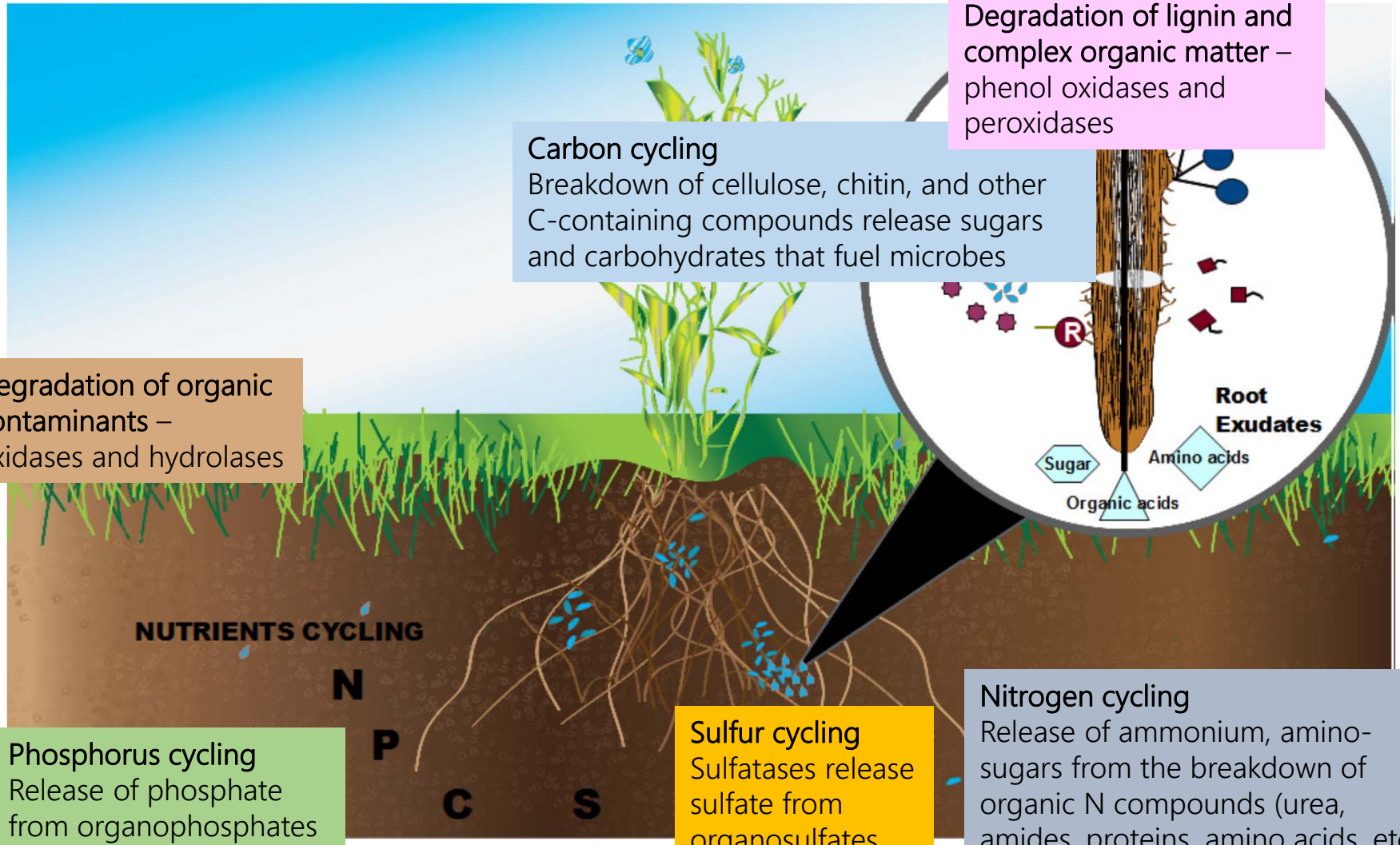
What are enzymes?

- Proteins that increase the rate of a reaction.
- Enzymes act on a substrate (e.g., cellulose) and release a product (e.g., glucose)
- In soils, these enzymes help increase how quickly plant residues decompose and release plant nutrients





Soil Enzyme Activities: What do they tell us?



