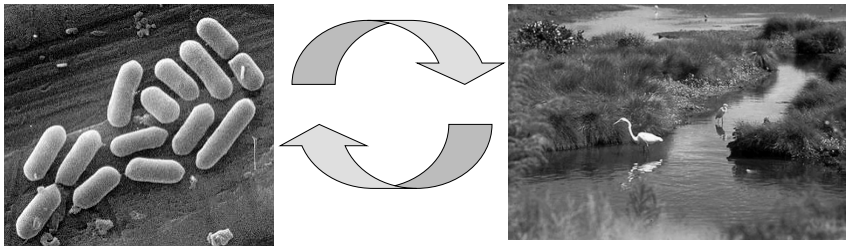
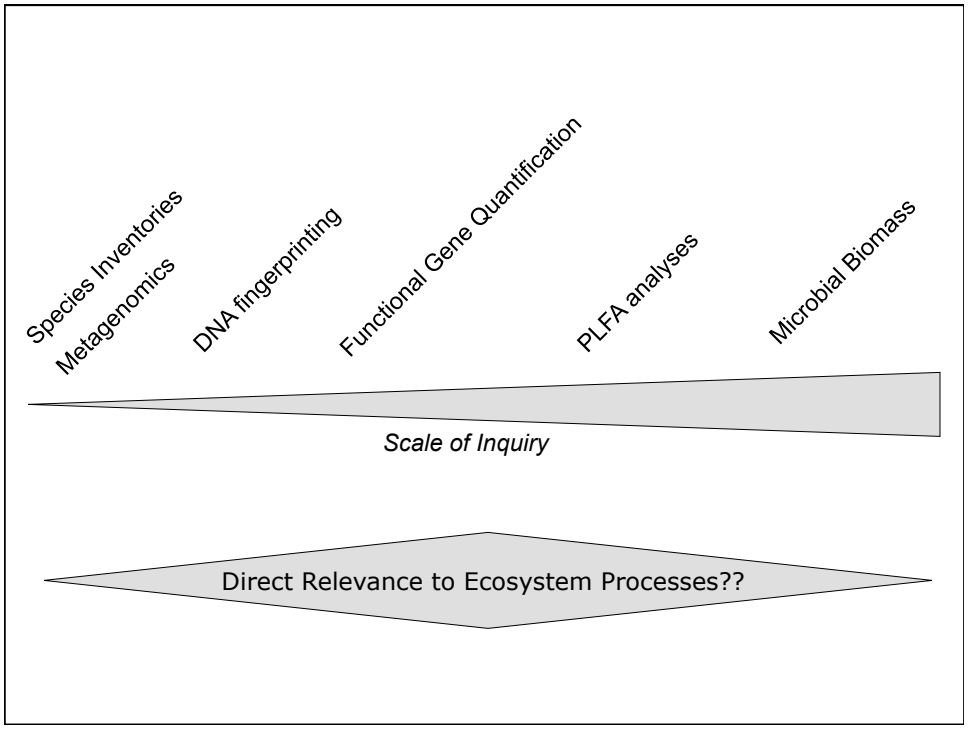
Strategies for Investigating Microbial Community Composition and Links to Ecosystem Function



Matthew Wallenstein

What can molecular techniques tell us?

- Who's there?
- Who's actively growing?
- What are they doing?
  - What substrates are they eating?
  - What genes are they expressing?
  - What proteins are they translating?



Microbiological and Biochemical Techniques

- Culture-based techniques
- Chloroform-fumigation biomass
- Ergosterol, Glomalin
- Phospholipid Fatty Acid analyses (PLFA)

Culture-based techniques

Advantages:

-Low cost

-Provides model organisms

-Fun!

Still relevant?

Disadvantages:

-Inefficient

-Biased sample, misses 99% of microbes***

Chloroform-fumigation based biomass estimates

Advantages

- Very commonly used index, relatively easy and comfortable for soil scientists

Disadvantages

- Cannot distinguish active from dormant cells
- Very low resolution

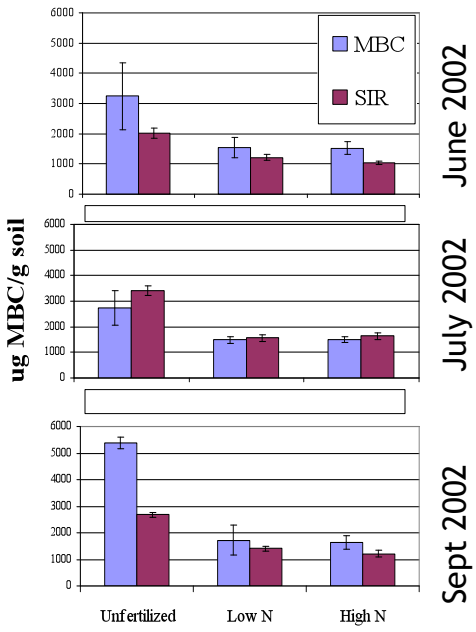
Substrate Induced Respiration

Advantages

- Relatively easy, cheap
- Consistent indicator
- Measures active biomass

Disadvantages

- Single, simple substrate
- Probably misses mycorrhizal fungi
- Doesn't measure dormant microbes



Mt. Ascutney (VT)

organic soil horizon

Egrosterol and Glomalin

Advantages

- Chemistry based technique
- Accurate
- Ergosterol produced by basidiomycetes, ascomycetes.
- Glomalin produced by AM fungi.

Disadvantages

- Different fungi produce different amounts
- Low resolution

PLFA

Phospholipid Fatty Acid Profiles

Advantages

Quantitative

Some Taxonomic Resolution

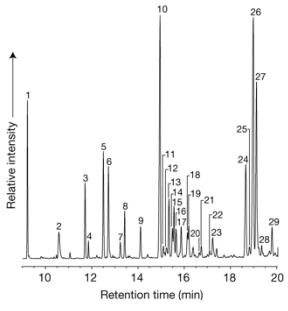
Can be coupled with 13-C assays to trace substrate use

