Microbial Studies of the Fermilab Chronosequence

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Why do we need to understand soil microbial communities?

- **Microbial biomass is a potential carbon store**
- **Changes in relative abundance of microbial functional groups may be important control point**
  - ***Fungi use carbon more efficiently than do bacteria***
  - ***Fungi more difficult to decompose based on cell wall chemistry***
  - ***Need to consider inputs from saprophytic hyphae vs. mycorrhizal hyphae***
    - **Saprophytic fungi prefer organic particulates**
    - **AMF associated with rhizosphere (direct access to plant c)**
- **Microbial community via its activity controls nutrient availability**

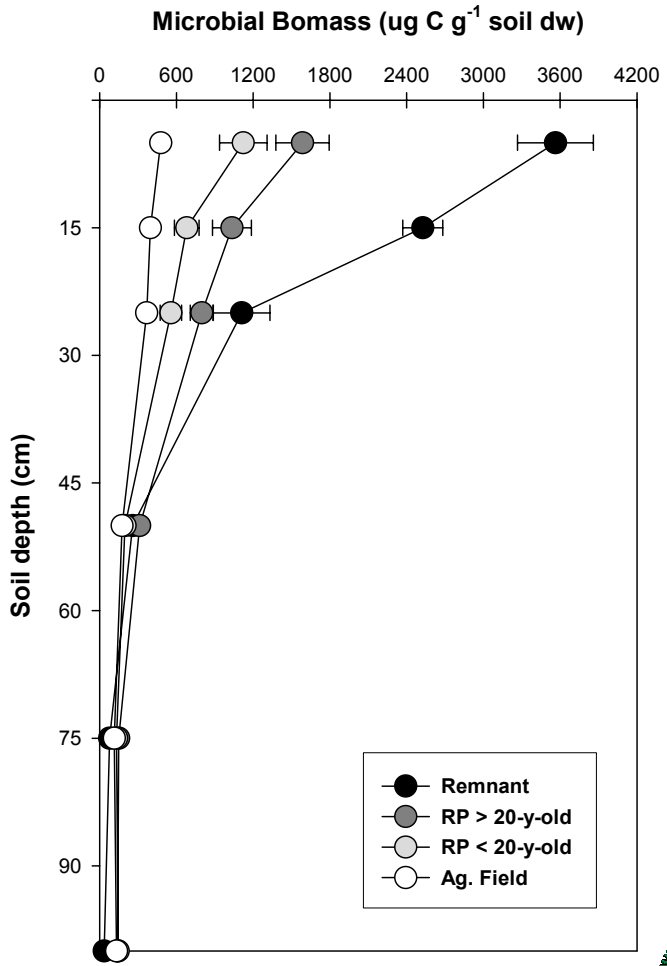
Some key questions:

Using the Fermi chronosequence:

- ***Can we detect changes in the size of the microbial community in an organic matter aggrading prairie restoration chronosequence?***
- ***Does a change in microbial community composition occur along the chronosequence?***
- ***Are changes in microbial community structure related to changes in plant or soil characteristics?***
- ***Are changes in microbial community structure associated with changes in SOC?***

Microbial biomass along the chronosequence

- The greatest differences in microbial biomass are found in the surface 0-5 cm depth.
- Deepest depths have similar biomass levels.
- Although biomass differences are evident, this does not tell us much about microbial community functional groups.



How can we measure the microbial community?

- **Traditional methods problematic:**
  - ***Can only detect microbes that can be plated***
  - ***Resting stages make it difficult to assess active microbial biomass***
  - ***Difficult to define an individual***

- **Phospholipid fatty acid analysis:**
  - ***Detects viable biomass***
    - **Does not require plating**
    - **Distinguishes between broad microbial functional groups**
  - ***Major membrane components***
  - ***Made up of glycerol, fatty acids, plus a phosphorus-containing group***

PLFA Functional groups

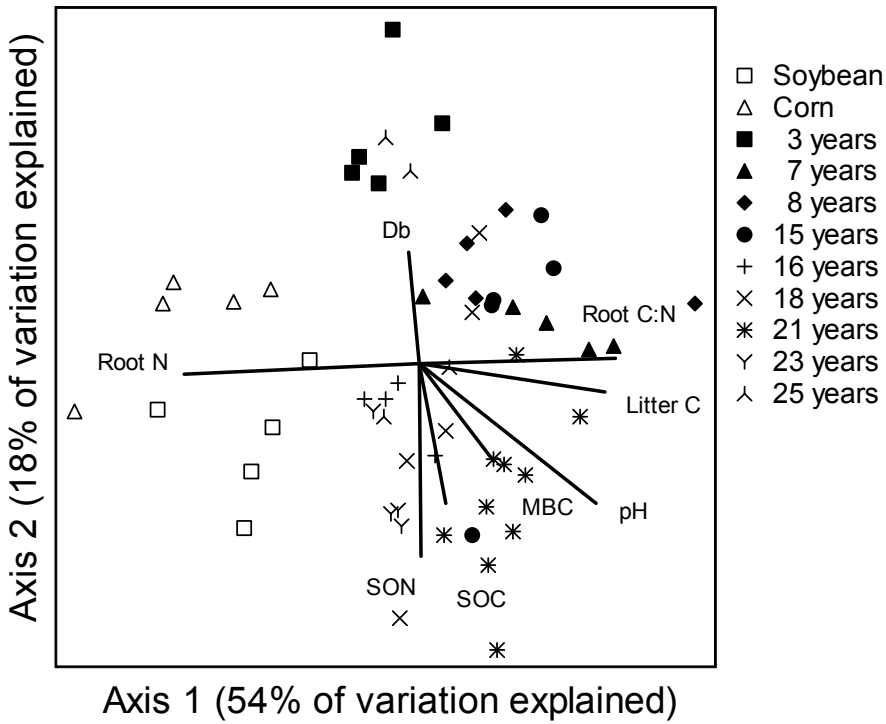
Func. group	*Signature PLFAs*					
Actinomycetes	10Me16:0	10Me18:0				
Bacteria	14:0	a15:0	16:1ω9c	i17:0	a17:0	cy19:0
Gram +ve	cy17:0	18:1ω7c				
Gram-ve	i15:0	i16:0				
Fungi	18:2ω6	18:1ω9c				
AMF	16:1ω5c	20:1ω9				
Protozoa	20:4 ω6					

Experimental approach

- Used the Drummer soil chronosequence (space-for-time)
- Reporting 0-5 cm depth
  - Emphasizes interface between soil and litter
  - Deeper depths currently undergoing analysis
- Using reciprocal averaging and regression procedures

Reciprocal Averaging of Soil and Plant measures

Axis 2 is most strongly correlated with soil characters, especially bulk density ($R^2 = 0.29$, $p \leq 0.0001$), pH ($R^2 = 0.31$, $p \leq 0.0001$), soil N ($R^2 = 0.42$, $p \leq 0.0001$) and soil organic C content ($R^2 = 0.30$, $p \leq 0.0001$).

