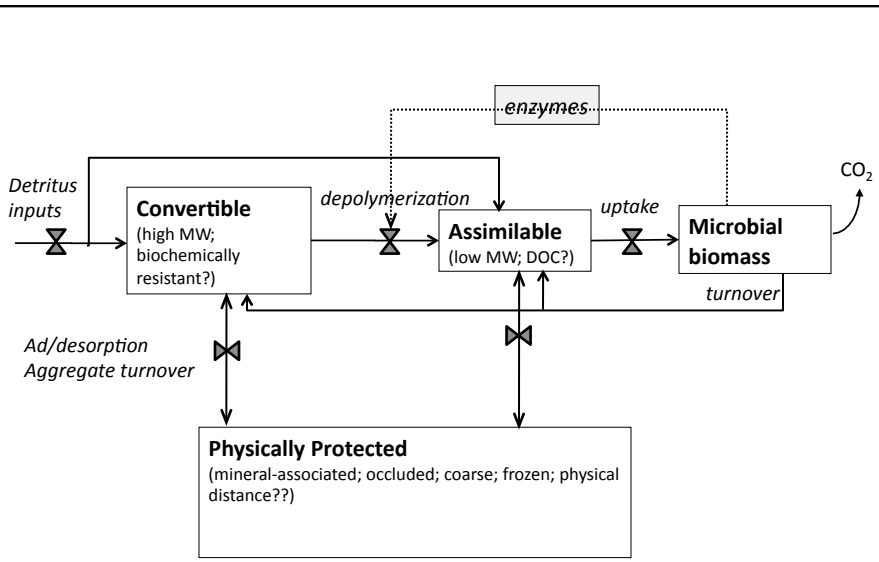
DECOMPOSITION AND ENZYMES



What gets decomposed?

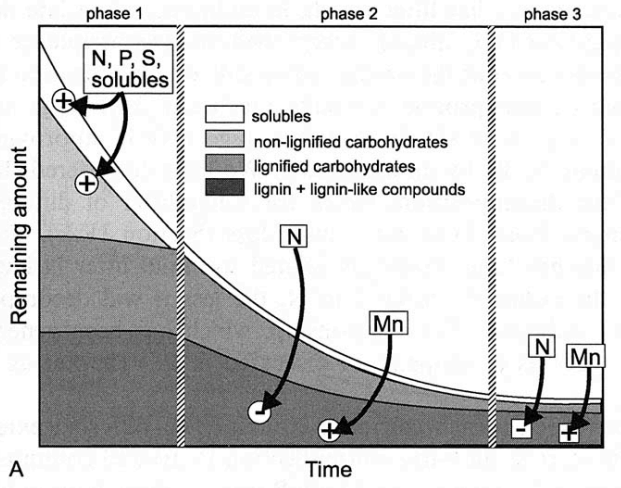
What is the difference between different types of detritus?

Where does decomposition happen?

How does decomposition happen?

1. Leaching of soluble organic matter
2. Physical shredding
3. Enzymatic breakdown

The classical litter decomposition pathway



Berg and Laskowski, 2006

Leaching

- Moves water-soluble compounds away from decomposing material
- Begins while leaves are still on plant
- Most important early in decomposition

Fragmentation

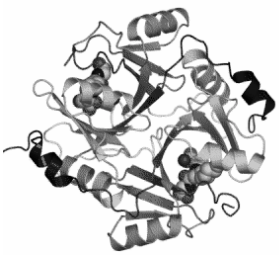
- Fresh litter is protected from microbial attack
  - Bark, epidermis or skin on exterior
  - Plant cells protected by lignin in cell walls
- Carried out mainly by soil animals
- Increases surface area for microbial attack
- Important in aquatic and terrestrial ecosystems

Chemical alteration

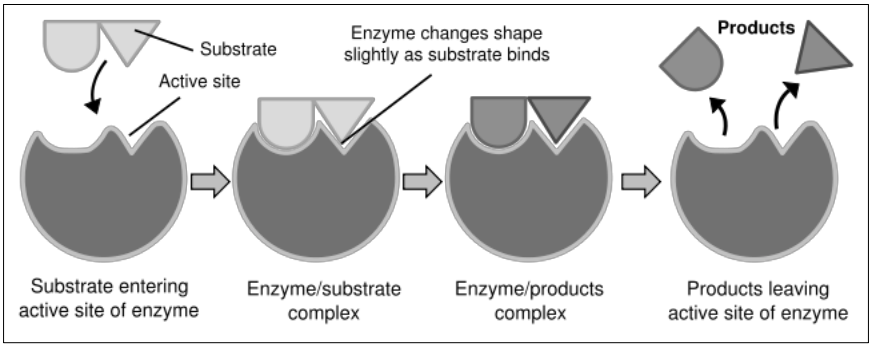
- Most organic compounds are polymers and cannot be directly assimilated (eaten) by microbes
- Microbes produce extracellular enzymes to depolymerize these compounds so they can assimilate them
  - Enzymes are proteins, which contain C and N
    - Thus, the production of enzymes is constrained by N availability
    - Proteins are also 'food' for microbes, and are broken down by protease enzymes