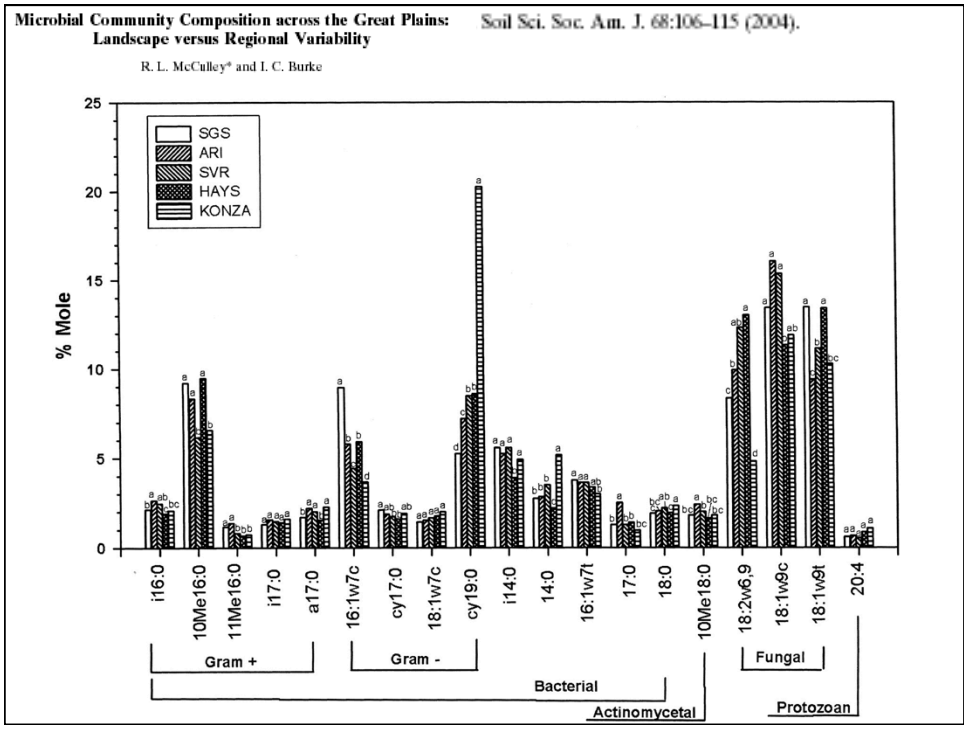
Disadvantages

Limited resolution

Dead-end

Labor-intensive





Physiological response profiles

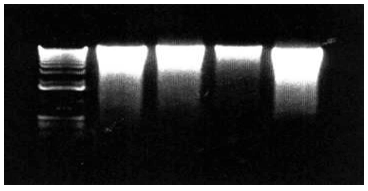
- BIOLOG physiological profiling
- In-situ physiological profiling
- MicroResp

Nucleic-acid based techniques

- DNA and RNA extraction
- Taxonomic versus Functional Gene targeting
- Polymerase Chain Reaction (PCR)
- Clone library construction (sequencing)
- Pyrosequencing
- Metagenomics
- Microarray hybridization
- Fingerprinting approaches
  - Terminal Restriction Fragment Length Polymorphism (TRFLP)
  - Denaturing Gradient Gel Electrophoresis (DGGE)

DNA and RNA Extraction

- Routine for most soils using commercially available kits (~$4/sample)
- RNA extraction is more difficult, but kits are now available.



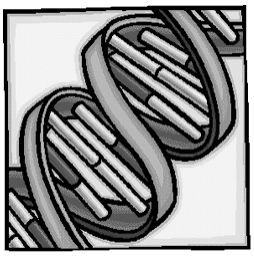
DNA

- Easy to extract, stable
- Genes may be present but not expressed

RNA

- Difficult to extract, unstable
- Closely linked to enzyme activities

What gene should I target?



- Functional genes code for enzymes involved in metabolic processes; useful when main question concerns process.
- rRNA genes can be used to infer taxonomic identities; useful for describing community structure.

Functional Genes

- May not be well conserved
- Difficult to design primers to target all genes
- Limited database

rRNA Genes

- Well conserved
- Good (and growing) database

Linking community structure to function using functional genes

Gene	Function
nifH	N fixation
amoA	ammonia oxidation (nitrification)
nirS, nirK	nitrite reduction
nosZ	nitrous oxide reduction

Linking community structure to function using functional genes

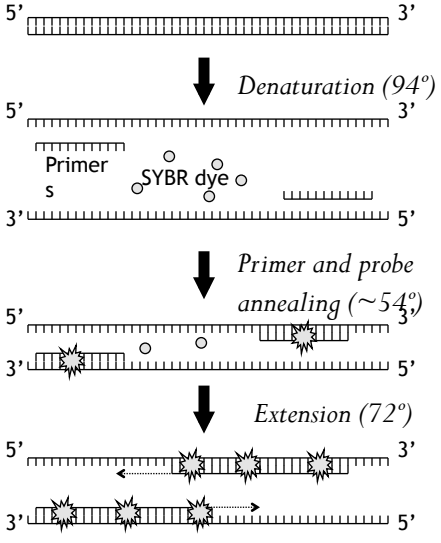
Nitrification

$$NH_4^+ \xrightarrow{AmoA} NO_2^- \rightarrow NO_3^-$$

Denitrification

$$NO_3^- \rightarrow NO_2^- \xrightarrow{Nir} NO \rightarrow N_2O \rightarrow N_2$$

Real-Time PCR amplification and sequence detection

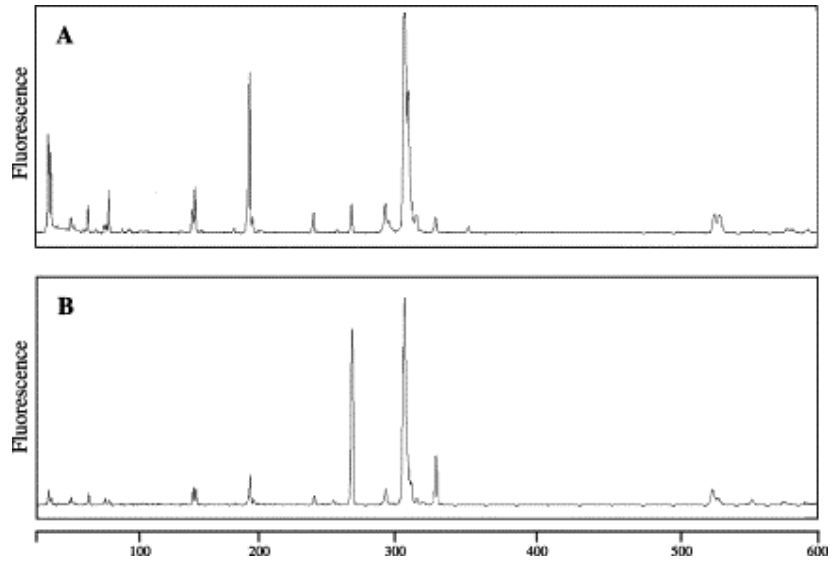


Double-stranded rDNA extracted from soil

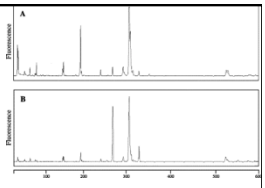
Primers designed to anneal to conserved target sequences