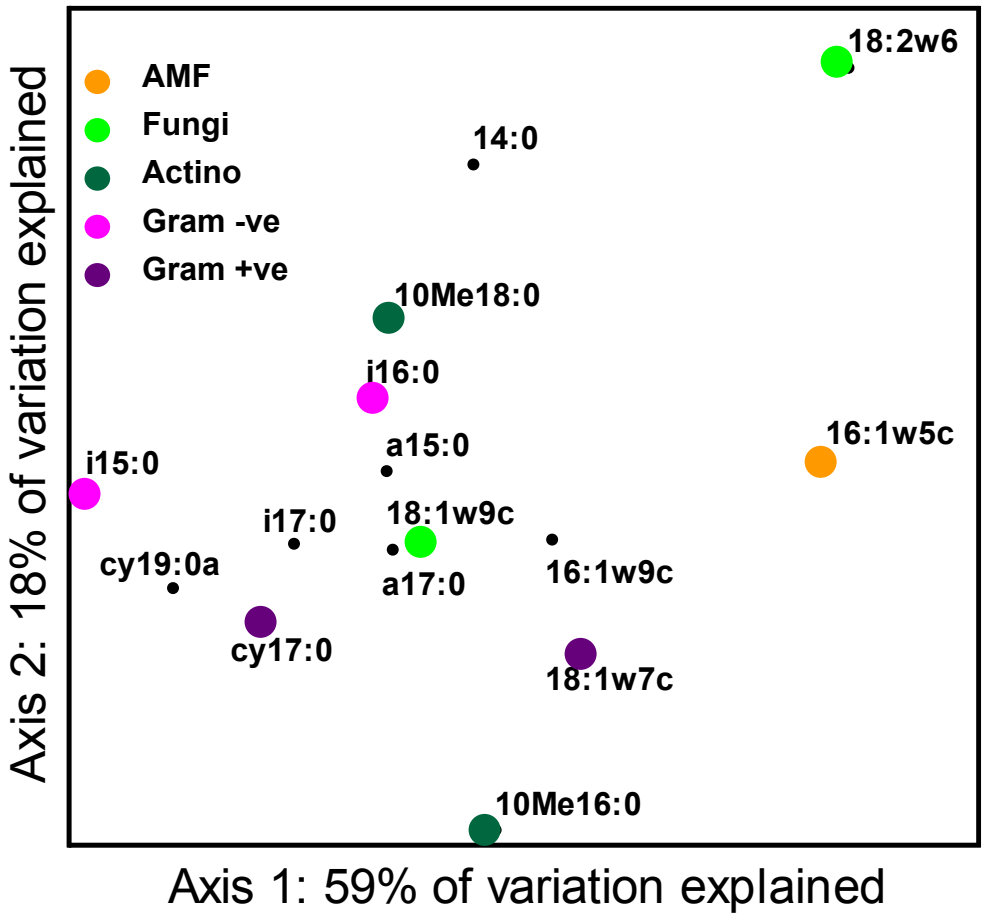


Axis 1 is most strongly correlated with changes in vegetation characters, especially root biomass ($R^2 = 0.55$, $p \leq 0.0001$), root C:N ratio ($R^2 = 0.41$, $p \leq 0.0001$), soil pH ($R^2 = 0.38$, $p \leq 0.0001$) and soil C:N ratio ($R^2 = 0.25$, $p \leq 0.0001$). Basically the left side of the graph is represented by soils agriculture and the right side by the restored prairie plots.

Reciprocal averaging of PLFAs

Analysis

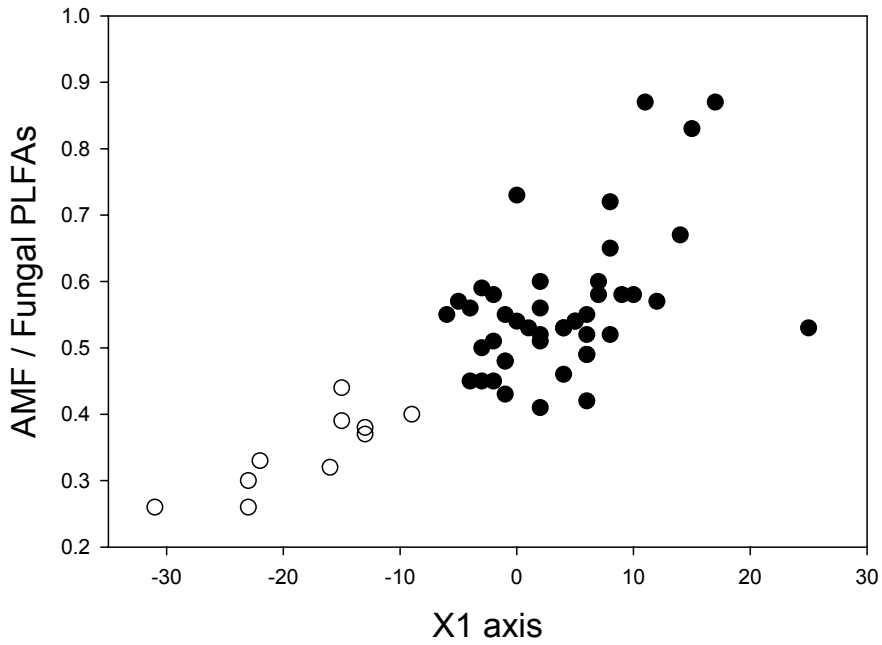
Microbial community signature phospholipid fatty acids were summarized using reciprocal averaging (RA) analysis. The position of each sample depends on the relative abundance of 15 signature fatty acids. Sample position along each axis was subsequently related to environmental variables by linear and nonlinear regression.