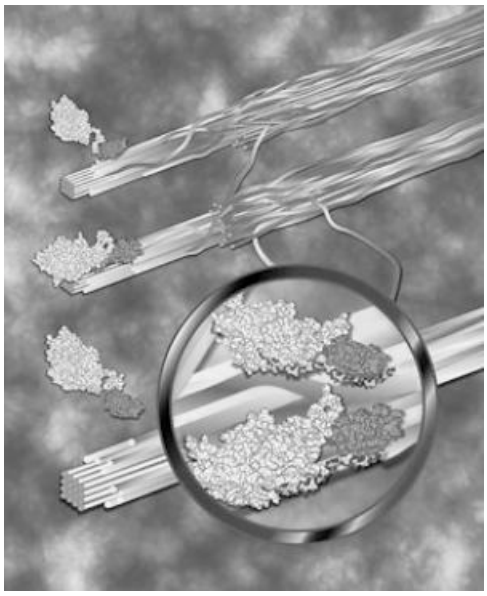
Types of enzymes

- Oxidative
  - Catalyze reduction-oxidation reactions
    - Phenol oxidase, Peroxidase
      - Primarily degrade lignin
      - Non-specific
- Hydrolytic
  - Specialized for each substrate
    - Cellulase degrades cellulose
    - Chitinase degrades chitin
    - Glucosidase degrades starch



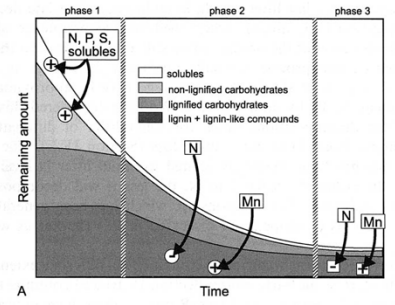
Enzyme Activity





"Who" are the decomposers?

"Who" are the decomposers?



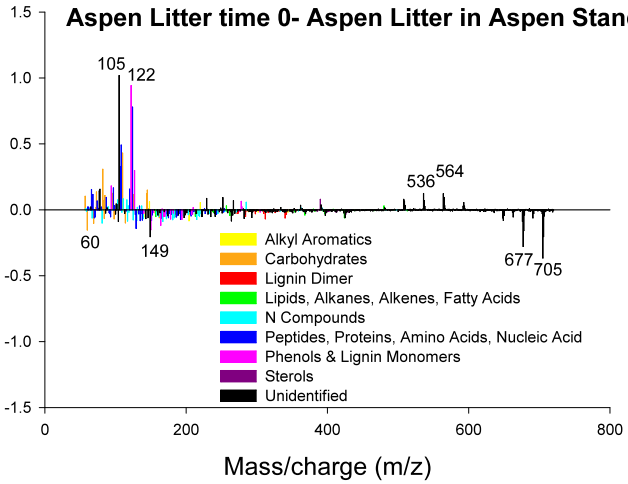
Berg and Laskowski, 2006

Phase 1 (leaching): fast growing microbes that eat solubles- already present on fallen litter.

Phase 2: medium fast growers that degrade starches and sugars.

Phase 3: slow growers that degrade lignin. Includes fungi whose hyphae extend into the soil.

Chemical changes of litter during decomposition

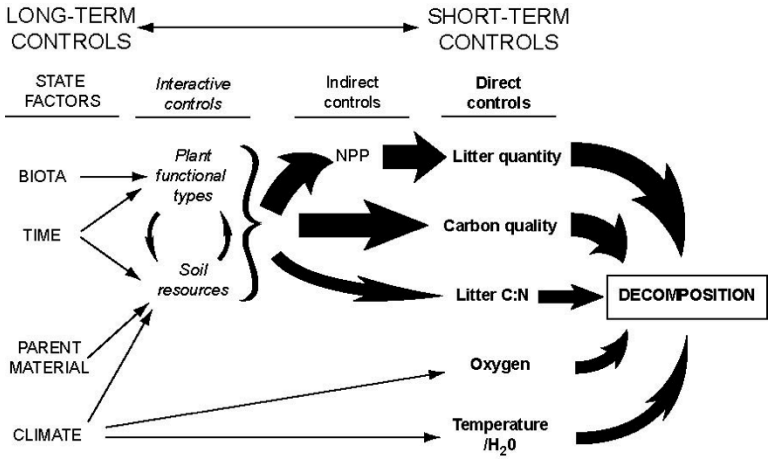


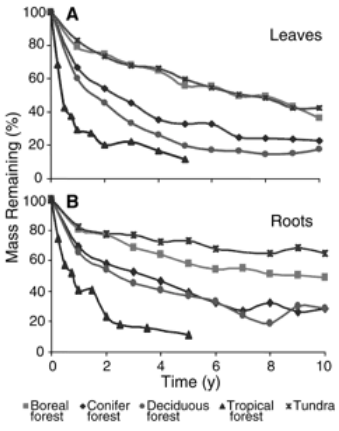
Why do they decompose?

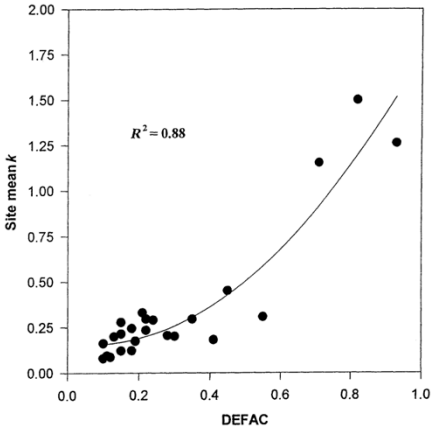
What controls decomposition rates?