Degradation of lignin and complex organic matter – phenol oxidases and peroxidases

Carbon cycling
Breakdown of cellulose, chitin, and other C-containing compounds release sugars and carbohydrates that fuel microbes

Degradation of organic contaminants – oxidases and hydrolases

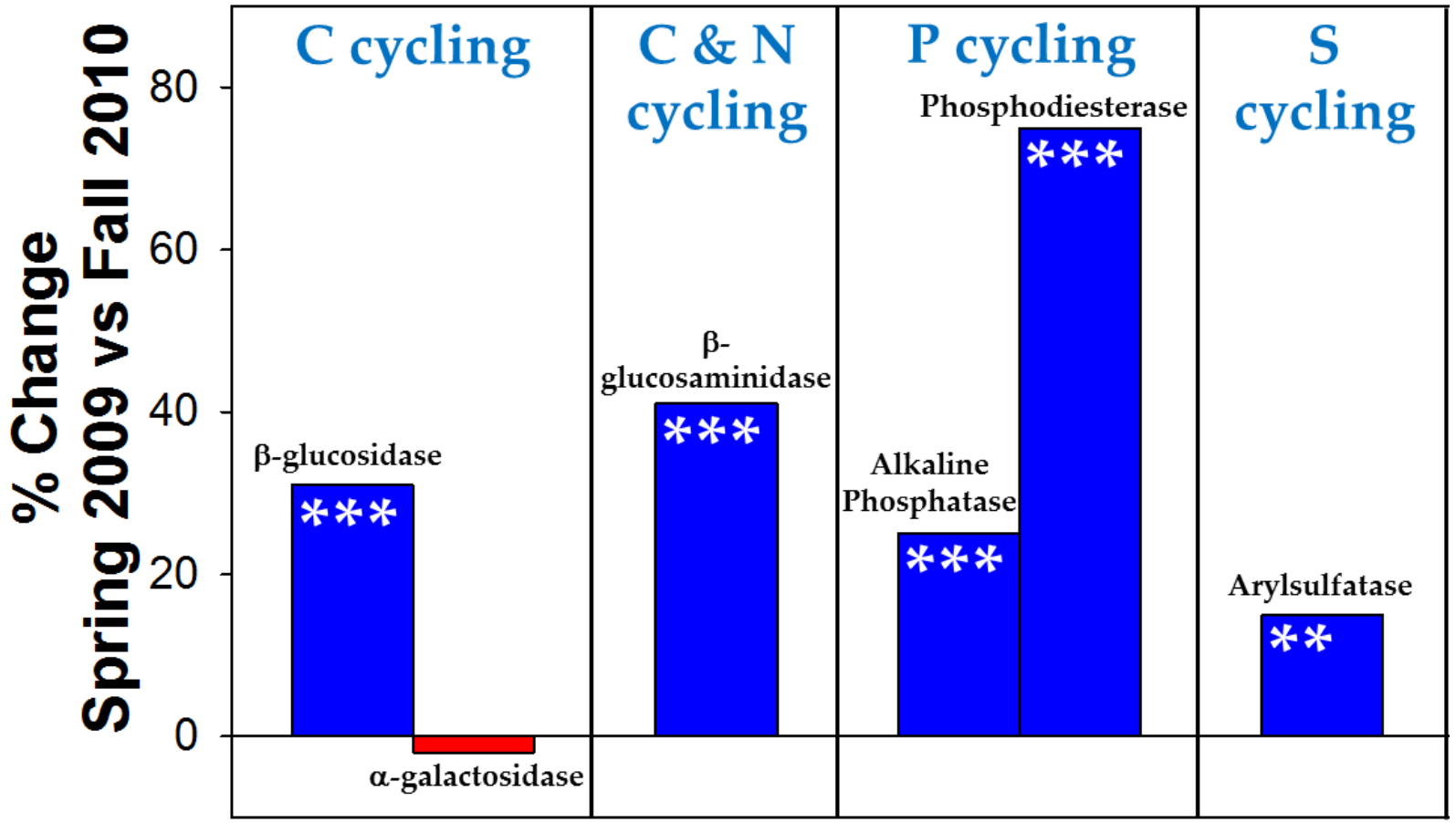
Phosphorus cycling
Release of phosphate from organophosphates

Sulfur cycling
Sulfatases release sulfate from organosulfates

Nitrogen cycling
Release of ammonium, amino-sugars from the breakdown of organic N compounds (urea, amides, proteins, amino acids, etc.)