AMF/Fungal PLFAs versus X1 axis

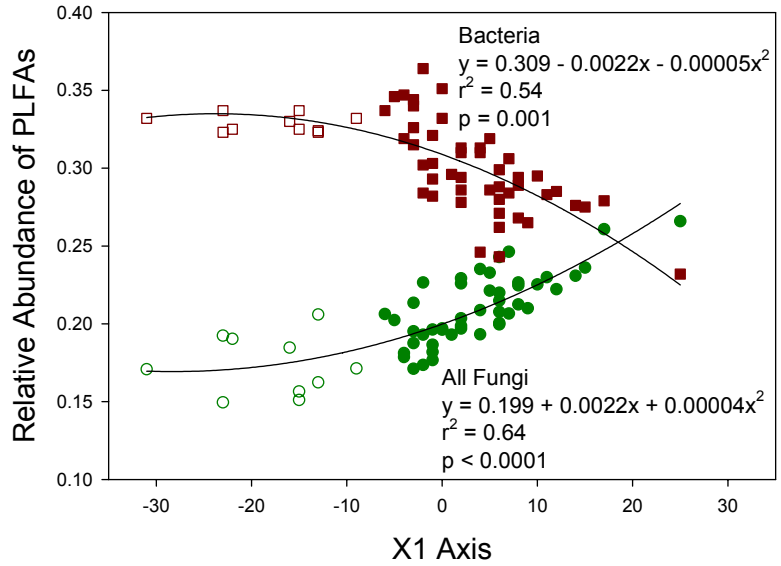
The chronosequence represents a soil carbon gradient where mycorrhizal fungi (AMF) become the dominant form of fungi within the system.

- a direct consequence of the development of a rhizospheric dominated soil.
- Suggests that as SOC accumulates a greater proportion of the fungal biomass will be from AMF.
- Could lead to a reduction in the efficiency of the fungal population to utilize the accumulated carbon, viz. an increase in AMF rather than saprophytic fungi (AMF are not able to degrade detritus carbon).

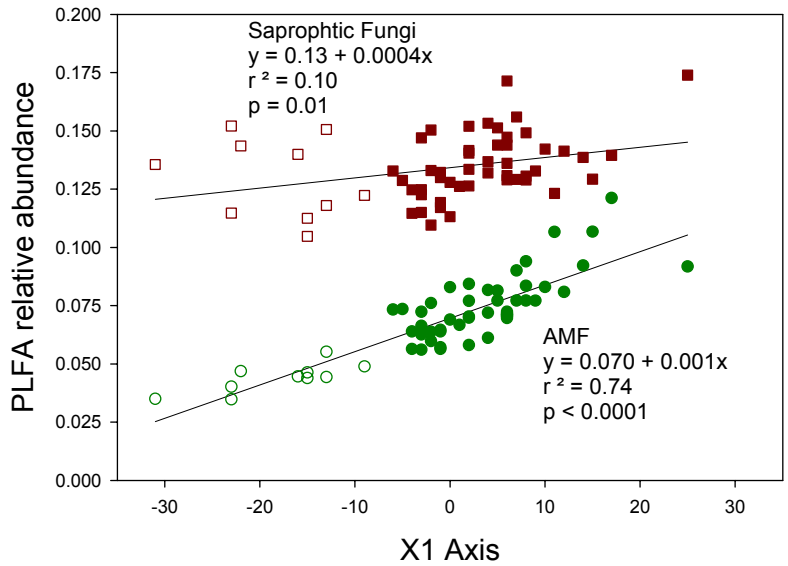


Relative abundance of bacteria and fungi versus X1 axis

The major change in microbial composition is summarized by axis 1 of the ordination: this corresponds to a decline in relative abundance of bacteria, but an increase in relative abundance of fungi along the chronosequence.



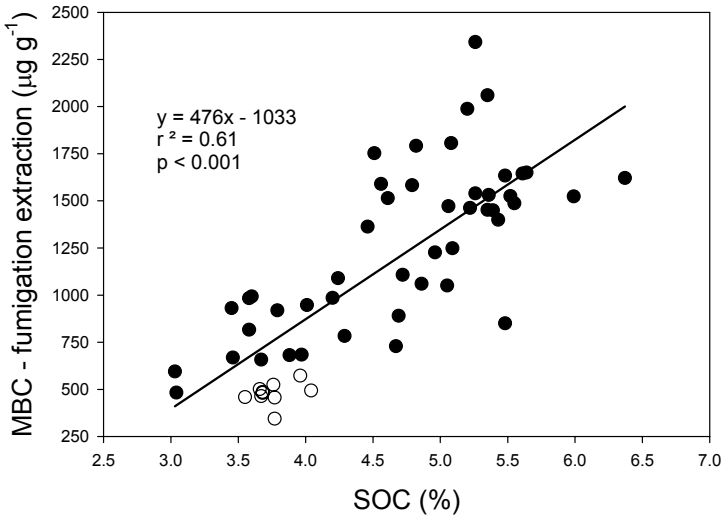
The increase in fungal PLFA relative abundance is proportionally larger for AMF than for saprophytic fungi.

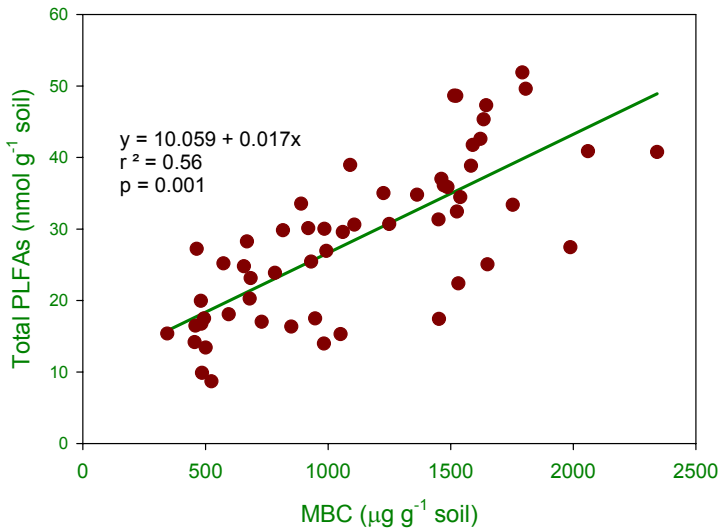


Relationship of MBC with SOC and total PLFA

A positive linear relationship exists between MBC and the amount of SOC along the restoration chronosequence (R^2 = 0.61; p <0.001).