Controls over decomposition range from long-to-short term
- Long-term: State factors
- Intermediate: Interactive controls
- Short-term: Indirect and direct physiological controls

Direct controls: Environment and substrate quality







Gholz et al 1996, Parton et al 2007

Direct temperature effects

- 1. Effects on microbial activity
  - Growth rates
  - Enzyme activity rates
  - Assimilation rates
- 2. Effect of temperature fluctuations
  - e.g., freeze-thaw
  - Kills microbes
  - Spill their guts into the soil, where they become substrate for other decomposers

Indirect temperature effects

- Effects on evaporation and soil moisture
- Effects on quantity and quality of litter inputs

Moisture effects

- Decomposition has similar shape of moisture response as does NPP
  - Declines at extremely low and high moisture
- Less sensitive to low moisture than is NPP (no litter accumulation in deserts)
- More sensitive to high moisture than is NPP (SOM accumulation in waterlogged soils)

Other environmental effects

- pH
  - bacteria predominate at high pH
  - Low growth efficiency promotes breakdown
- Soil texture
  - Protection of SOM by clays
  - Aggregate structure (anaerobic microsites)
- UV-B
  - Direct photodegradation
  - Important in arid ecosystems (especially grasslands)

Substrate quality

- Susceptibility to decomposition
- May be the predominant control over decomposition rates
  - Climate exerts large effect on substrate quality through effects on vegetation

Substrate quality depends on:

- 1. Size of molecule
- 2. Types of chemical bonds
- 3. Regularity of structure
- 4. Toxicity
- 5. Nutrient concentrations

Substrate quality depends on:

- 1. Size of molecule
  - Large molecules must be broken down outside of cells
  - Limits metabolic control that microbes can exert over breakdown process
  - Requires production of exoenzymes

Substrate quality depends on:

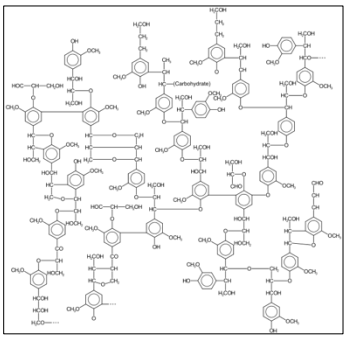
- 2. Types of chemical bonds
  - Some bonds are easier to break than others
    - e.g., peptide bonds compared to aromatic rings
    - Most of litter nitrogen (80%?) is in protein
    - Most N in old SOM is in aromatic rings
    - High N concentration in these two types of SOM means very different things to microbes

Substrate quality depends on:

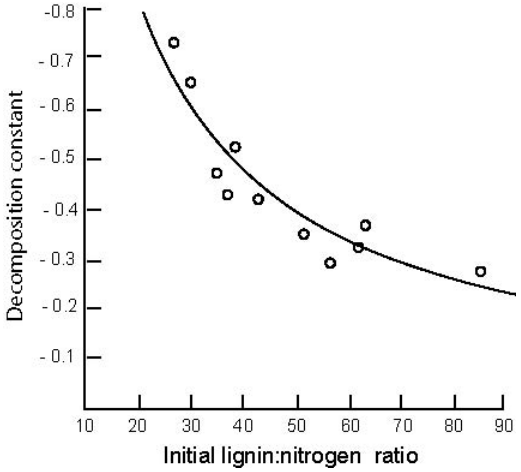
- 3. Regularity of structure
  - Lignin and humus have irregular structure
- 4. Toxicity
  - Phenolics evolved to protect plants from herbivores and pathogens
  - Also affect decomposers;
  - Importance of this effect is uncertain
- 5. Nutrient concentrations
  - Nutrients are essential to support microbial growth

Predictors of decomposition

- C:N ratio
  - Index of ratio of cytoplasm to cell walls
  - Measure of nitrogen concentration
  - Directly affects decomposition ONLY in presence of labile C
- Lignin:N ratio
  - Integrated measure of N concentration and substrate size/complexity

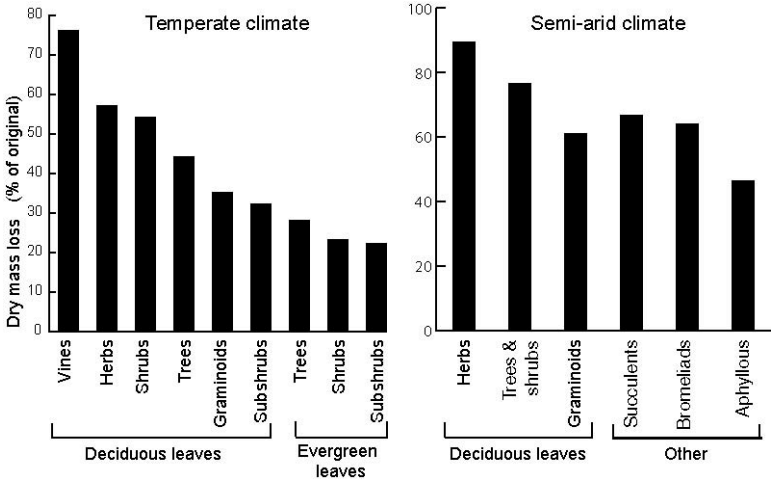


A possible lignin structure



Plant species differ predictably in litter quality

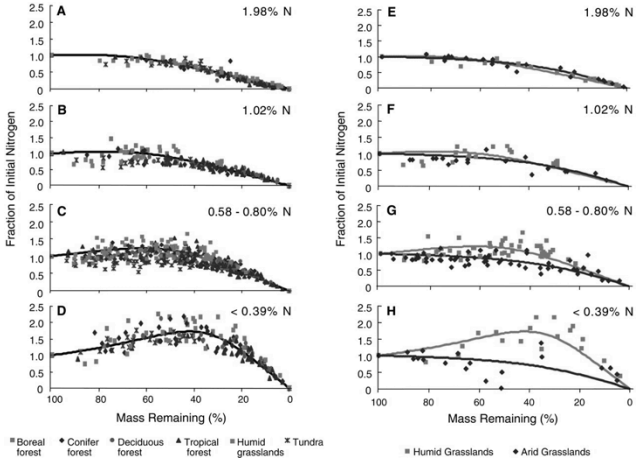
High-resource-adapted leaves decompose quickly due to higher concentrations of labile C



