Microbial Ecology and C&N Dynamics

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Microbial Effects: Rationale

- **Soil microbial biomass (bacteria and fungi) is critical to:**
  - **Cycling of C and nutrients**
  - **Degradation of organic compounds in soil**
  - **Conversion of soil C to recalcitrant forms**
  - **Stabilization of soil aggregates**

- **Bacteria and fungi cycle C differently**
  - **Fungal biomass more resistant to degradation**
  - **Bacteria have lower efficiency for assimilating C than fungi**
  - **Management favoring fungal population and activity may enhance longevity and retention of soil C**

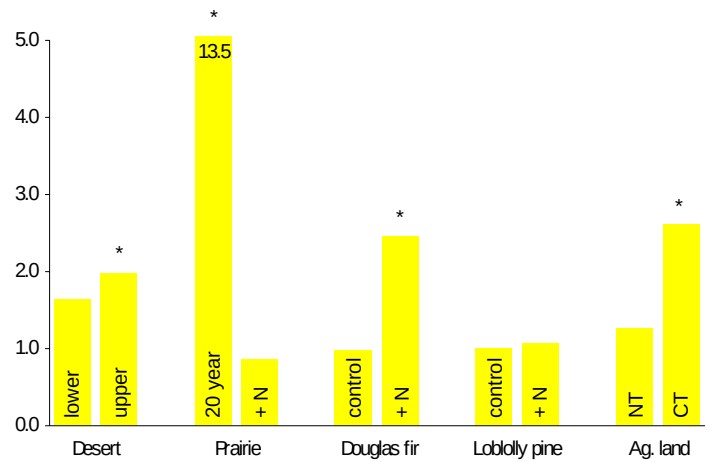
Linkages

- **Use field sites and management systems studied in other tasks**
  - **Prairie restoration (Fermilab NERP)**
  - **Industry forest nutrition cooperatives**
  - **No-Till (Washington)**
  - **ALE NERP**

- **Integrate data and experiments with other tasks**
  - **Grassland restoration from cropland**
  - **Stabilization of soil organic C**

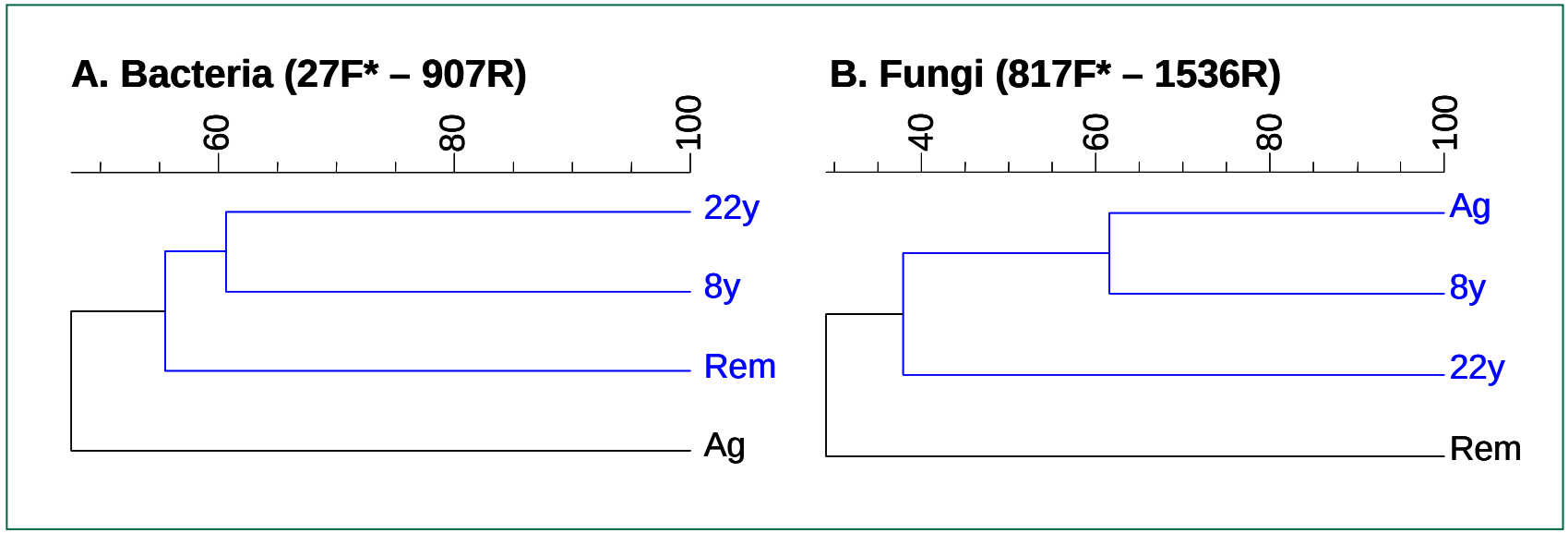
Early hypotheses

- **Management affects the structure and function of soil microbial communities**
  - **the active microbial community will differ in soils that are managed for C sequestration compared to soils that are not.**



Bailey, V. L., J. L. Smith, and H. Bolton, Jr. 2002. Soil Biol. Biochem. 34:997-1007.