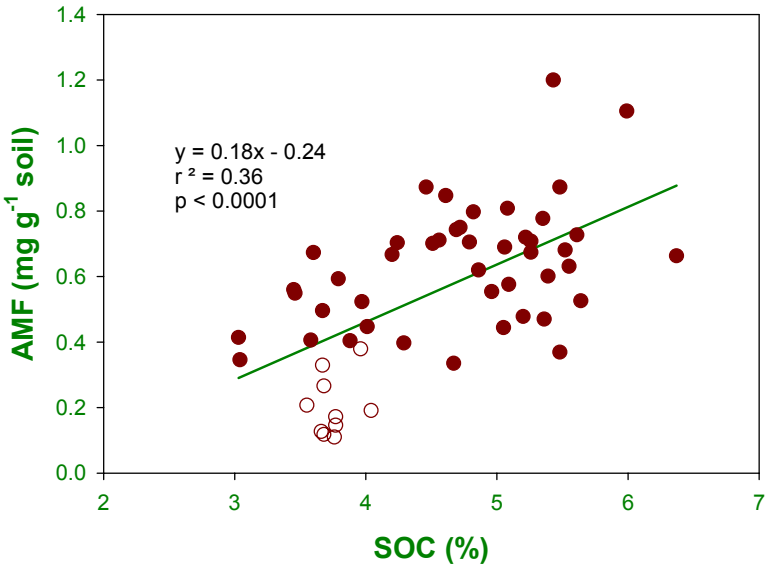
A similar positive relationship exists for MBC and total PLFA (R^2 = 0.56; p<0.001)

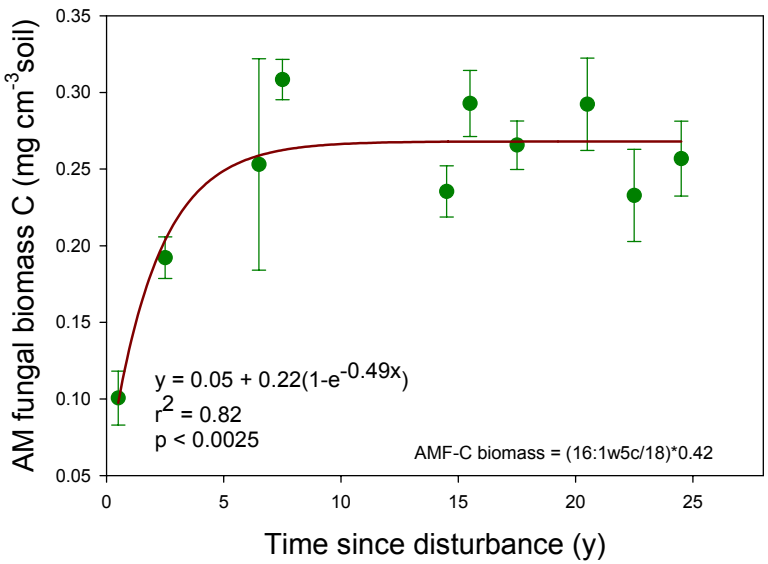




AMF biomass associations

The amount of the AM fungal marker PLFA is positively associated with the accumulation of SOC within the restoration chronosequence (not on a hyphal C basis).





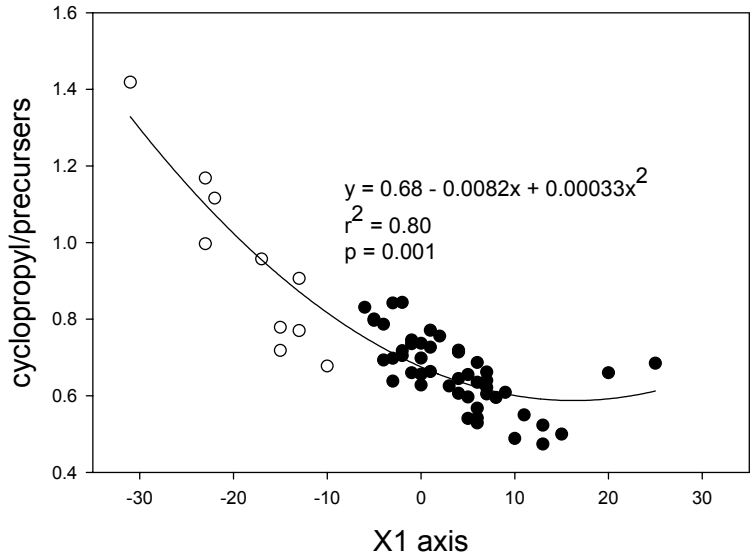
Changes in the amount of soil 16:1w5c PLFA (0-5 cm depth) AM fungi appear to be a major contributor to the microbial biomass AMF biomass approaches equilibrium at around 10 y from cropping.