Substrate quality of SOM

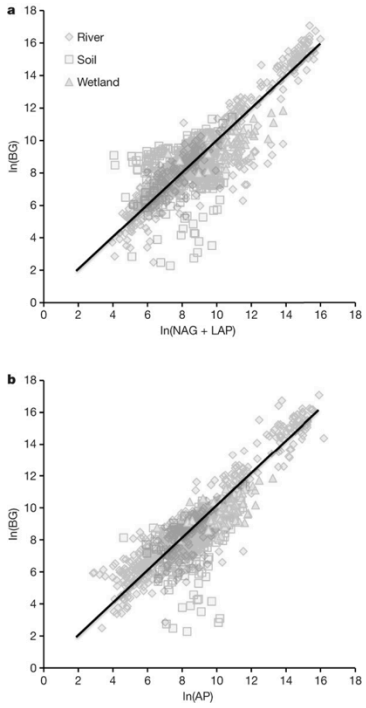
- Much of SOM is old and recalcitrant
- Consists of "leftovers" and microbial products
- Binds to clay minerals
- Bulk soil is a "nutritional desert"

Stoichiometry of litter decomposition



Parton, W., W. L. Silver, I. C. Burke, L. Grassens, M. E. Harmon, W. S. Currie, J. Y. King, E. C. Adair, L. A. Brandt, S. C. Hart, and B. Fasth. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science **315:361-364.**

Organic nitrogen (N) acquisition activity and organic phosphorus (P) acquisition activity in relation to carbon (C) acquisition.



RL Sinsabaugh *et al. Nature* **462**, 795-798 (2009) doi:10.1038/nature08632

What is the ultimate fate of decomposed detritus?

Abiotic decomposition: the effect of solar radiation

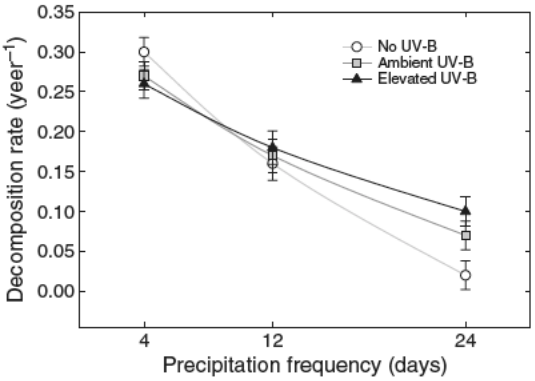


Fig. 4 Constants of decomposition or decay rates (years^{-1}) observed within the control soil treatment during the litter decomposition experiment. Decomposition rates were calculated from parameter estimates made by the interaction model. Error bars, ± SEM.

Smith, W. K., W. Gao, H. Steltzer, M. D. Wallenstein, and R. Tree. 2010. Moisture availability influences the effect of ultraviolet-B radiation on leaf litter decomposition. Global Change Biology **16:484-495.**

Abiotic decomposition: the effect of solar radiation

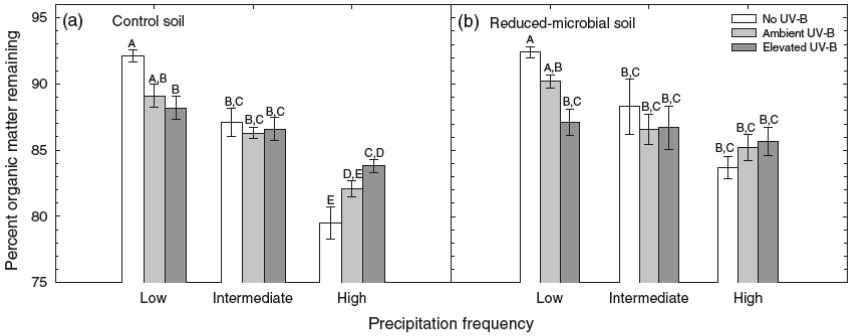
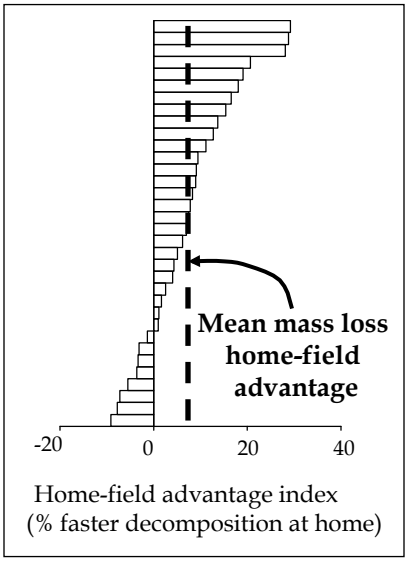
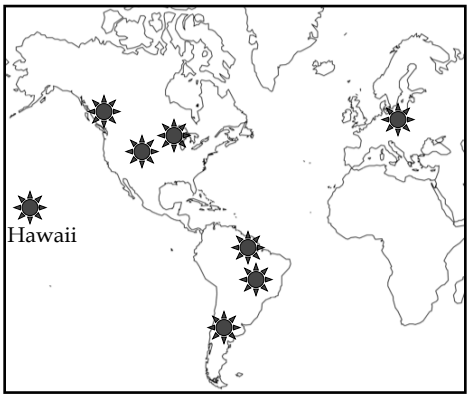