DNA fingerprinting of Fermi soils

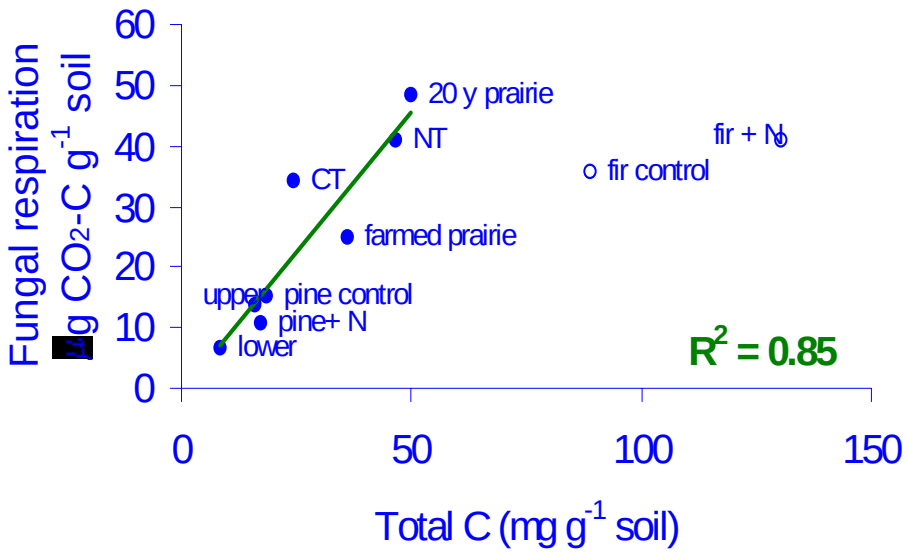


- **Bacterial community composition recovers faster from agriculture to native conditions than the fungal**
- **At Fermi, it is the recovering, less diverse fungal activities that are more associated with C storage**

Shutthanandan, J. F. J. Brockman, H. Bolton, Jr., and V. L. Bailey. 2004. Micro. Ecol. Submitted.

Fungi and C Storage

- **The storage of C is expected to be more labile when mediated by the bacterial biomass and more persistent when mediated by the fungal biomass.**



Thus, management activities or site characteristics that favor fungi may also favor enhanced storage of C in soil.

Bailey, V. L., A. D. Peacock, J. L. Smith, and H. Bolton, Jr. 2002. Soil Biol. Biochem. 34:1385-1389.

Bailey, V. L., J. L. Smith, and H. Bolton, Jr. 2003. Biol. Fertil. Soils 38:154-160.

C Dynamics in Tillage Treatments

- **Observed a priming effect that suggested C inputs have a greater effect on mineralization of residual ^{14}C compared to disturbance**
  - **endogenous metabolism appears to be the source of primed C**
  - **becomes more pronounced as the F:B ratio increases.**

Bell, J. M., J. L. Smith. V. L. Bailey, and H. Bolton, Jr. 2003. Biol. Fertil. Soils 37:237-244.

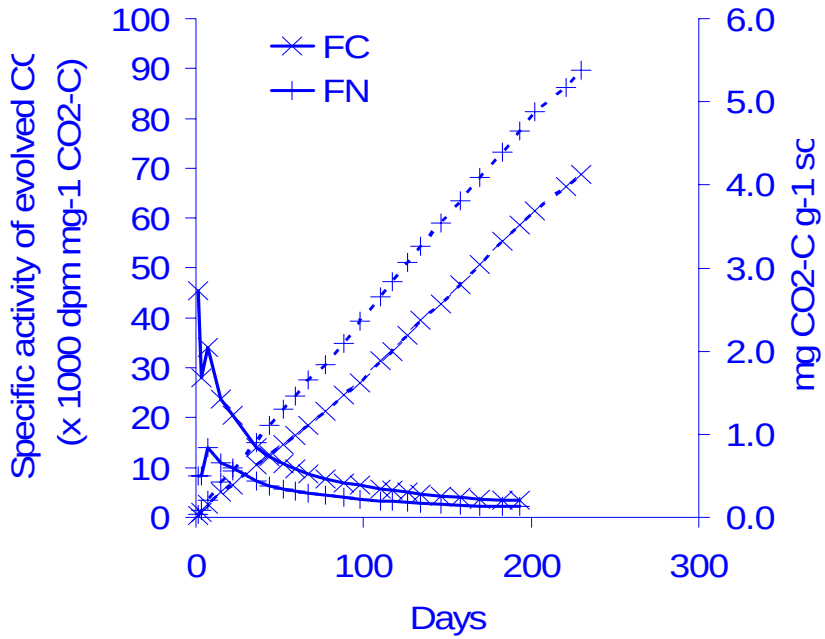
Detection and Prediction of C Sequestration

Objectives

- ⇨ **to search for differences in the metabolism of a complex substrate in contrasting soils over 8 months**
- ⇨ **to identify short-term indicators of long-term C distribution**

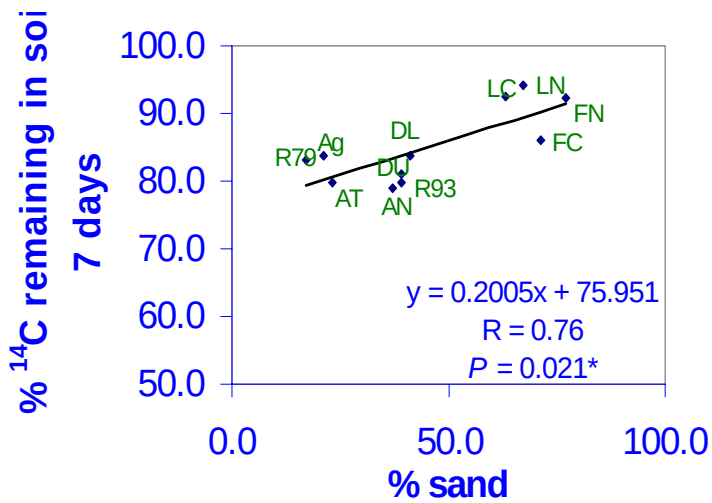
- **Recalcitrant substrate**
  - **lignocellulose**
  - **Bailey et al. Biol. Fertil. Soil (submitted)**
- **Labile substrates**
  - **glucose, acetate, oxalic acid, urea, arginine, phenylalanine**
  - **In prep**