Measures of stress

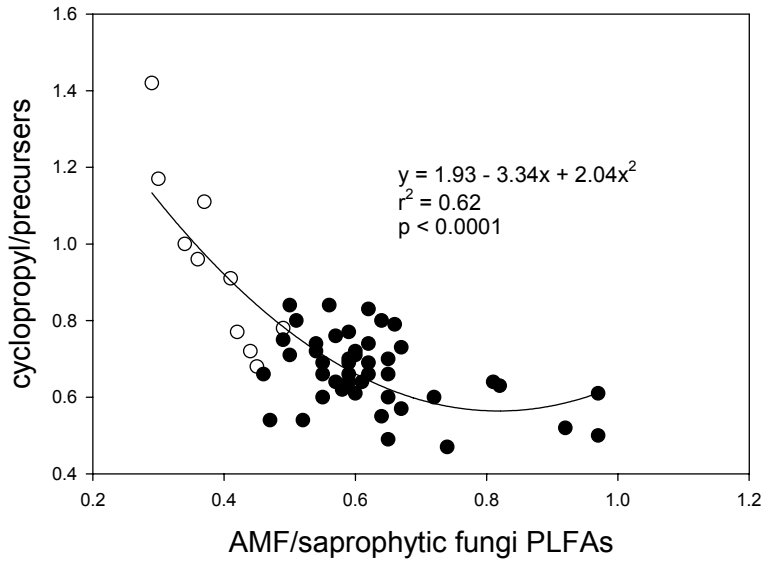
The ratio of cyclopropyl fatty acids *cy*17:0 and *cy*19:0 relative to their precursors, 16:1ω7c and 18:1ω7c, declines following conversion to prairie (represented by X1 axis).

Indicates an increase in proportion of bacterial cells in log rather than stationary phase of growth.

Suggests bacterial communities in the agricultural soils may be carbon limited.

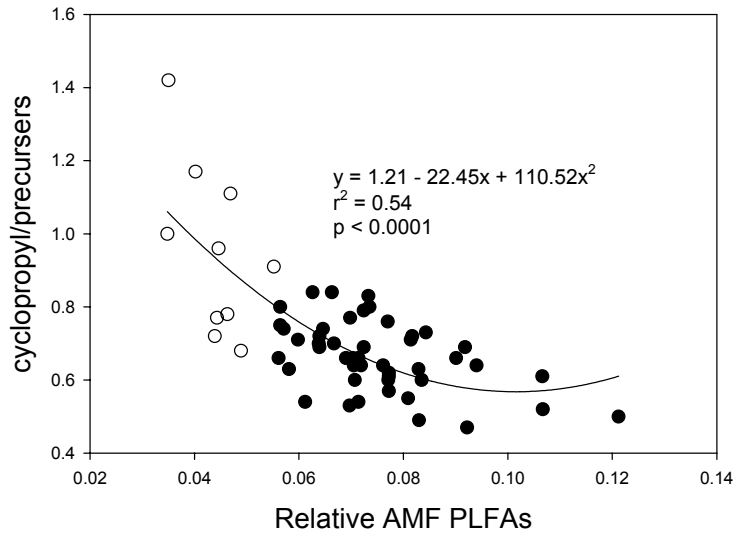


A strong negative relationship for the ratio of AMF PLFA 16:1ω5c with saprophytic fungal PLFA 18:2ω6,9 and the cyclopropyl fatty acid to precursor ratio suggest amelioration of stress is evident for bacteria as the amount of AMF increases.

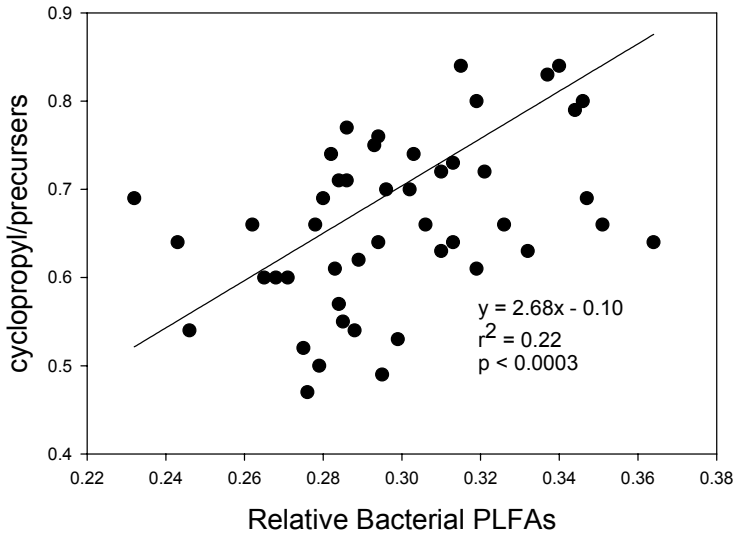


Measures of stress

The relative amount of AM Fungal PLFA is negatively associated with the cyclopropyl / precursor ratio indicating that as the proportion of AMF fungi increases as a greater proportion of bacterial cells are in log phase growth.