Fig. 5 (a) Percent organic matter remaining observed at the final collection period ($t = 0.53$ years) for the control soil treatment. Letters indicate significant pair-wise differences (Tukey's HSD; $F = 22.5$, $P < 0.0001$). Error bars, ± SEM. (b) Percent organic matter remaining observed at the final collection period ($t = 0.53$ years) for the reduced-microbial soil treatment. Letters indicate significant pair-wise differences (Tukey's HSD; $F = 6.14$, $P = 0.0002$). Error bars, ± SEM.

Smith, W. K., W. Gao, H. Steltzer, M. D. Wallenstein, and R. Tree. 2010. Moisture availability influences the effect of ultraviolet-B radiation on leaf litter decomposition. Global Change Biology **16:484-495.**

Litter decomposes faster in it's 'home-field'.





Litter decomposes 8% faster at home than away

***p* < 0.01**

Metabolomes differed with microbial community structure

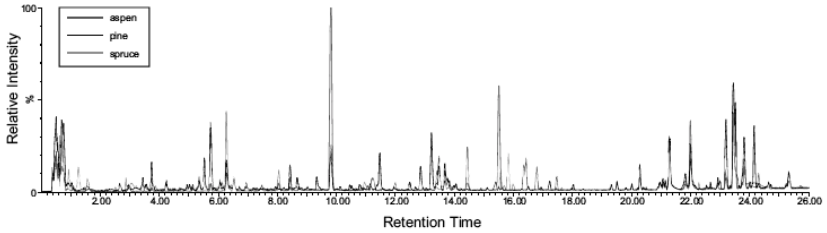


Fig. 2. Representative UPLC chromatograms of metabolites from aspen leaves decomposed under adjacent aspen, pine, and spruce stands.

Wallenstein, M. D., A. M. Hess, M. R. Lewis, H. Steltzer, and E. Ayres. 2010. Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. Soil Biology and Biochemistry **42:484-490.**

Metabolomics of aspen litter

Aspen litter decomposed in stands of aspen, pine, or spruce

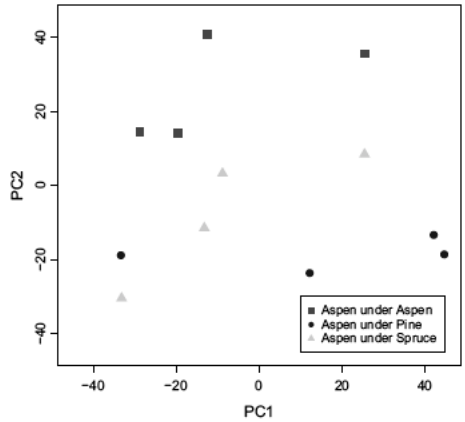


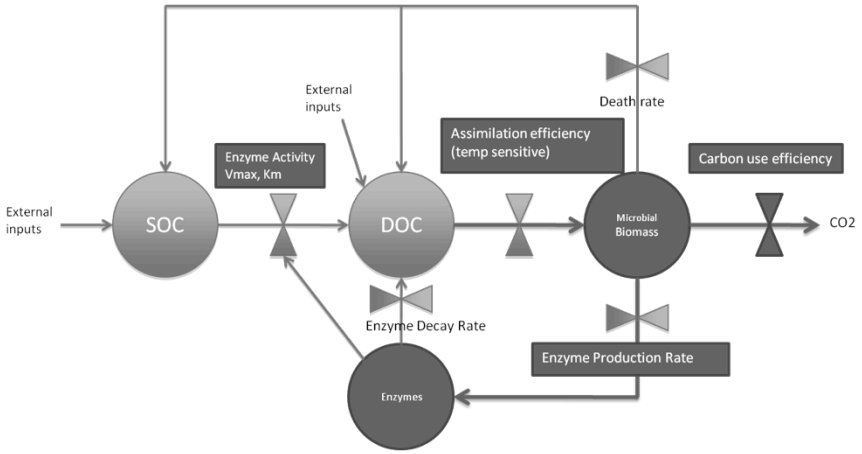
Fig. 4. A 2-D plot of the first three principal components of *P. tremuloides* leaf litter metabolomes that were decomposed in aspen, pine, and spruce stands. The amounts of variation explained by PC1 is 31.6% and by PC2 is 20.5%.

Wallenstein, M. D., A. M. Hess, M. R. Lewis, H. Steltzer, and E. Ayres. 2010. Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. Soil Biology and Biochemistry **42:484-490.**

Key Concepts

- The chemical composition of litter affects decomposition rates
- Moisture and Temperature affect microbial activity, and thus decomposition rates
- The activity of enzymes chemically degrades detritus
- Decomposer communities are complex and diverse. Both fungi and bacteria are involved.

Enzymes are the proximate drivers of decomposition

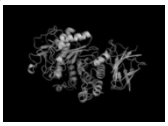


Allison, S., M. Wallenstein, M. Bradford, 2010. Nature Geosci.