Polymerization by Taq polymerase, detection of SYBR fluorescence

TRFLP



TRFLP



Advantages

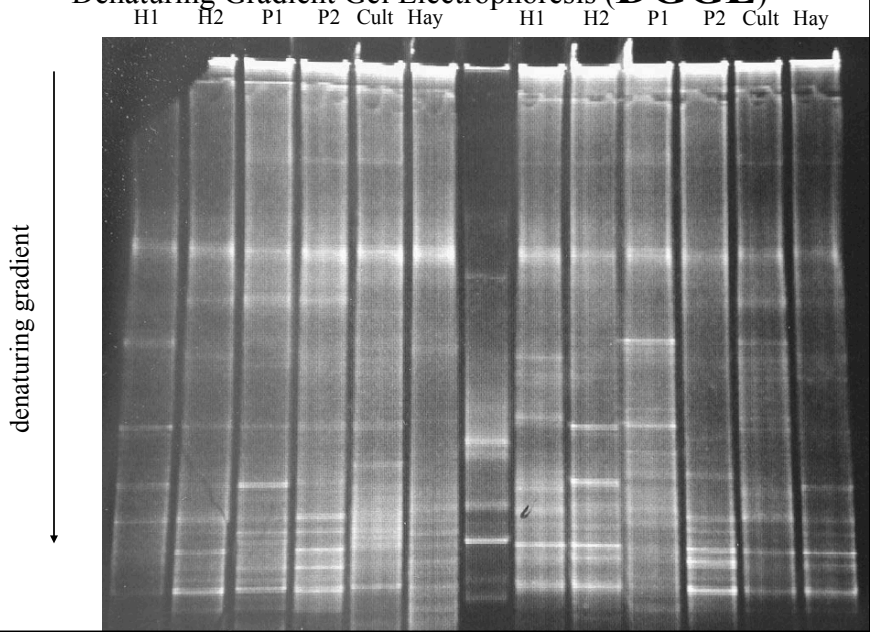
- Quantitative, precise
- <$1/ sample
- Can potentially be used to identify sequences with extensive database

Disadvantages

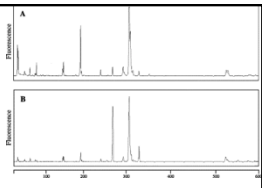
- Usually gives fingerprint only, no identification

Calhoun soils - 18S rDNA diversity

Denaturing Gradient Gel Electrophoresis (**DGGE**)



DGGE



Advantages

- Fairly high throughput
- Bands can be extracted and sequenced

Disadvantages

- Usually get smears
- Difficult to analyze images
- Non-quantitative

DNA Microarrays

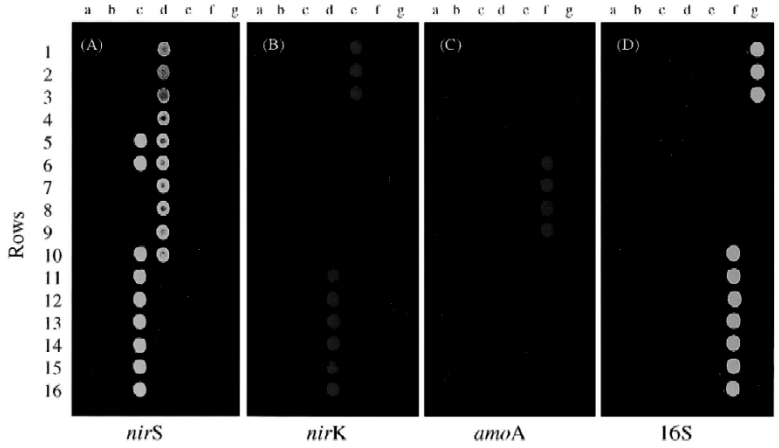
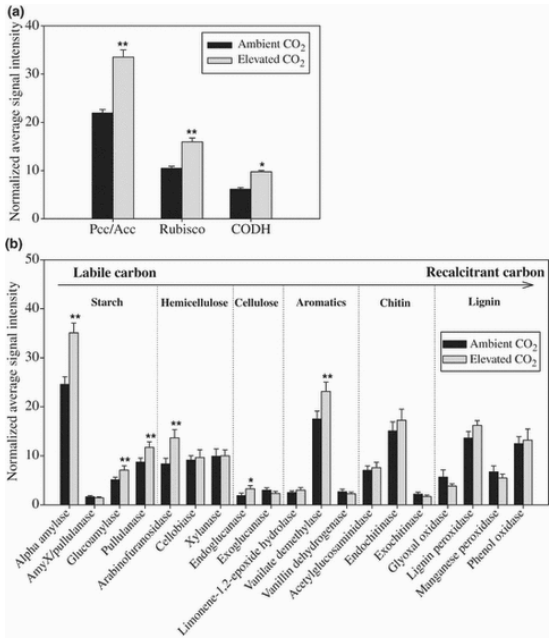
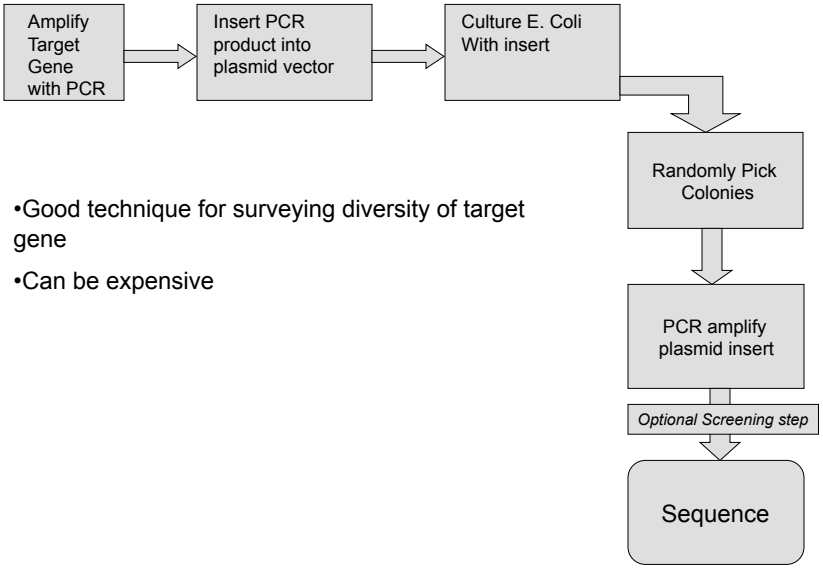


FIG. 1. Fluorescence images showing the specificity of *nirS*, *nirK*, *amoA*, and 16S rRNA target genes in DNA microarray hybridization. Target DNA was labeled with either Cy3 (green pseudocolor; *nirS* and 16S rRNA genes from pure cultures) or Cy5 (red pseudocolor; *nirK* and *amoA* genes from pure cultures) using the method of PCR amplification and hybridized separately at high stringency (65°C) to functional gene arrays containing *nirS*, *nirK*, and *amoA* gene probes from both pure bacterial cultures and environmental clones. 16S rRNA and yeast genes served as positive and negative controls, respectively. Shown are *nirS* (A), *nirK* (B), *amoA* (C), and 16S rDNA (D).