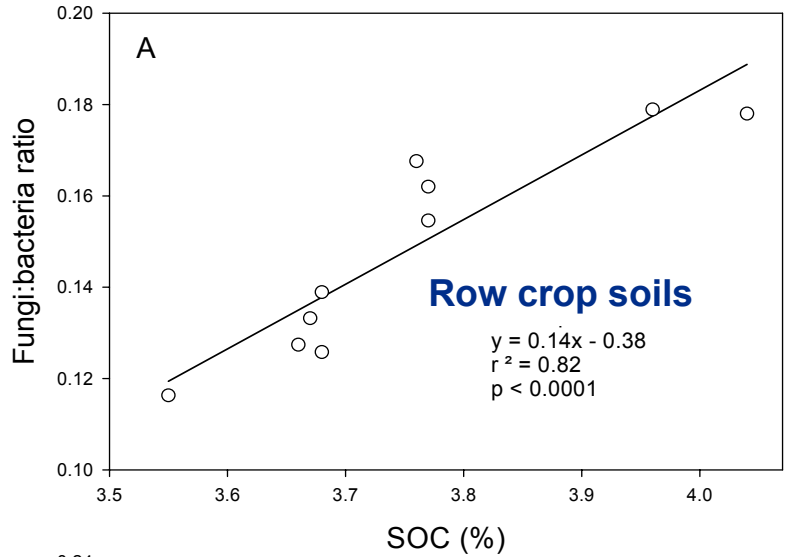


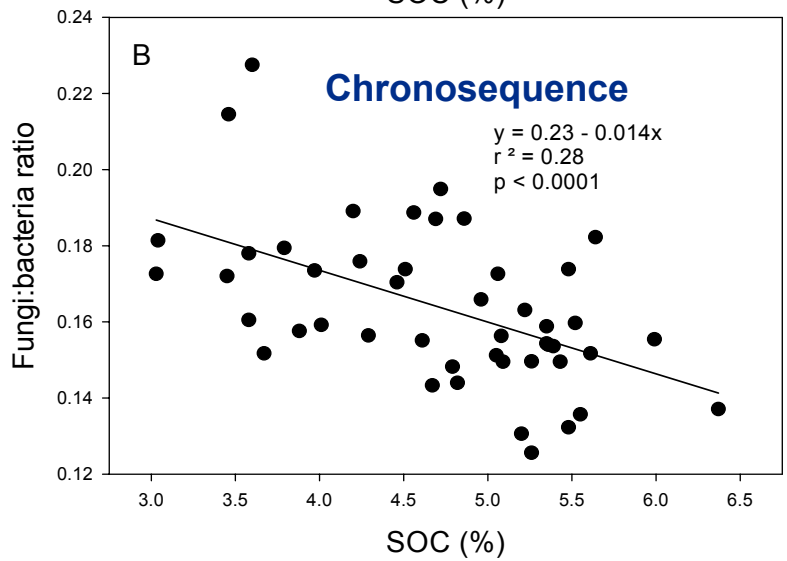
Within the restoration chronosequence the relative proportion of bacterial cells increases with the cyclopropyl to precursor ratio increases indicating as the relative density of bacteria increases a greater proportion of them are in stationary phase growth – an indicator of stress.



Fungi/bacteria ratio relationship with SOC

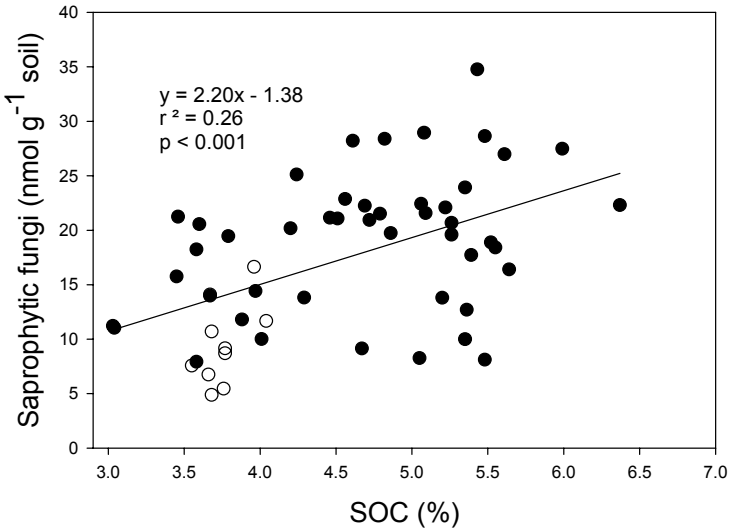
The proportion of fungal-to-bacterial PLFAs shows a positive relationship with SOC in the row crop soils, while the ratio decreases within the chronosequence suggesting that in an aggrading system the amount/activity of saprophytic fungi decrease as C content increases.





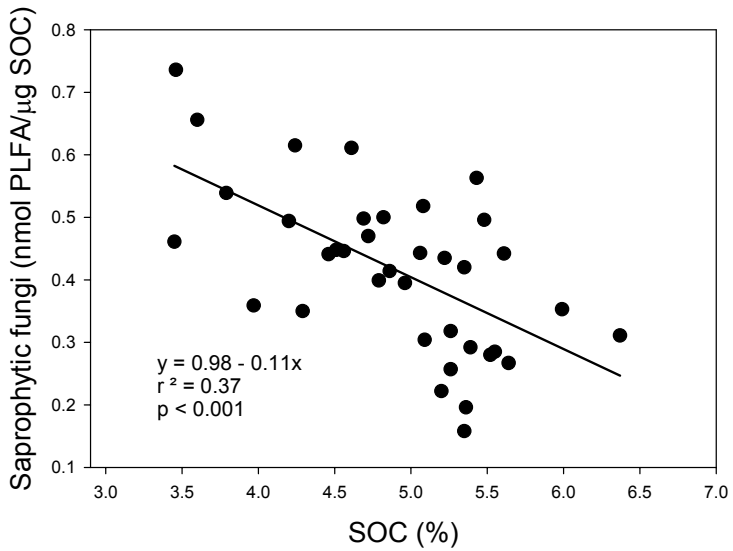
Saprophytic fungi and SOC

The total amount of saprophytic fungal PLFAs increases as SOC accumulates within the restoration chronosequence. A similar response with SOC also exists for bacterial PLFAs.

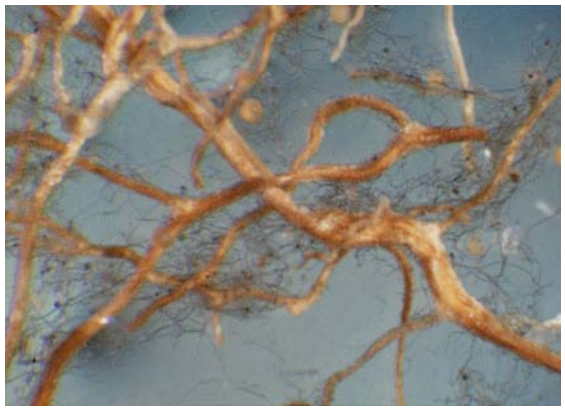


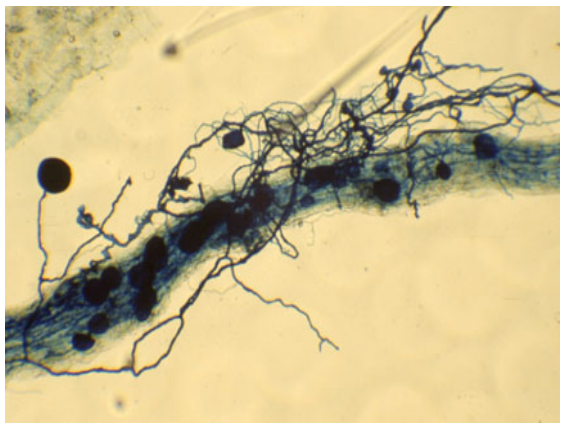
If saprophytic fungal PLFA is expressed on a soil carbon basis rather than by soil dry wt a significant negative association exists between saprophytic fungi and SOC .