Complex substrates - Ligno-cellulose

(i) the easy bit - cellulose to glucose

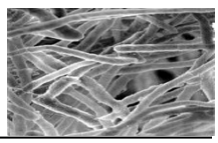
- **1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4)**
- **1,4-β-D-glucan cellobiohydrolases (EC 3.2.1.91)**
- **1,4-β-D-glucosidases (EC 3.2.1.21)**



But there are dozens of enzymes and isoenzymes in each group based on: reaction kinetics, substrate specificity, pH and temperature optima, molecular mass, stability, active site topology, catalytic domains, etc.

Why? the substrate and the microbial microenvironment is ever changing…

The classic cellulose degrader, *Trichoderma reesei* has >30 cellulases and a secretome of >100 proteins



Ligno-cellulose the impossible substrate (ii) the difficult bit - lignins to quinones, phenols, aldehydes etc

- **manganese peroxidases (EC 1.11.1.13)**
- **lignin peroxidases (EC 1.11.1.14)**
- **laccases (EC 1.10.3.2)**
- **(and lots of other 1.10.3s and 1.11.1s)**
- **cellobiose dehydrogenases (EC 1.1.99.18)**
- **pyranose-2-oxidases (EC 1.1.3.10)**
- **glyoxal oxidases (EC 1.1.3. -)**
- **superoxide dismutases (EC 1.15.1.1)**
- **aryl alcohol oxidases (EC 1.1.3.7)**

ad infinitum

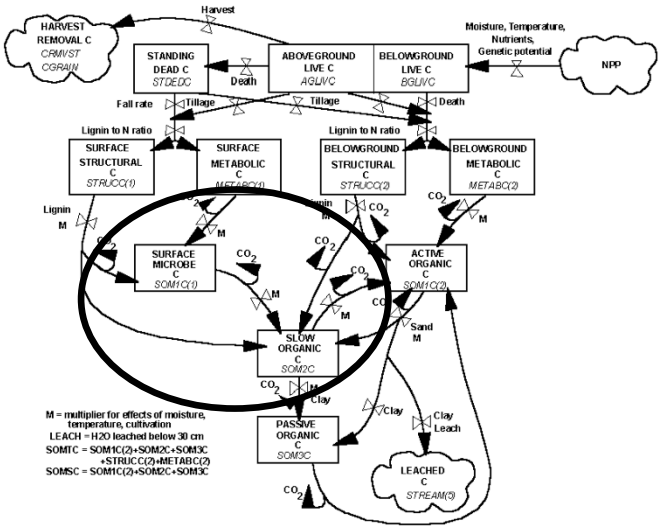
Coprinopsis cinerea **has a secretome of 1769 proteins of which at least 100 are enzymes**

Phanerochaete chrysosporium a white rot fungus that may have solved the problems

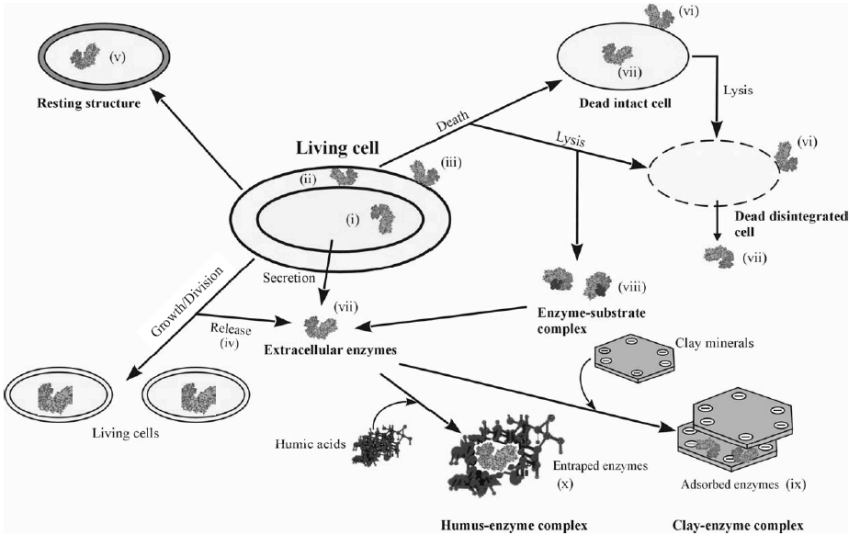
P. chrysosporium **degrades cellulose, hemicellulose and lignin and has 87+ genes for extracellular glycosyl hydrolases, 103+ for 'ligninases' and a total predicted secretome of 790 proteins!**

P c **also has bioremediation potential: PAHs, PCBs, PCPs, organo-chlorine and phenoxyacid pesticides, phthalates, dioxins, TNT and so on**

Enzymes in current terrestrial ecosystem models



Where are microbial enzymes located in soil?

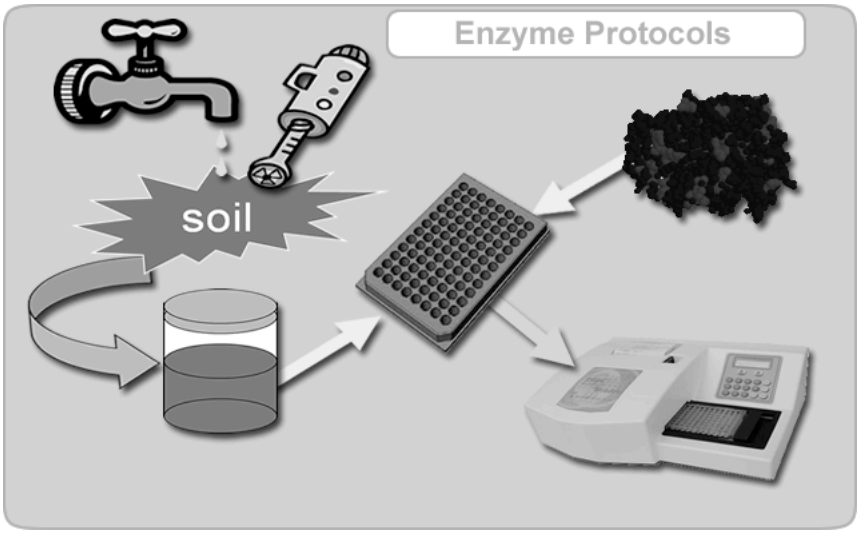


Wallenstein MD & Burns RG (2010)