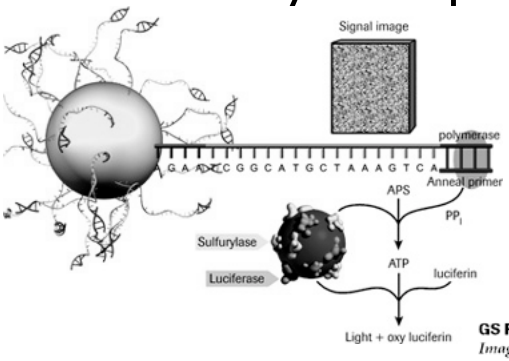
He et al, 2010, Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. Ecology Letters.

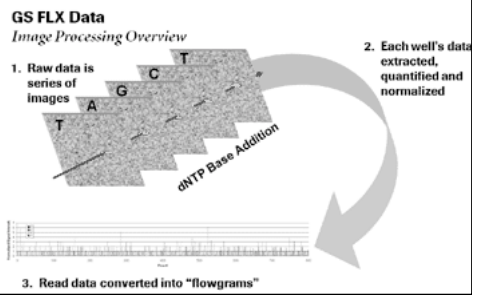
Clone Library Construction



- Good technique for surveying diversity of target gene
- Can be expensive

Pyrosequencing



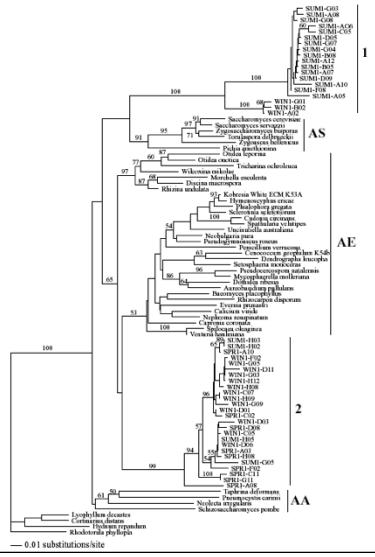


Sequence Identification/ Bioinformatics

- Alignment

```
                          **********
 1 gnl|UG|At#S163   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 2 gnl|UG|At#S202   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 3 gnl|UG|At#S116   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 4 gi|74036196|em   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 5 gnl|UG|At#S306   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 6 gnl|UG|At#S306   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 7 gi|89473615|gb   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
 8 reverse_primer   ---------GAGGAGTTCGTCGGAGAA---------
 9 ref|NC_003075.   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
10 gnl|UG|At#S117   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
11 gi|82397589|em   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
12 gnl|UG|At#S117   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
13 gi|52747721|em   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
14 gi|53794189|gb   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
15 gnl|UG|At#S261   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
16 gnl|UG|At#S311   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
17 gi|86611480|gb   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
18 gi|3907538|gb|   AATATACAGAGGAGTTCGTCGGAGAAACTCCGGT
   ruler            ....390.......400.......410.....
```

Sequence Identification/ Bioinformatics

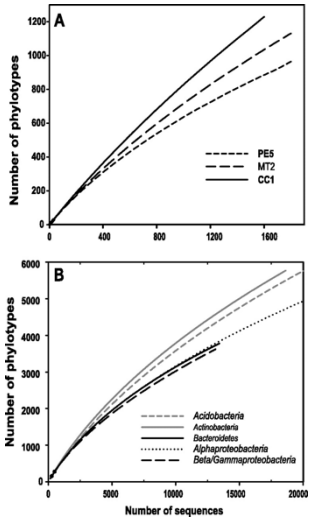


Seasonal Dynamics of Previously Unknown Fungal Lineages in Tundra Soils

Christopher W. Schadt,[1]* Andrew P. Martin,[1] David A. Lipson,[2] Steven K. Schmidt[1]†

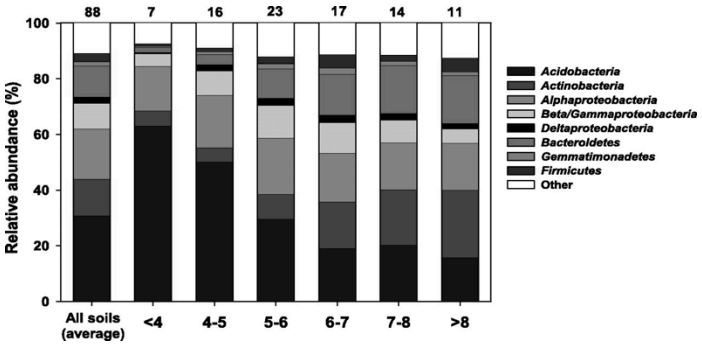
(2003). *Science* **301,** 1359-1361.