Although not presented, a marginal relationship exists for bacterial PLFAs and SOC (p = 0.072).



Extraradical hyphae (ERH) of AMF represent a considerable portion of the biomass in soil





In a restored prairie community soil (Miller et al. 1995; Allison et al., in press)

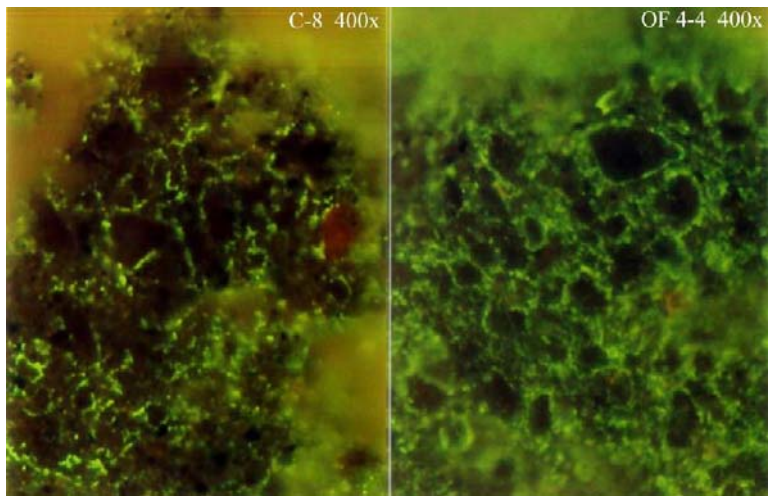
- ⇨ *Peak Extraradical hyphal C (ERH-C) =*

  215 μg cm^{-3} soil (110 m cm^{-3} soil)
- ⇨ *Peak MBC (1068 μg cm^{-3} soil)*
- ⇨ *Production of ERH-C = 84 μg cm^{-3} soil*
- ⇨ *ERH-C/MB-C = 0.23*
- ⇨ *16:1ω5c/18:2ω6 $\approx$ 0.50*
- ⇨ *ERH-C turnover* **($T = P/I_{Bmax}$)** *= 2.42 y*

Contributions of Glomalin

Glomalin Story

- Mycorrhizal hyphae as a sticky-string-bag (*The sticky on the string*)
- Glycoprotein production
  - hydrophobic nature – desiccant protector?
    - structural integrity
    - growth across pore space in soil
  - glue for stabilizing soil aggregates
  - Contributes to soil carbon and nitrogen pools
  - Chelater of metals, especially iron, zinc



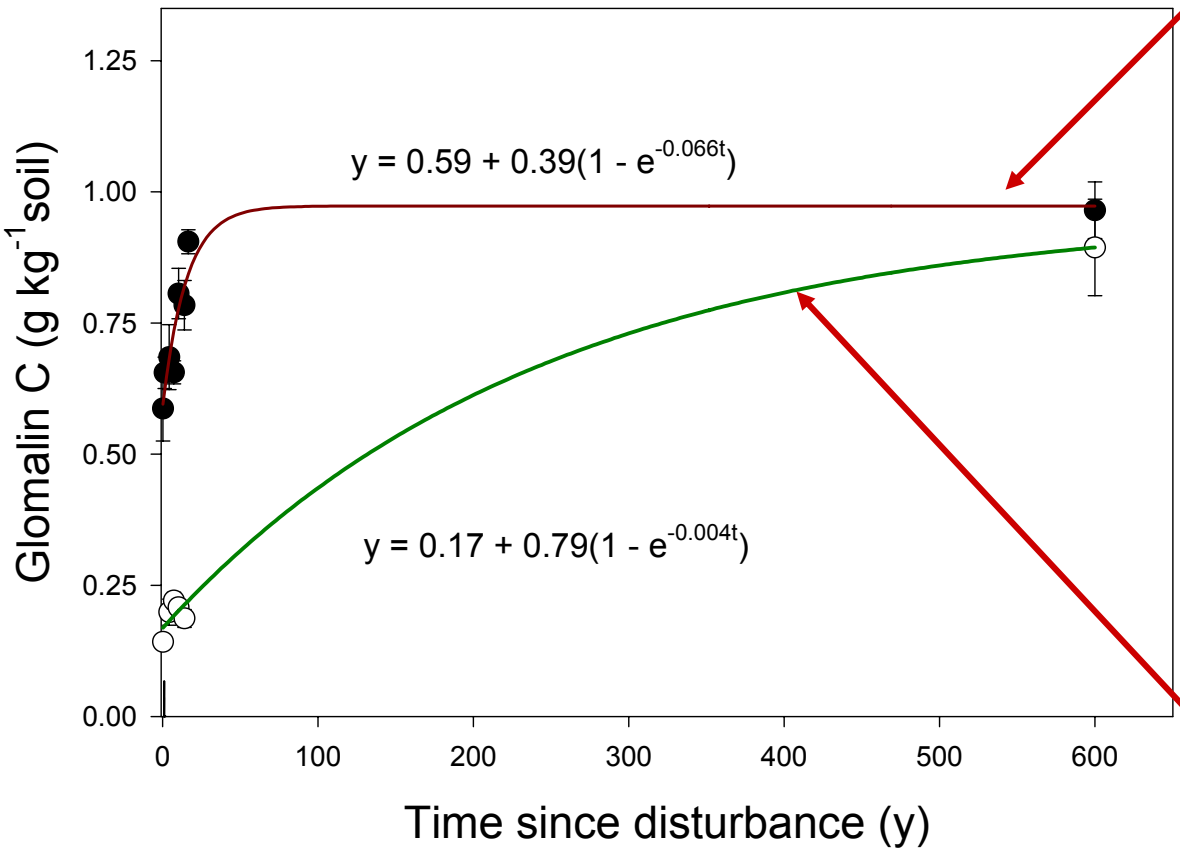
(Wright & Upadhyaya, Soil Science 161: 575-586, 1996; Wright et al, Plant & Soil 181:193-203, 1996; Rillig et al. Nature 400, 628, 1999;Miller & Jastrow, 2000)