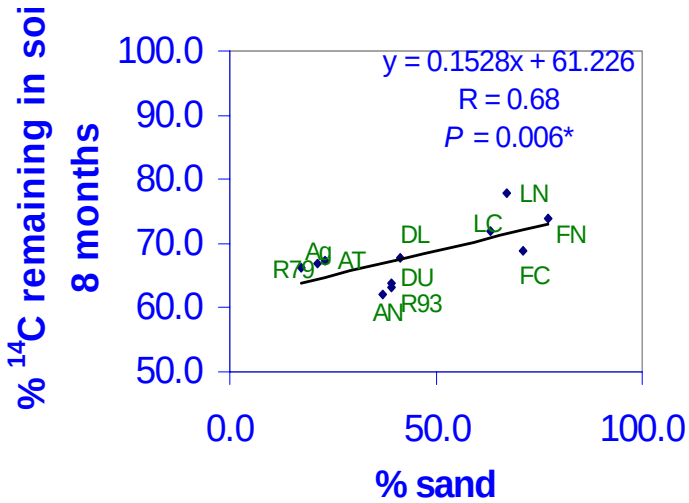
Recalcitrant substrate



- **The soils that rapidly metabolized freshly added C exploited endogenous and newly transformed C to a lesser degree.**
  - **These tended to be soils hypothesized to not favor C storage**
  - **Microbial communities in these soils may be less able to metabolize the recalcitrant endogenous C remaining in the soil**
  - **Communities in the richer soils at the same site may be more diverse or competent to use the native C following stimulation of the biomass by freshly added C**

Predicting new C storage from soil texture

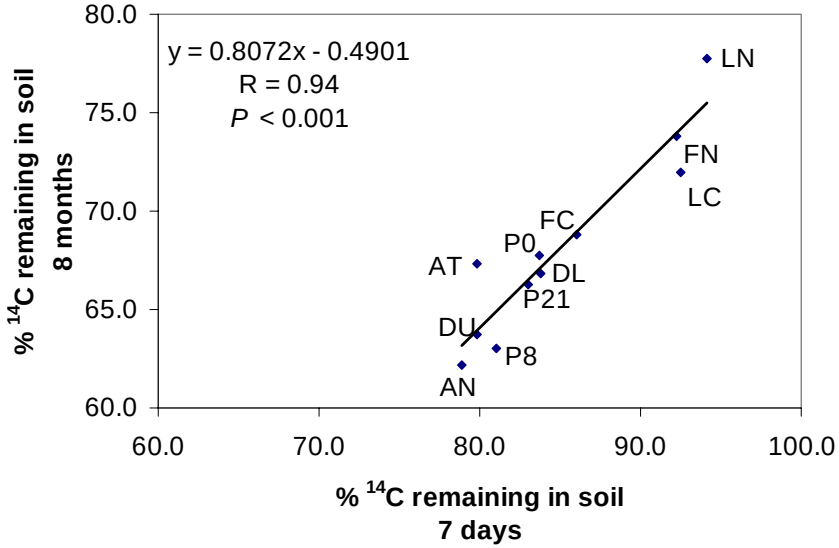




Soil sand content was correlated with the amount of freshly added C remaining in the soils after 7d and 8 months.

Predicting long term storage from short term metrics

The short-term mineralization of lignocellulose-C (measured as C remaining in the soil) was also found to be predictive of the longer-term storage of C, after 8 months.



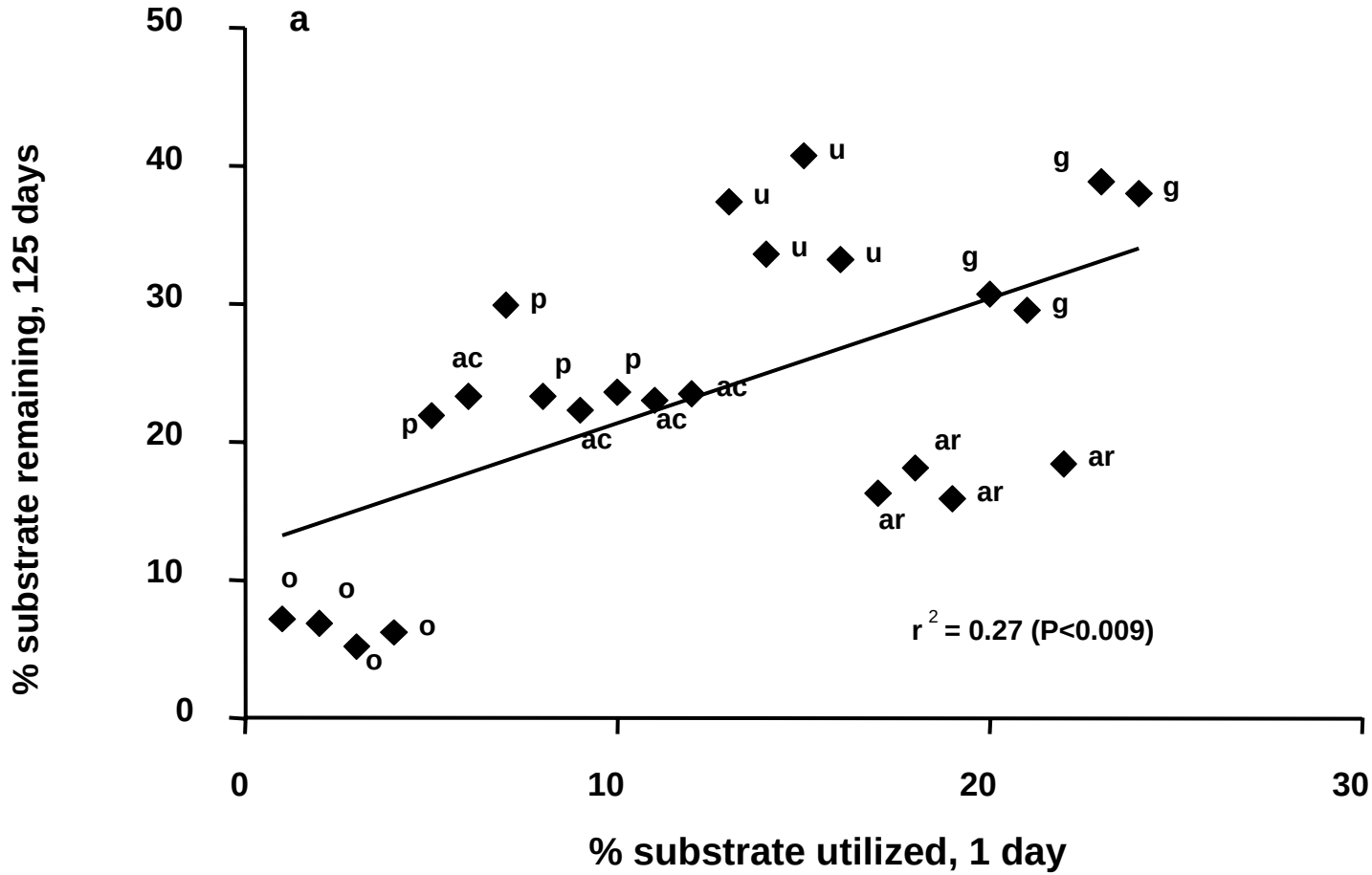
Both of these predictors may be valuable tools in C management systems