Picture from: Rincon-Florez, V., et al. (2013). Diversity 5(3): 581-612.

Enzyme activities increased 17-75% following transition from cotton to biofuel sorghum (after only 1 year!)

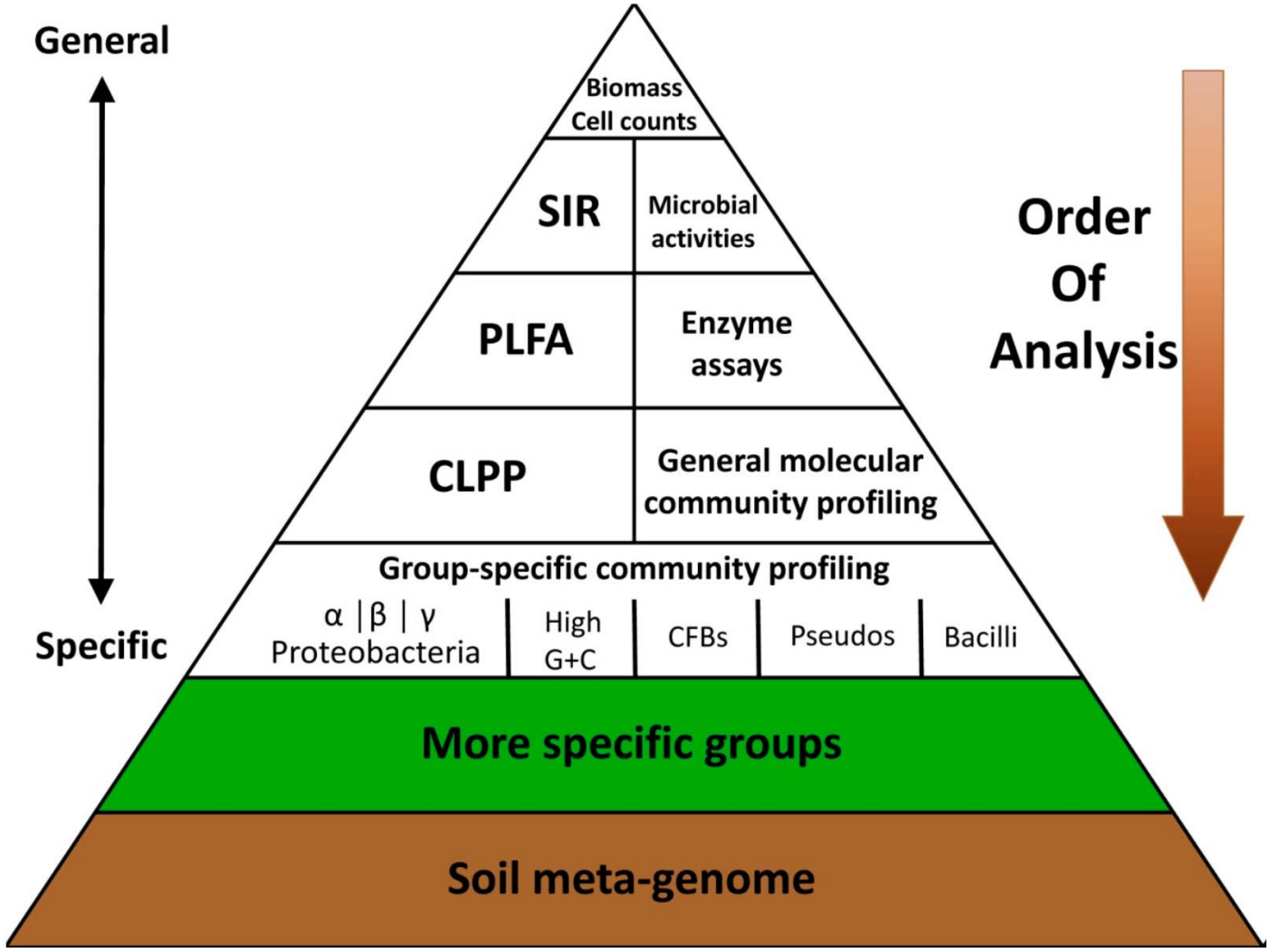


Cotton et al., 2013. Biol Fert Soils 49:403-413

Enzyme Activities as Biological Indicators: Cautions

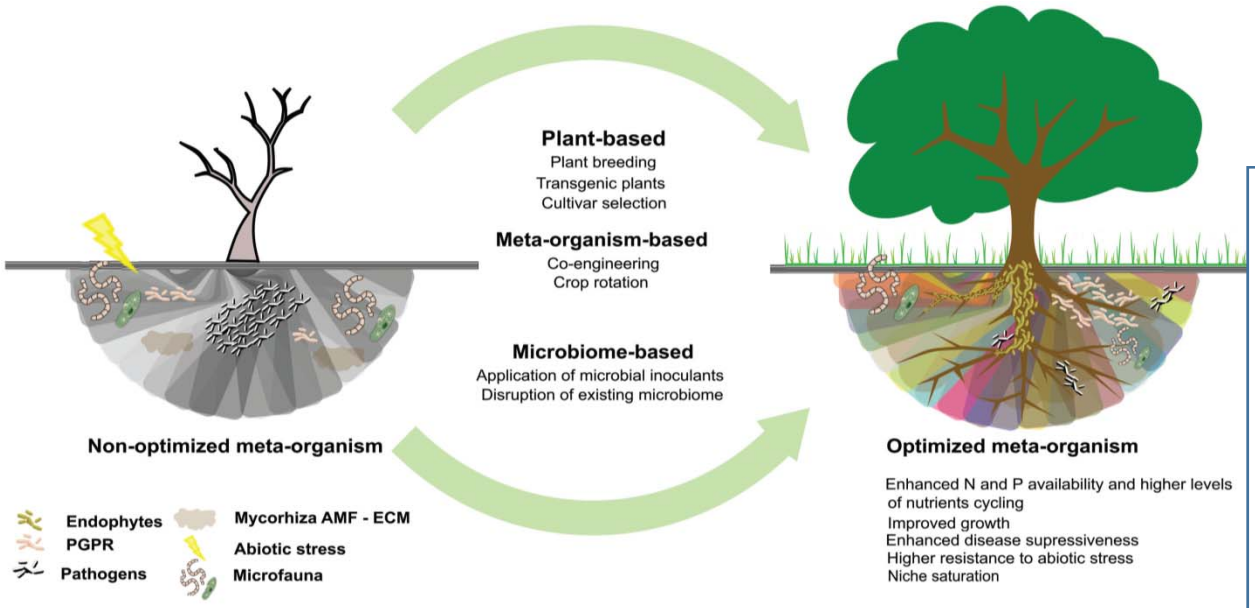
- Similar to microbial biomass, individual numbers are challenging to interpret
  - Data exists for beta-glucosidase and is part of a soil health assessment
- Not currently available commercially but methods are relatively simple and quick
- Fertilizer applications may affect some enzyme activities complicating interpretative power
- High spatial and temporal variability
- Sample handling and storage may affect results but air-drying is possible for most enzyme tests

Future hope in molecular tools?



Lehman, R. M., et al. (2015). "Understanding and Enhancing Soil Biological Health: The Solution for Reversing Soil Degradation." Sustainability 7(1): 988-1027.

Manipulation of Soil Biota or Manipulation of Management?