Fundamental questions in soil enzymology

- What factors determine the production of enzymes by plants and microbes?
- What is the turnover time of enzymes after they are released?
- How much activity is maintained by stabilized enzymes?
- 'Who' produces different types of enzymes?

Enzyme Assays



How do we interpret patterns in enzyme activities?

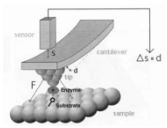
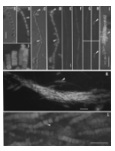
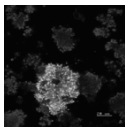
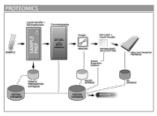
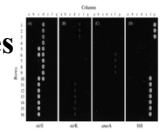
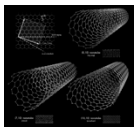
- Assumptions:
  - The abundance of enzymes that degrade C-rich substrates reflects the abundance of the substrate
  - The abundance of phosphatase, chitinase, proteases, etc reflect stoichiometric demands for P and N
  - Enzyme activities measured in lab assays indicate potential in situ activities

Contribution of each enzyme compartment to the total substrate catalysis will change in time (and space)

- **The one hour assay = existing pre-generated enzyme (accumulated, mural, solution, etc)**
- **The six hour assay = the above plus new enzymes from *r*-strategists and zymogenous bugs**
- **The 24-hour assay = the above plus new enzymes from *K*-strategists and autochthonous bugs**
- **The 72-hour assay = the above plus activity due to selection pressures and resulting *new* interactive microbial communities**

Exciting new ways to reveal the origins, location and activities of enzymes in soil

- Atomic force microscopy
- Immunofluorescence
- Autofluorescence
- 'Omics galore
- Functional gene probes
- Nano-sensors

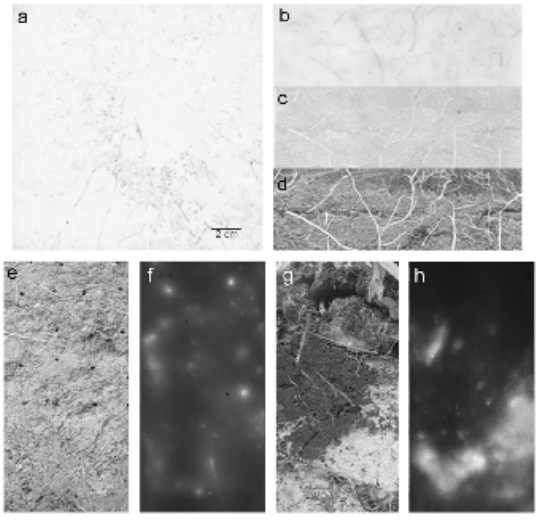
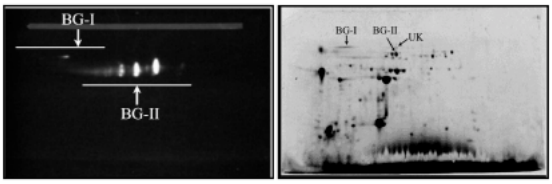
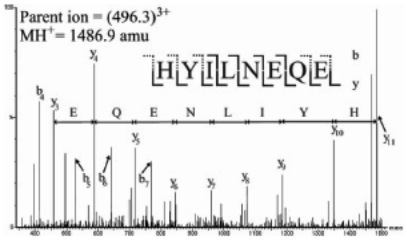


Fig. 2. Imprints and soil profiles from root windows in interior Douglas-fir stands near Barriere, British Columbia, Canada. (a) phosphatase imprint; (b) aminopeptidase imprint, (c) soil image overlain with the same imprint, (d) image of soil profile; (f) β-glucosidase imprint and (e)associated soil image; (h) chitinase imprint and (g) associated soil image. All images are at the same scale.

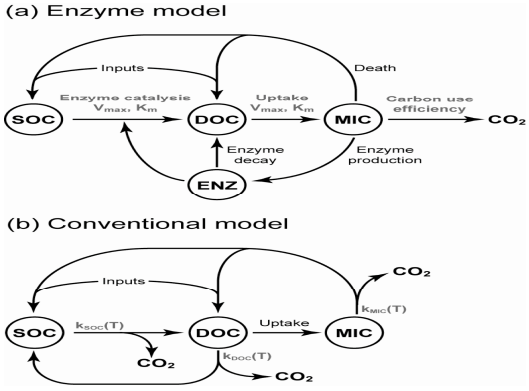
Proteomics





Kim, K.-H., Brown, K. M., Harris, P. V., Langston, J. A., Cherry, J. R., 2007. A Proteomics Strategy To Discover B-Glucosidases from Aspergillus fumigatus with Two-Dimensional Page In-Gel Activity Assay and Tandem Mass Spectrometry. J. Proteome Res. 6, 4749-4757.