- **predicting 8-month C storage with the 7-day metric was a strong, significant function**
- **the association of sand with C storage is a rapid assessment that can be made based on standard soil characterization analyses.**

Labile substrates

- 4 soils of the ALE elevation transect (semi-arid shrub steppe)
- 6 ^{14}C labeled substrates
  - Glucose, acetate, oxalic acid, urea, arginine, phenylalanine
- 2 temperatures 24 and 34°C
- Measure $^{14}CO_2$, biomass ^{14}C

Elevation (m)	pH	EC[1] dS m^{-1}	Organic N g N kg^{-1} soil	Organic C g C kg^{-1} soil
A 313	6.5	.23	2.7	13
B 598	6.1	.33	3.1	15
C 751	5.7	.42	3.5	22
D 840	5.5	.50	4.0	28


a
% substrate remaining, 125 days
% substrate utilized, 1 day
0
10
20
30
40
50
0
10
20
30
u
u
u
u
g
g
g
g
p
p
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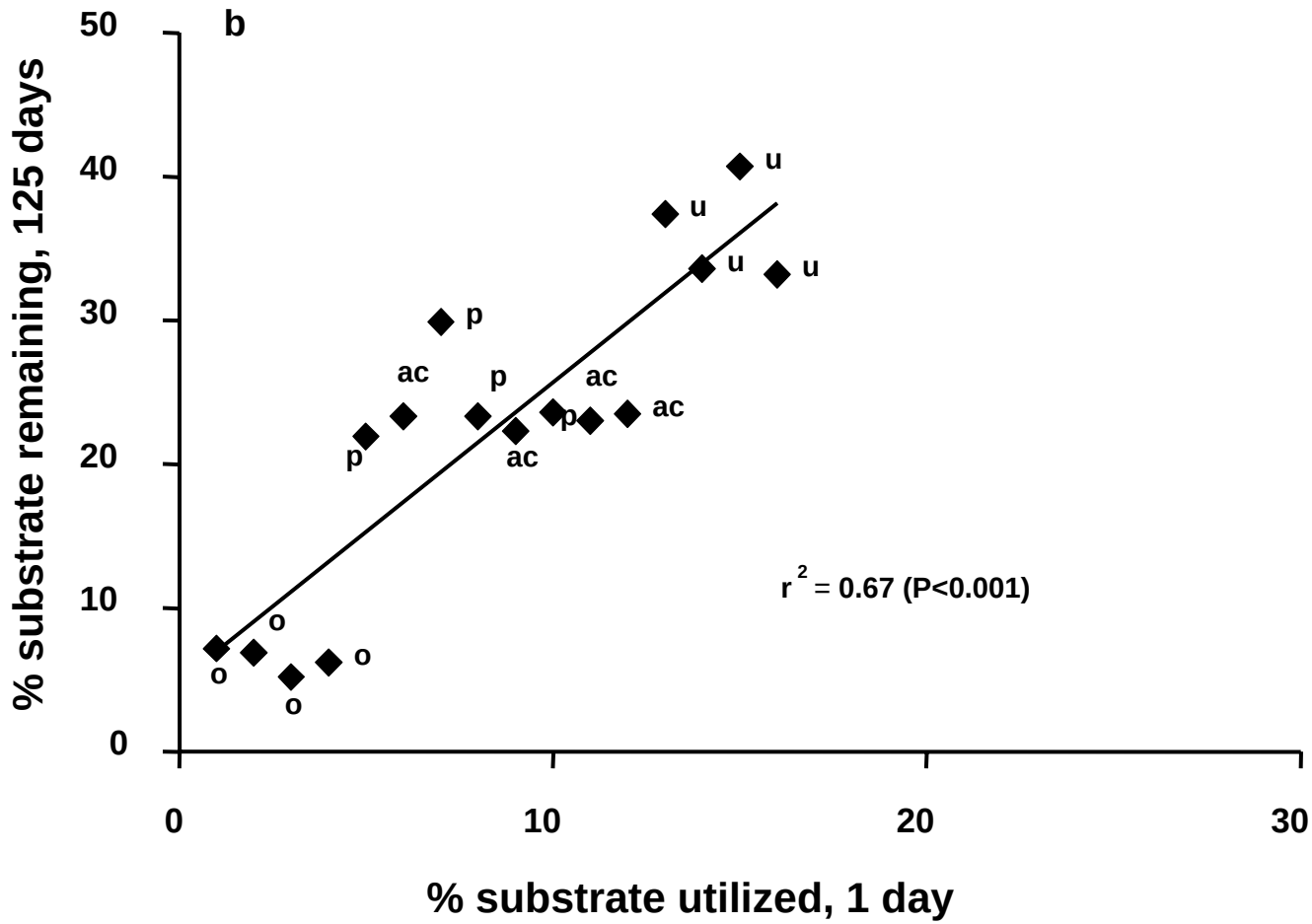
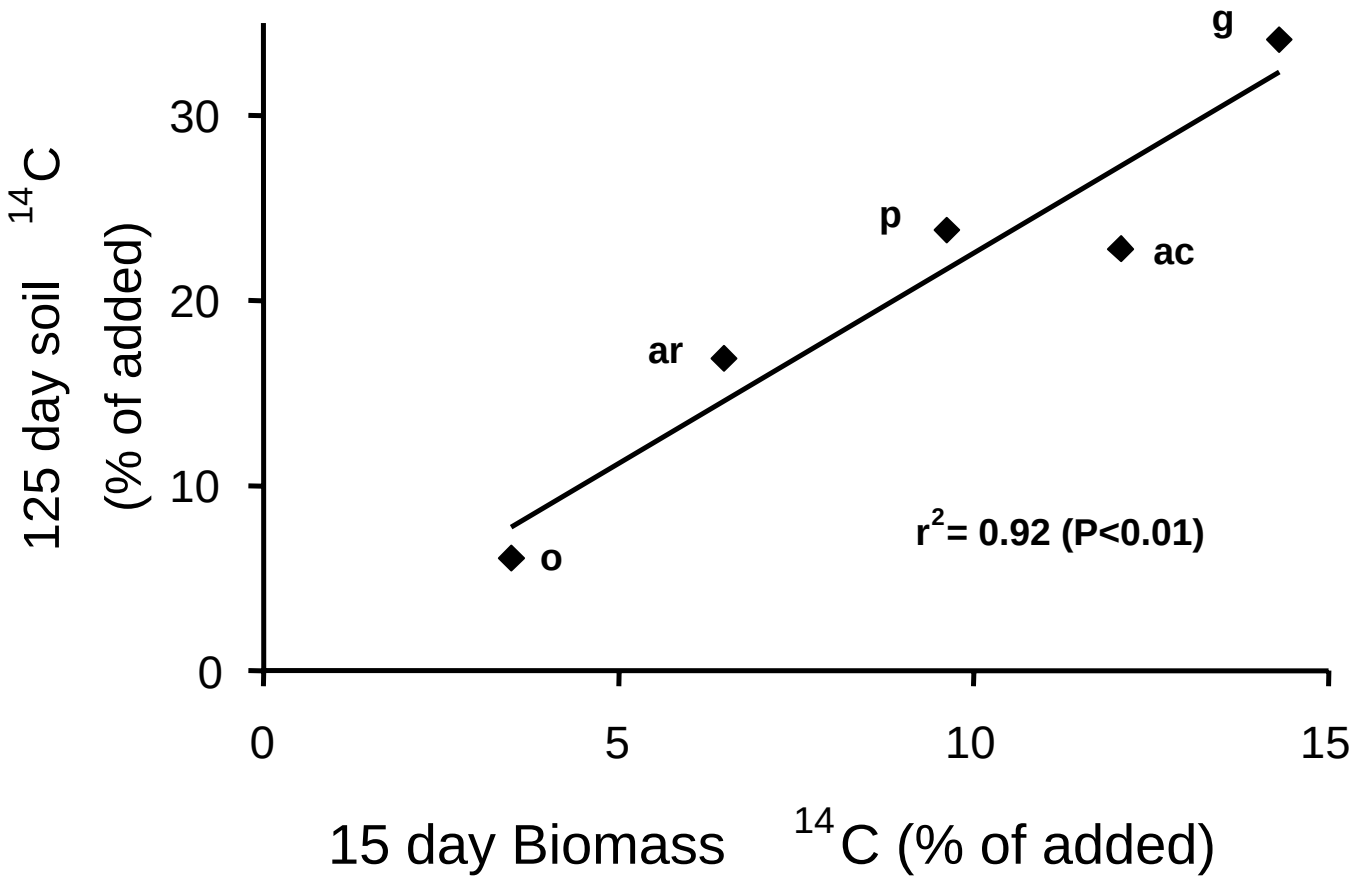
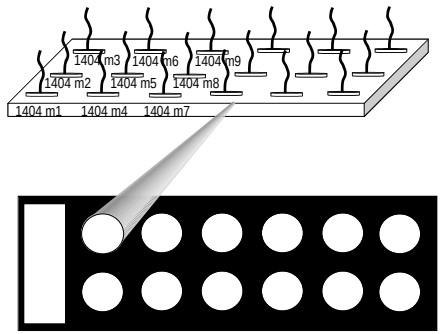
o
r 2 = 0.27 (P<0.009)


b
% substrate remaining, 125 days
% substrate utilized, 1 day
0
10
20
30
40
50
0
10
20
30
u
u
u
u
p
ac
p
ac
p
ac
p
ac
o
o
o
o
r 2 = 0.67 (P<0.001)


g
p
ac
ar
o
r^2= 0.92 (P<0.01)
30
20
10
0
0
5
10
15
125 day soil ^{14}C (% of added)
15 day Biomass ^{14}C (% of added)

More Direct Approaches

- Longer-term ^{15}N cycling
- Pool dilution
- Microarray development



^{15}N Plots

1g ^{15}N

1g ^{15}N

1g ^{15}N

Long-term 15N cycling

- **The 1979 restored prairie has a higher enrichment of ^{15}N in the SOM and MB than the 1993 restored prairie**
- **The data for the repeated sampling has just been completed**