FIG. 1. (A) Rarefaction results for soils with low (PE5), average (MT2), and high (CC1) levels of diversity



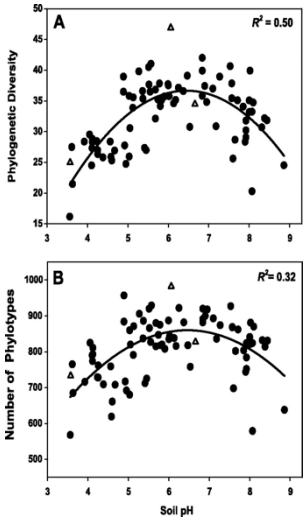
Lauber, C. L. et al. 2009. Appl. Environ. Microbiol. 75(15):5111-5120

FIG. 2. Relative abundances of dominant bacterial taxa in all soils combined and in soils with different pH levels



Lauber, C. L. et al. 2009. Appl. Environ. Microbiol. 75(15):5111-5120

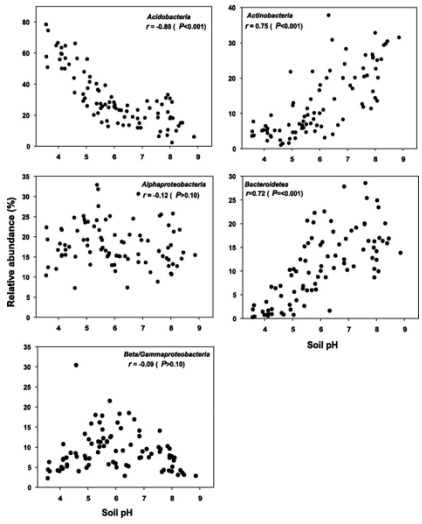
FIG. 3. Relationship between soil pH and soil bacterial diversity, measuring using Faith's PD (A) and the number of phylotypes (B), with phylotypes defined at the 97% sequence similarity level



Lauber, C. L. et al. 2009. Appl. Environ. Microbiol. 75(15):5111-5120

Applied and Environmental Microbiology

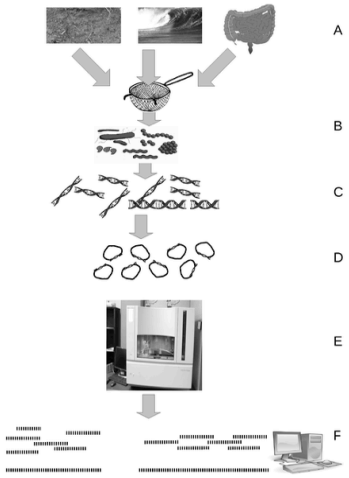
FIG. 5. Correlations between relative abundances of the five dominant bacterial phyla and soil pH



Lauber, C. L. et al. 2009. Appl. Environ. Microbiol. 75(15):5111-5120

Applied and Environmental Microbiology

Metagenomics



Wooley, J. C., A. Godzik, and I. Friedberg. 2010. A Primer on Metagenomics. PLoS Computational Biology **6.**

Table 2 Number of different gene variants retrieved in the metagenomic data sets for different functions in the active layer soil and the 2-m permafrost

Function	*Active*	*2 m*
Methane		
F420-dependent methylene-H_4 MPT reductase (EC 1.5.99.11)	7	1
Formylmethanofuran dehydrogenase (EC 1.2.99.5)	2	1
Formylmethanofuran-H_4 MPT *N*-formyltransferase (EC 2.3.1.101)	2	0
Methyl-H_4 MPT:CoM methyltransferase (EC 2.1.1.86)	2	0
Carbohydrates		
α-Amylase (EC 3.2.1.1)	35	14
α-L-fucosidase (EC 3.2.1.51)	23	13
α-*N*-acetylglucosaminidase (EC 3.2.1.50)	6	1
β-Galactosidase (EC 3.2.1.23)	67	35
β-Glucosidase (EC 3.2.1.21)	30	21
Chitinase (EC 3.2.1.14)	34	8
Trehalase (EC 3.2.1.28)	18	2
Nitrogen		
Ammonia monooxygenase	2	0
Copper-containing nitrite reductase (EC 1.7.2.1)	8	1
Cytochrome *c* nitrite reductase (EC 1.7.2.1)	1	0
Nitric-oxide reductase (EC 1.7.99.7)	9	5
Nitrite reductase (NAD(P)H) (EC 1.7.1.4)	19	6
Nitrogenase (EC 1.18.6.1)	1	1
Nitrous-oxide reductase (EC 1.7.99.6)	7	2
Respiratory nitrate reductase (EC 1.7.99.4)	13	12

Abbreviation: MPT, methanopterin.

Yergeau, E., H. Hogues, L. G. Whyte, and C. W. Greer. 2010. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J.

Linking Microbial Community Structure to Ecosystem Processes

Linking Microbial Community Structure to Ecosystem Processes

Stable Isotope Probing

Can be combined with PLFA or DNA techniques

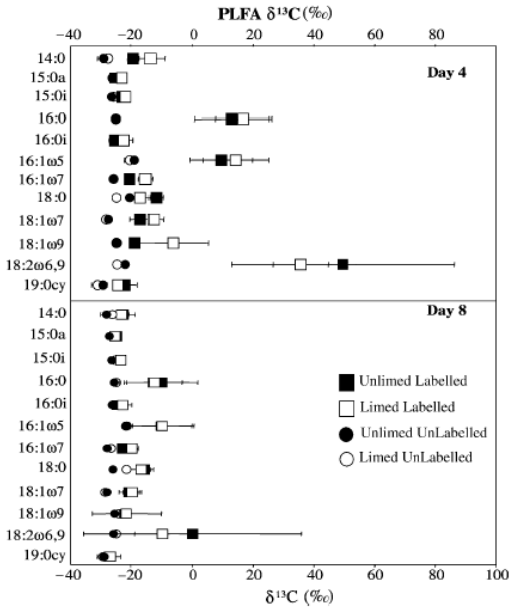


Fig. 2. PLFA $\delta^{13}C$ values. a, 4 d after pulse-labelling. b, 8 d after pulse-labelling. Error bars show one standard error ($n = 3$). ■, unlimed, labelled; ■, lime, labelled; ●, unlimed, unlabelled; ▲, lime, unlabelled.

Soil Biology & Biochemistry 36 (2004) 533–537

Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs

Amy M. Treonis[a,*], Nick J. Ostle[b], Andrew W. Stott[b], Ruth Primrose[a], Sue J. Grayston[a], Philip Ineson[c]

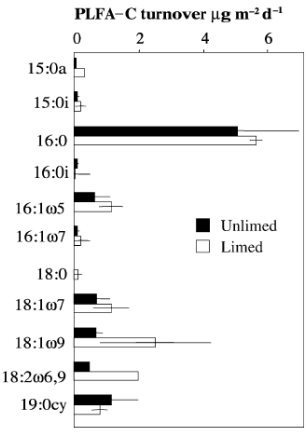


Fig. 3. Turnover estimates of PLFA-C in limed and unlimed grassland soils. (An estimate is not shown for 14:0 due to missing values.) Error bars show one standard error ($n = 3$).