Roots, hyphae, glomalin and soil aggregation – Fermi chronosequence

	SOC	Clay	Percent macro - aggregates	Aggregates <210µm diameter
Immuno-reactive glomalin	**0.93*** **	*0.44***	0.28*	0.19 ns
Extraradical hyphal length	-0.14 ns	0.02 ns	**0.60*** **	**-0.50*** **
Fibrous root length	*0.33***	0.07ns	**0.61*** **	*-0.41***

*Pearson r coefficients and significance levels: p<0.001***, p<0.01**, p<0.05*, ns=not significant*

Two pool model for determining glomalin turnover time



Labile pool =TG – IRTG
(fast turnover pool)

	1/k
Labile pool	*15 y*
Slow pool	*200 y*

Slow pool = IRTG

Carbon Inputs by AM Fungi to SOC

The input of carbon to the soil organic carbon pool is defined as

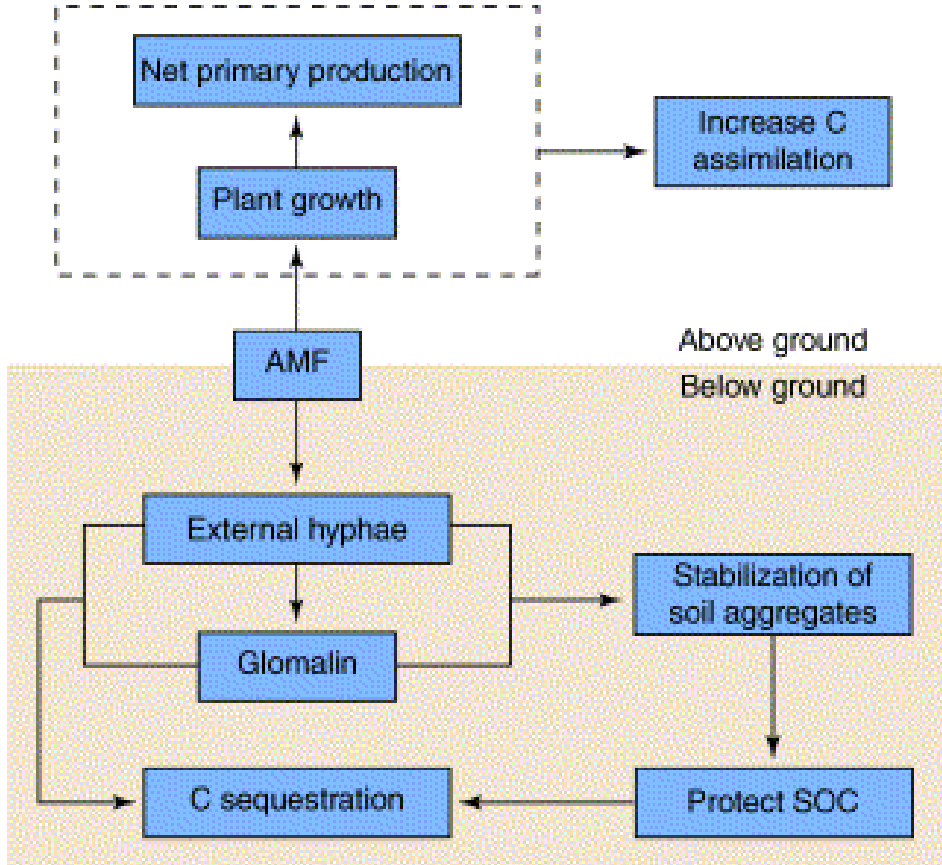
$$I = C_e \times k$$

where C_e is the equilibrium carbon inventory and k is the first order rate constant for loss.

	Inputs
SOC	0.860 g kg^{-1} y^{-1}
Hyphae -C	0.049 g kg^{-1} y^{-1}
Glomalin-C	0.005 g kg^{-1} y^{-1}
AMF-C	0.051 g kg^{-1} y^{-1}
AMF-C/SOC	5.9 %

Contributions of AMF hyphae to soil carbon sequestration (Zhu & Miller, 2003)

- **Influence of AMF on soil carbon sequestration**
  - **Nutrient effects on Host**
    - **Plant growth**
    - **NPP**
    - **C assimilation**
  - **AMF biomass**
    - **External hyphae**
      - **Chitin cell wall**
      - **glomalin**
    - **Protected SOC**
  - **AMF efficiency – direct access to photosynthate**



TRENDS in Plant Science

Conclusions:

Factors influencing Microbial community structure

- Soil microbial biomass increases with time since restoration
  - Proportion of fungal biomass (AMF+Sapros) increases with time
  - Proportion of bacteria decreases
  - Total amount of both bacteria and fungi increases with time
- Correlated most strongly with increased soil C and N, declining bulk density, and increasing soil moisture
- Community composition is affected most strongly by root production (development of a rhizosphere) and surface litter biomass
- AMF are a major contributor to the change in microbial community composition when agriculture ceases (development of a rhizosphere)
- Management changes can potentially increase carbon storage by increasing both microbial biomass and fungal dominance

Fermilab Restoration Chronosequence

Publications:

1. Miller, R.M. and J.D. Jastrow. 2000. Mycorrhizal fungi influence soil structure. In: *Arbuscular Mycorrhizas: physiology and function*, Y. Kapulnik and D. Douds, eds. Kluwer Academic Publishers, Dordrecht, p 4-18.
2. Zhu Y.G., and R.M. Miller. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends in Plant Science* 8: 407-409.
3. Allison V.J., and R.M. Miller. 2004. Using fatty acids to quantify arbuscular mycorrhizal fungi. In: *Basic Research and Applications of Mycorrhizae*, G. Podila and A Varma. Eds. I.K. International Pvt. Ltd. New Delhi, pp 141-161.
4. Allison V.J. and R.M. Miller. 2005. Sample size and grinding affect fatty acid extraction efficiency and relative abundances in soil. *Soil Science Society America Journal* 69:(in press).
5. Allison V.J., Miller R.M., Jastrow J.D., Matamala R., and D.R. Zak. 2005. Characterization of environmental and edaphic factors affecting soil microbial community structure in a tallgrass prairie chronosequence. *Soil Science Society Journal* 69: (in press).