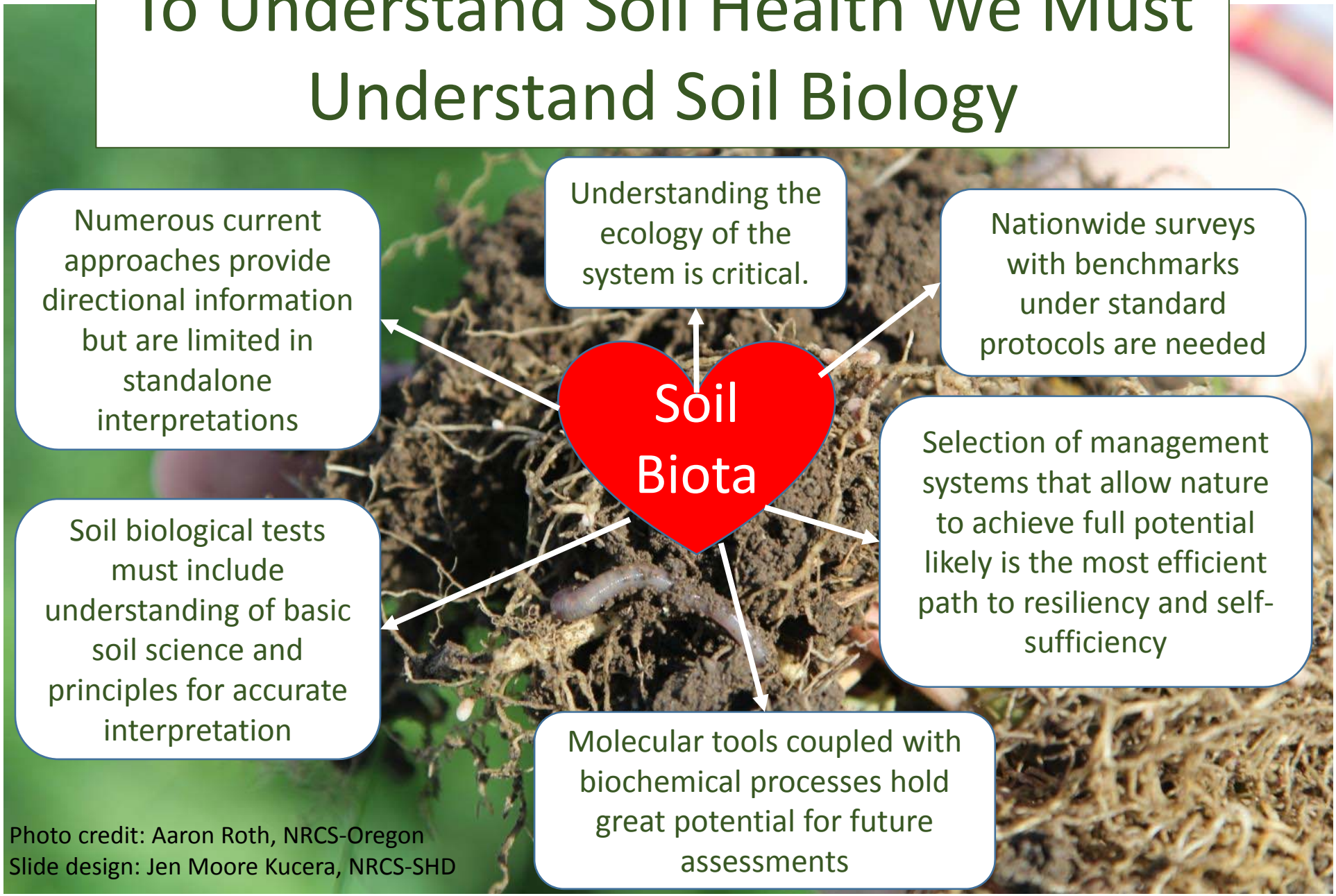
Microbial Manipulations

Management (Agronomic) Manipulations

- No-till/ conservation tillage; IPM
- Crop rotations; livestock incorporation
- Cover crops; relay crops; crop residue retention
- Perennial crops
- Organic fertilizers
- Weed control by mulching, shading, competition

Quiza, L., et al. (2015) Frontiers in Plant Science 6, Article 507; Lehman, R. M., et al. (2015). Journal of Soil and Water Conservation 70(1): 12a-18a; Lehman, R. M., et al. (2015) Sustainability 7(1): 988-1027; Bender et al. 2016. Trends Ecol Evol.

To Understand Soil Health We Must Understand Soil Biology



Understanding the ecology of the system is critical.

Nationwide surveys with benchmarks under standard protocols are needed

Numerous current approaches provide directional information but are limited in standalone interpretations

Selection of management systems that allow nature to achieve full potential likely is the most efficient path to resiliency and self-sufficiency

Soil biological tests must include understanding of basic soil science and principles for accurate interpretation

Molecular tools coupled with biochemical processes hold great potential for future assessments

Photo credit: Aaron Roth, NRCS-Oregon
Slide design: Jen Moore Kucera, NRCS-SHD

Thank You!

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