RNA-SIP

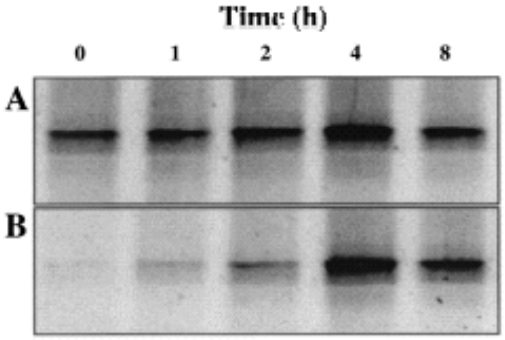
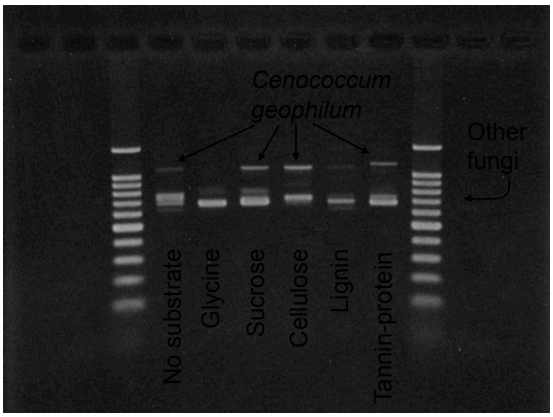


FIG. 4. Increasing buoyant density of the dominant band in 16S rRNA community profiles throughout the duration of the phenol-$^{13}C_6$ pulse. (A) The intensity of the dominant DGGE band remains constant in fraction 10 over time. (B) The intensity of the dominant DGGE band increases in fraction 6 over time, confirming an increase in the buoyant density of RNA from this species as phenol-$^{13}C_6$ is consumed.

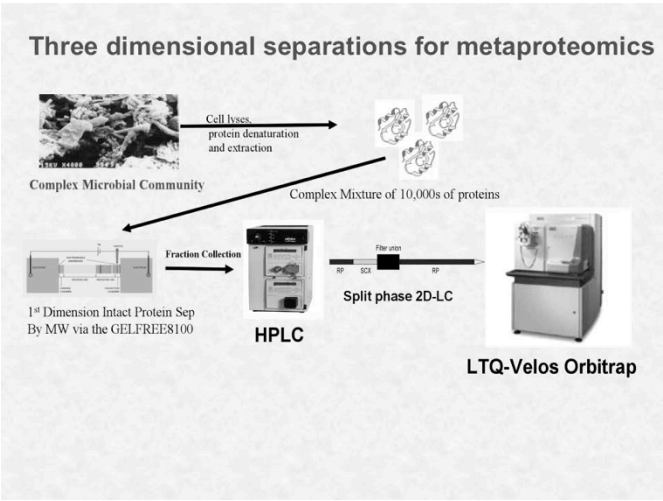
BrdU labeling



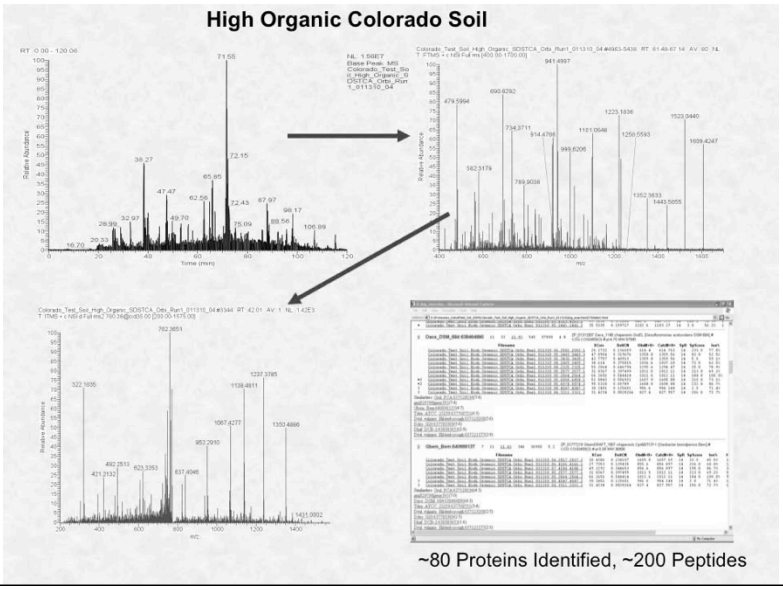
- **Glycine**
  - *Mortierella cf. hyalina*
  - Zygomycete sp. GFI 1
- **Sucrose**
  - *Amanita pantherina*
- **Cellulose**
  - *Entoloma prunuloides*
- **Lignin**
  - *Reniforma strues*
  - *Lecythophora* sp. Olrim22
  - *Lecythophora* sp. UBCtra1453C
  - Zygomycete sp. Olrim331
- **Tannin-protein**
  - Zygomycete sp. Olrim456

Hanson, C. A., S. D. Allison, M. A. Bradford, M. D. Wallenstein, and K. K. Treseder. 2008. Fungal Taxa Target Different Carbon Sources in Forest Soil. Ecosystems **11:1157-1167.**