Enzyme-explicit modeling



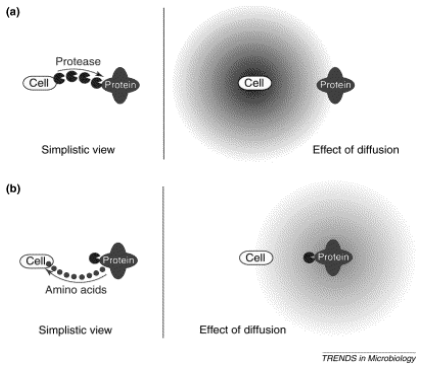
Allison, Wallenstein, and Bradford. Nature Geosciences 2010.

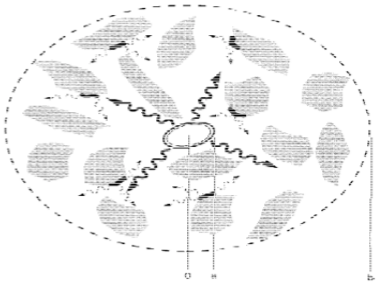
How do microbes and their enzymes locate and degrade natural and synthetic polymers in soil?

And should we call this soil enzyme (nano)ecology?

What are the various strategies for the effective functioning of these extracellular enzymes?

Strategy 1: microbes constitutively secrete enzymes to forage for substrates - *but* dilution and sorption reduce efficiency





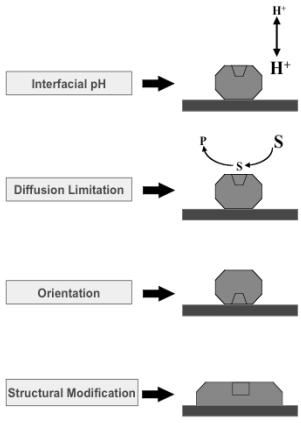
And many other factors that make enzyme secretion in soil a high risk strategy

1. **Attacked by proteolytic microbes**
2. **Substrate doesn't exist**
3. **Substrate never found (remote or hidden within microaggregates)**
4. **Wrong mix or sequence of enzymes**
5. **Others ('cheaters') cash in = no rewards**

Ways around these problems: (i) pre- or post-secretion modifications and behaviour will help stabilise enzymes in solution

- ***Stabilization*: conformational changes and polymerisation; disulphide bonds; complexing with glycoproteins and polysaccharides; proteolysis yielding active subunits; co-secretion with protective proteases and antibiotics**
- ***Optimization*: combining with other secreted enzymes (sequence) ; orientation at cellulose surface; binding modules; catalytic domains; and 2-D migration**

Ways around these problems: (ii) enzymes protected and retain activity when complexed with clays and humates



Quiquampoix & Burns (2007)

Are clay- and humic-immobilized enzymes a good strategy for substrate degradation?

Stable humic-*B*-glucosidase generates glucose from cellobiose

Synthesis and secretion of new microbial cellobiase induced or up-regulated after *signal recognition* and chemotaxis

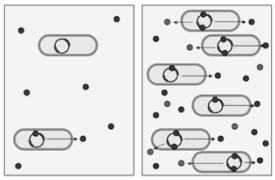
Stable humic-clay-urease rapidly hydrolyses urea

Ammonia provides energy for chemolithotrophs and then nitrate (good rhizosphere sequence)

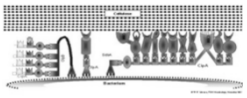
Difficult to see a key role in ligno-cellulose decay (although colloids are mobile/diffusive)

Strategy 2: take your time - don't secrete (many) enzymes until the substrate is detected

- **Many plant phytopathogens (the *Erwinia* story) only secrete cell wall depolymerases when substrate quality, quantity, and proximity is appropriate and when microbial density is high**
- **Homoserine lactones and oligopeptides provide one answer to this problem – *quorum sensing***



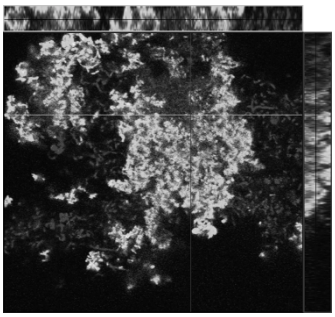
Strategy 3: keep the enzymes close (i) the 'somes'



- **Some soil bacteria and fungi retain their extracellular enzymes at the cell wall whilst others package cellulases within a 'cellulosome' (18-200nm, 100MDa)**
- **Cellulosomes are attached to the outside wall of the cell and contain numerous hydrolases (c, hc, pc) arranged on a protein scaffold to optimise attachment to substrate**
- **Other poysaccharide-degrading enzymes (e.g. xylanases) are contained in the cellulosome ('celluloxylanosome') or exist as separate 'somes (xylanosome, pectinosome)**
- **Are there 'peroxidosomes' and 'laccasosomes' and 'dehalagenosomes' and, if not, can we construct them?**

Strategy 3: keep the enzymes close (ii) the community biofilm

Motility - attachment - biofilms - community development - process competence - and then enzyme secretion



Conclusions

- A convergence of inquisitive questions and novel approaches – let's test our assumptions!
- Towards an integrated understanding of abiotic drivers of biological responses, interactions of enzymes with the soil environment, and substrate-enzyme interactions.
- As we improve our quantitative understanding of soil enzymology, they will become more prominent in the conceptual view of soil biogeochemistry.

Finally, a plug…

The Enzymes in the Environment Research Coordination Network
http://enzymes.nrel.colostate.edu