^{15}N mineralization

- **We now have ^{15}N labeled soil**
- **We know what pools it was incorporated into**
- **Thus we can now trace the N mineralizing out of the soil as a rate of mineralization and origin**

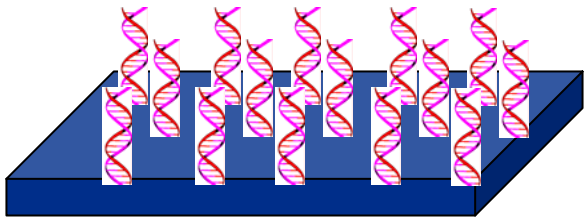
Isotope dilution

	min	NH4	SMB	MRT	C min
	ug N/g soil/d		ug C/g	days	kg/ha/y
Ag	22.2	17.5	1114	51	23
R-93	11.6	9.5	1408	19	225
R-79	4.3	9.7	2267	30	78
T000	1.8	3.9	735	44	-79
T110	7.6	10.4	617	9	53

- N cycling most rapid in the agricultural soil
- N accrual sustains plant productivity and thus increases C storage
- Need more help understanding what is going on!

DNA Microarrays

- **Microarrays:** Glass slides or other solid surface containing thousands of genes arrayed by automated equipment.



- **DNA microarrays:** Any length and composition of DNA fragments are attached to glass slides.
- **Oligo microarrays:** Oligonucleotides are synthesized either in situ on a small solid surface or by conventional synthesis followed by on-chip immobilization.

Oligonucleotide Array for C and N Cycling

- ⇨ **Nitrogen cycling: 5089**
- ⇨ **Carbon cycling: 9198**
- ⇨ **Sulfate reduction: 1006**
- ⇨ **Phosphorus utilization: 438**
- ⇨ **Organic contaminant degradation: 5359**
- ⇨ **Metal resistance and oxidation: 2303**
- ⇨ **Total: 23,408 genes**

Will be very useful for community and ecological studies

Details of probe design for N cycle related genes

Denitrification	# of input genes	# with Unq probes	# with Grp probes	% inclusion
nitrite reductase (nirS)	411	140	129	65.5
nitrite reductase (nirK)	952	139	132	28.5
nitrous oxide reductase (nosZ)	273	117	94	77.3
nitrate reductase (narG)	544	196	227	77.8
nitrate reductase (nasA)	120	57	55	93.3
nitric oxide reductase (norB)	68	46	13	86.8
nitrate reductase (narB)	34	10	22	94.1
nitrate reductase (napA)	145	56	81	94.5
subtotal	**2547**	**761**	**753**	**59.4**
Nitrification				
ammonium monooxygenase (amoA)	1243	49	349	32.0
hydroxylamine oxidoreductase (haoR)	15	2	12	93.3
subtotal	**1258**	**51**	**361**	**32.8**
Nitrogen Fixation				
dinitrogenase (nifD)	124	32	74	85.5
dinitrogenase reductase (nifH)	2038	435	549	48.3
subtotal	**2162**	**467**	**623**	**50.4**

Details of probe design for C cycle related genes

Carbon polymer degradation	# of input genes	# with Unq probes	# with Grp probes	% inclusion
laccase	190	97	79	92.6
chitinase	413	248	100	84.3
cellulase/endoglucanase	567	417	80	87.7
beta-mannosidase	73	66	4	95.9
polygalacturonase	112	86	25	99.1
glutamate dehydrogenase	112	88	10	87.5
subtotal	**1467**	**1002**	**298**	**88.6**

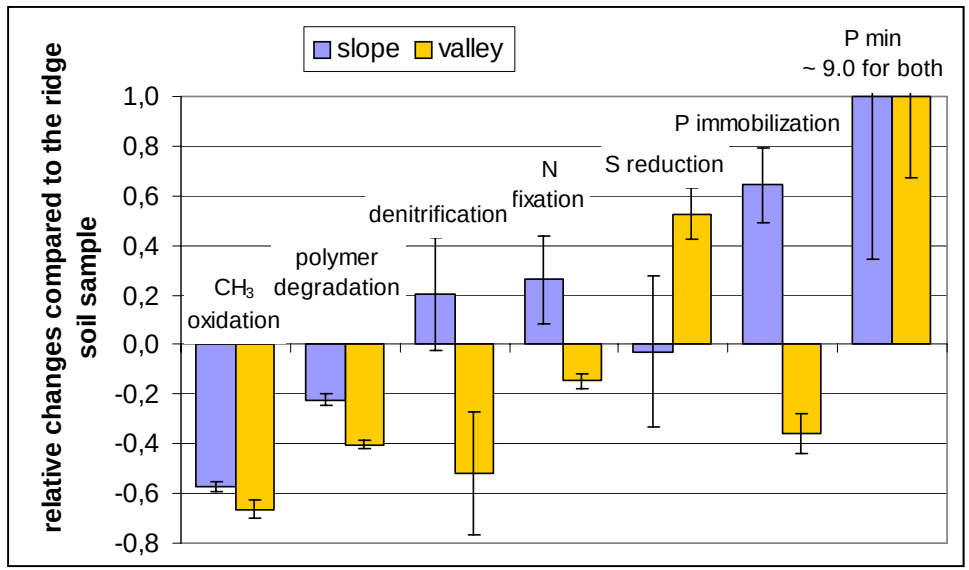
Carbon Fixation				
carbon monoxide dehydrogenase	10	10	0	100.0
formyltetrahydrofolate synthetase	155	52	87	89.7
rubisco large subunit	470	158	87	52.1
rubisco small subunit	299	16	85	33.8
subtotal	**934**	**236**	**259**	**53.0**