Metaproteomics



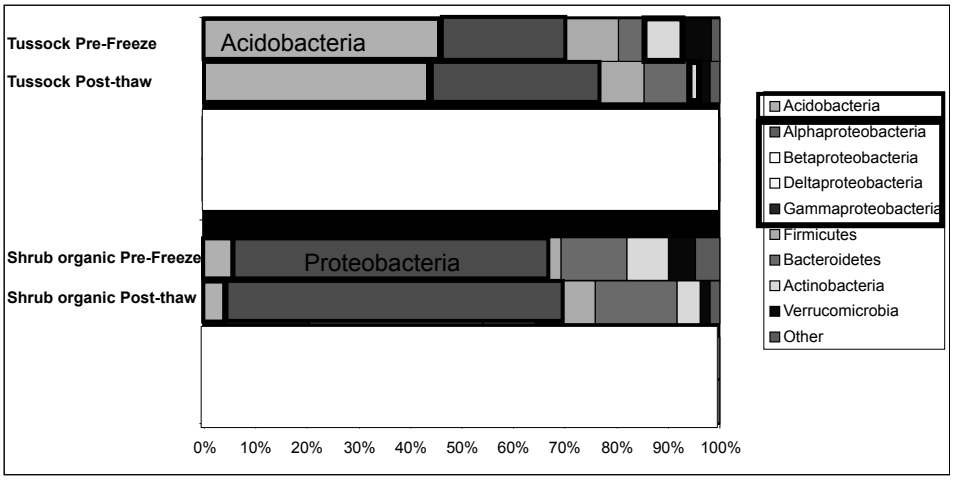
Metaproteomics



Conclusions

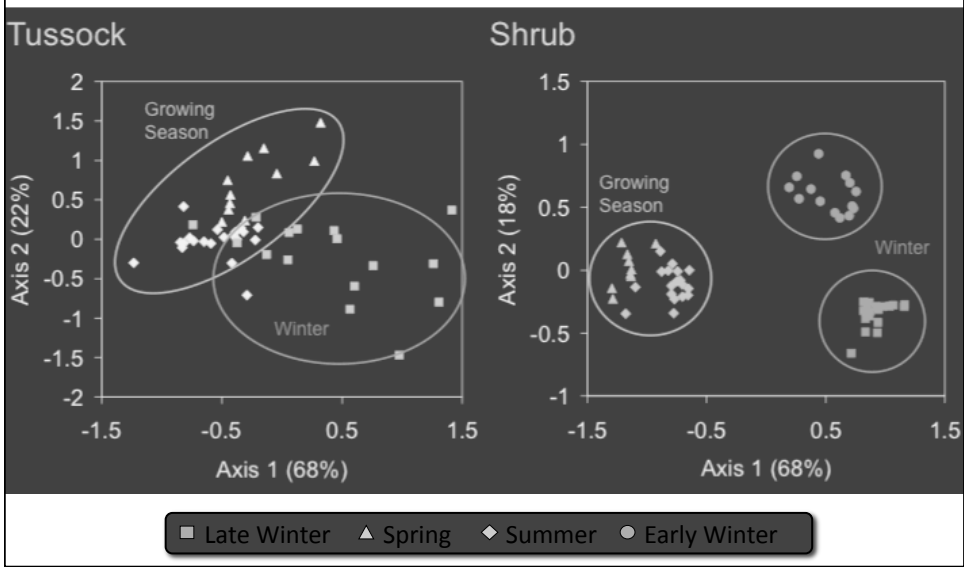
- It is critical to formulate a focused question before choosing the appropriate technique.
- Different techniques provide different levels of resolution
- Soil microbes do not live in isolation-it is a complex food web.

Bacterial community composition

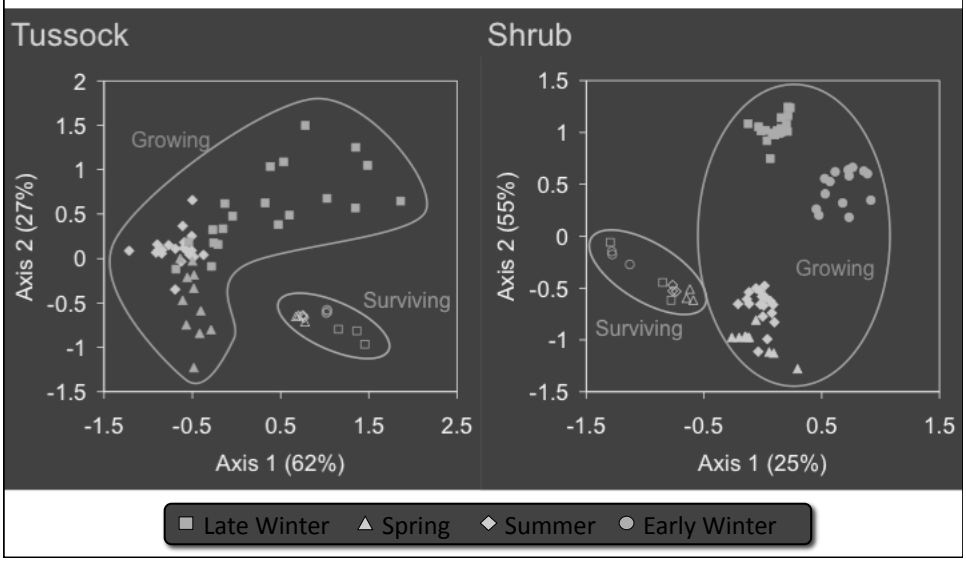


Wallenstein et al 2007 FEMS Micro Ecol

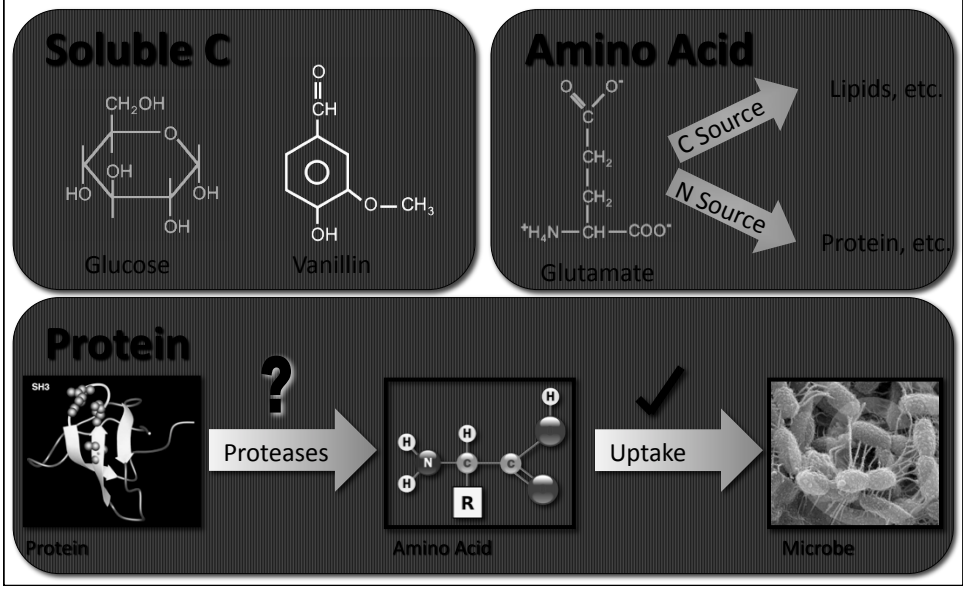
Winter & Growing Season Active Bacterial Communities Are Different



Active Communities are Different from Total Communities



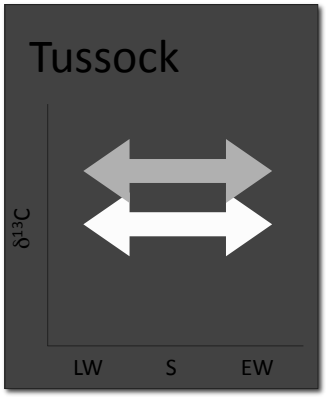
Three Metabolic Perspectives



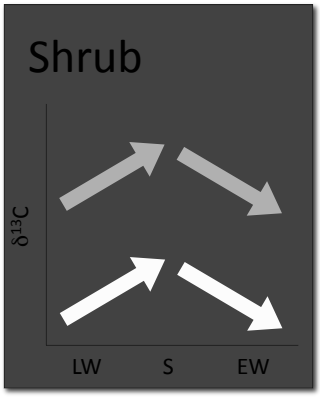
Applications of molecular techniques:

Arctic tundra soil microbial ecology

Soluble C Metabolism



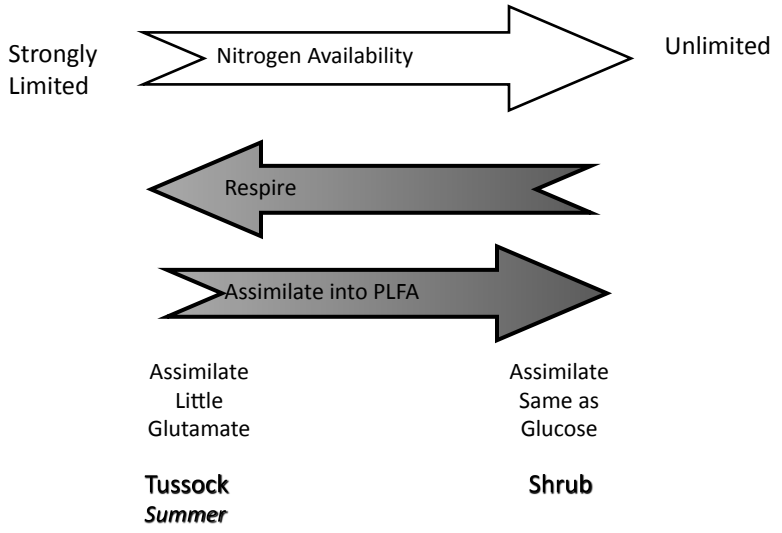
Bacteria: year-round
Fungi: more in winter
Glucose ≈ Vanillin



Bacteria: mostly summer
Fungi: more in winter
Hardly used vanillin

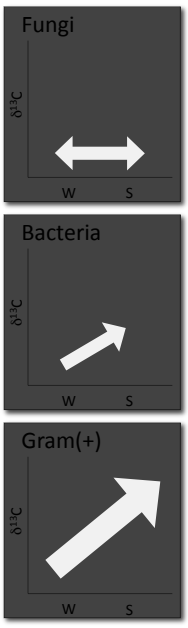
Glucose Vanillin

Glutamate Metabolism Relative to N Availability

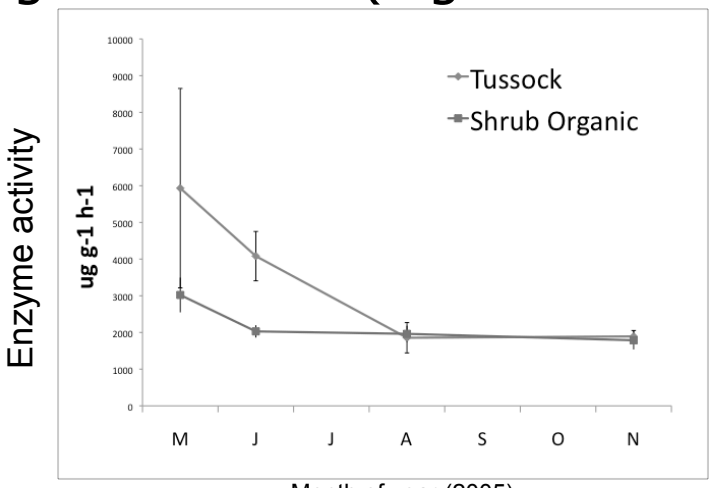


Protein Metabolism

- Fungi didn't use protein regardless of season
- Little to no protein incorporation in winter by most bacteria but significant summer bacterial use
- Specialist group of proteolytic Gram (+) bacteria



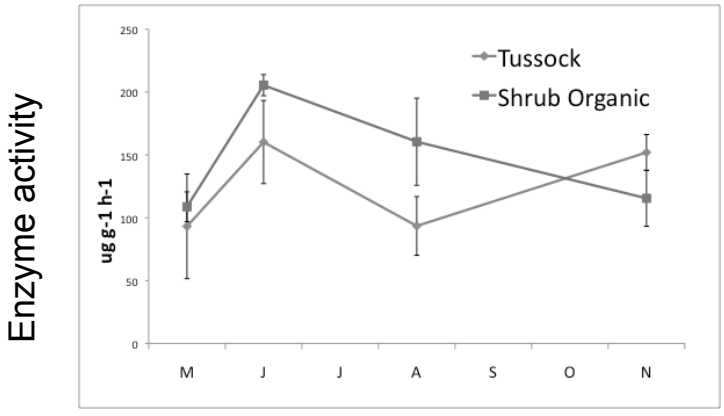
B-glucosidase (Lignocellulose)



Lignocellulose degrading enzymes peak in late winter.

Wallenstein, McMahon, and Schimel. 2009, Global Change Biology.

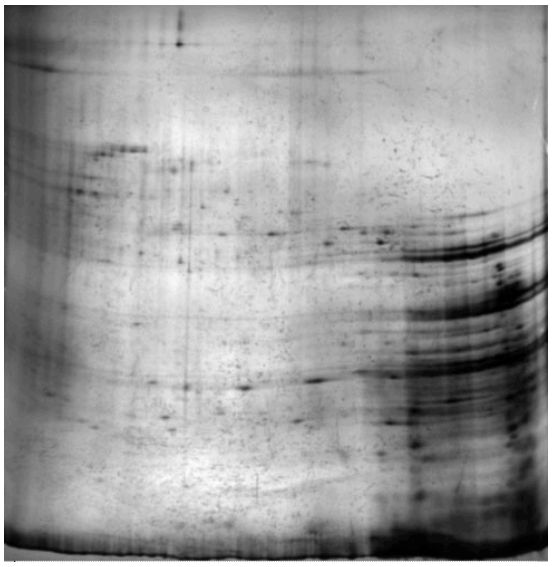
Protease



High protein availability following spring thaw?

Wallenstein, McMahon, and Schimel. 2009, Global Change Biology.

Environmental Proteomics



Winter

Fungi and Gram (-) bacteria
Degrading Lignocellulose

Summer

Diverse microbial community
Degrading chitin, protein, hemi-cellulose