Application of functional gene arrays to soil DNA

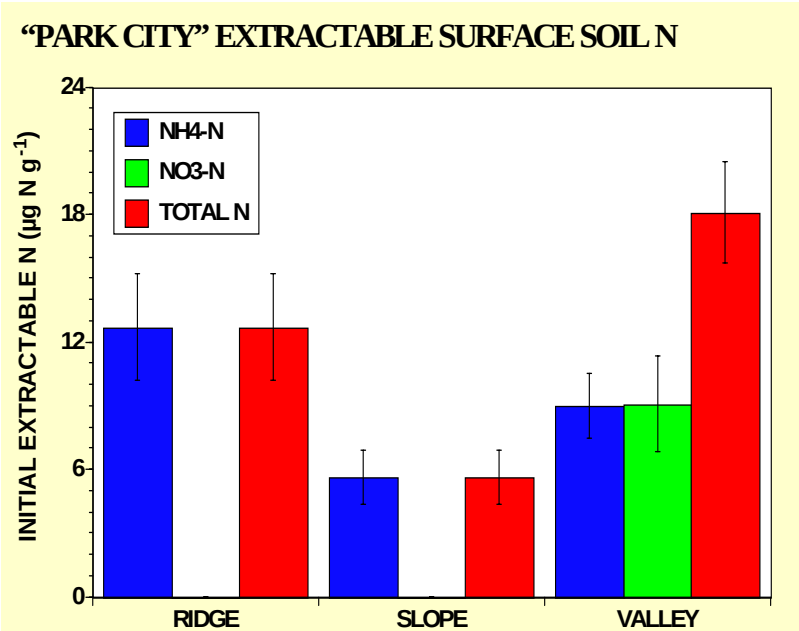
Approach

DNA from 3 different soil samples from the Oak Ridge Reservation (ridge, valley, slope) was extracted and applied to the functional gene arrays. This experiment contributes to the “Park Site” Field Experiment by Chuck Garten et al.

- Between 29 and 94 different genes can be detected on the microarrays for each soil sample.
- Detected differences in the microbial community composition between the sites, which can be related to soil chemistry data.
- After comparison of the data to a reference sample only very few significant different genes can be detected.

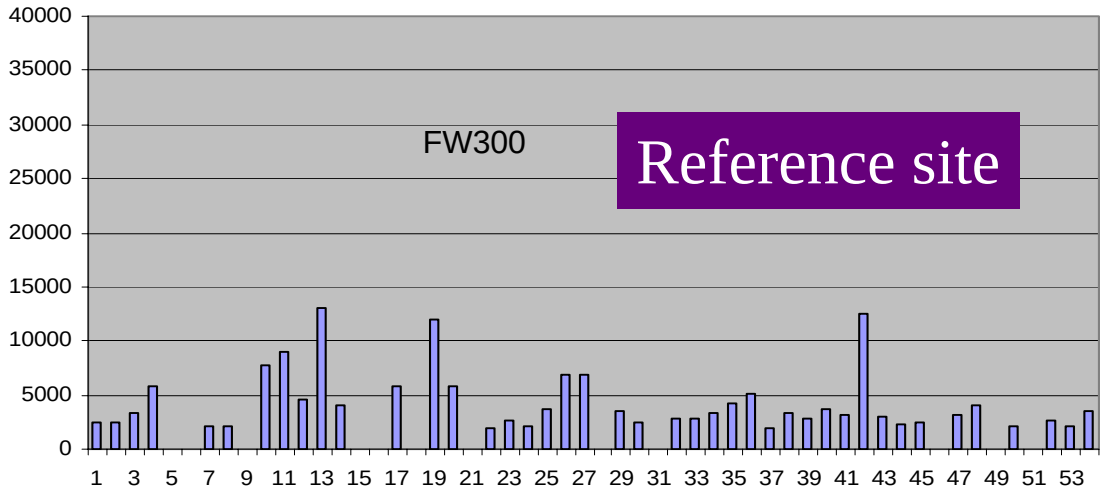


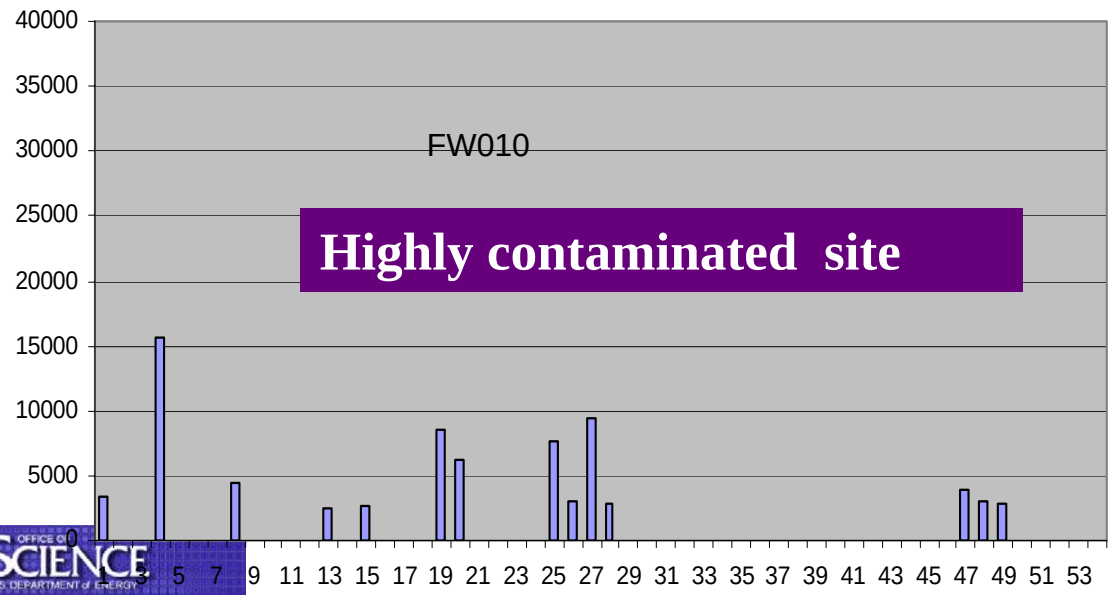
Nitrogen availability



- ⇨ No nitrate found in slope
  - High relative denitrification
- ⇨ High nitrate in valley
  - Low relative denitrification

Difference of functional genes in soils from NABIR Field Research Center





- Clear difference was observed among contaminated and noncontaminated sites.
- E.g., some genes are present in noncontaminated site but not in contaminated sites

Global microarray applications

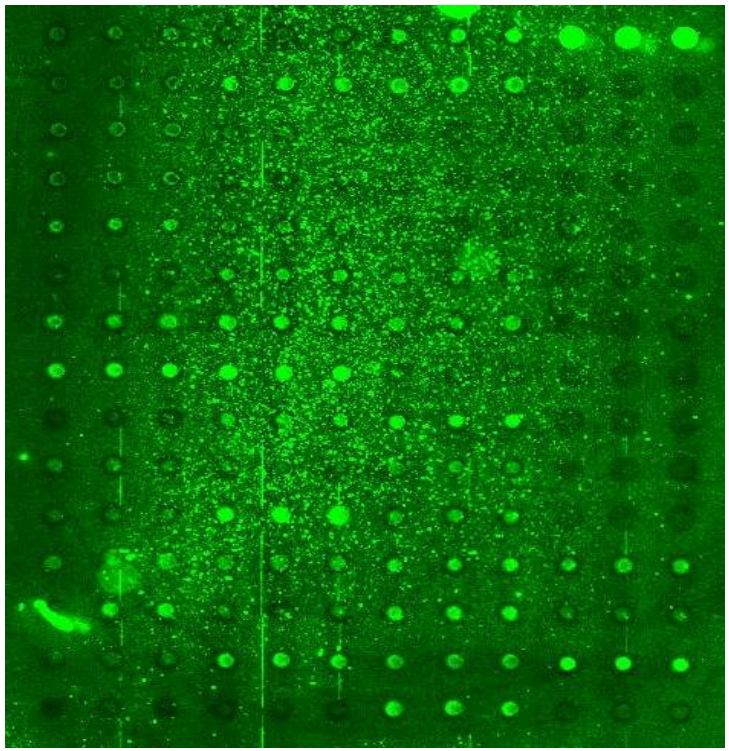
- Interrogate DNA from soils (fractions) of the Fermi chronosequence
  - identify where the key functional genes occur temporally and physically.
- Interrogate the RNA from different treatments from the same site
  - Identify which genes are regulated by the treatment
- Should be a useful tool for monitoring perturbations to the C cycle

Boutique arrays

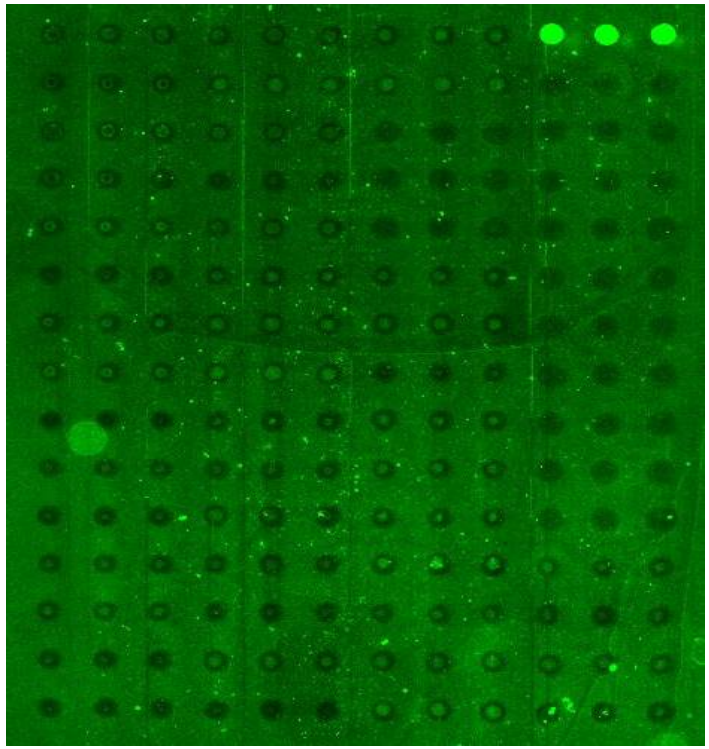
- **Attribute the enzyme activity to the functional microbial groups in the soil chronosequence using DNA microarrays**

- **Current research attributes almost all production of major lignin-degrading enzymes lignin peroxidase (*lip*), manganese peroxidase (*mnp*), laccase (*lac*), and glyoxal oxidase (*glox*) to fungi.**
  - **We are seeking to apply microarray technology to compare the lignin-degrading activities of soil fungal communities from different soils**

Lignocellulose boutique array



Tallgrass prairie soil
(more diverse)



Farmed soil
(less diverse)

Significance & Summary

- Fungi are strongly associated with C storage
  - May be a route to enhanced sequestration
- Community structure needs to be linked to function
  - We are also linking this to soil structure
- There are simple measurements that can be used to predict C storage
  - Why may these work?
- Resolving community structure vs. function requires new tools, integrated approaches
  - C and N cycling genes may offer insights to this black box
  - Still need process-based measurements

Persistent questions

- Do fungi cause, or are they an effect of C sequestration?
  - Which fungi? Which soils?
  - Experiment in progress
- Diversity, function, abundance, activity
  - Do these all affect C sequestration?
- Are certain soils or treatments more responsive; do they hold greater C sequestration potential?
  - Can we predict which soils?
- What C pools are most likely to show sequestration?