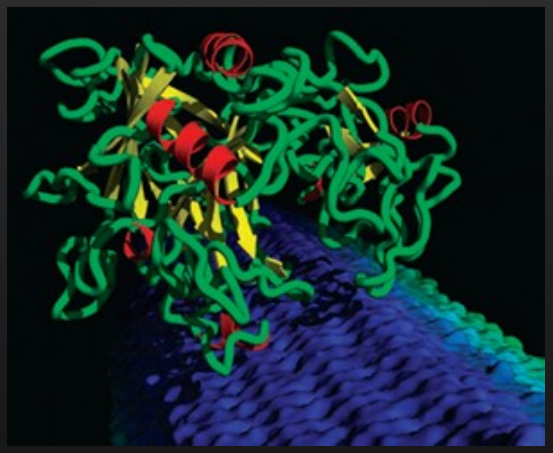
Integrating ‘omics into Environmental Enzymology: *The Next Frontier or Fool’s Gold?*



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The Problem

Current techniques only allow us to detect the activities of enzymes.

How can we interpret enzyme activities without a broad understanding of their ecology and biochemistry?

What do we want to know?

1. What controls enzyme production?

What is the potential for production of enzymes in any given environment?

How does enzyme production vary with abiotic conditions?

Which taxa produce enzymes under different conditions?

2. What controls in-situ enzyme activity?

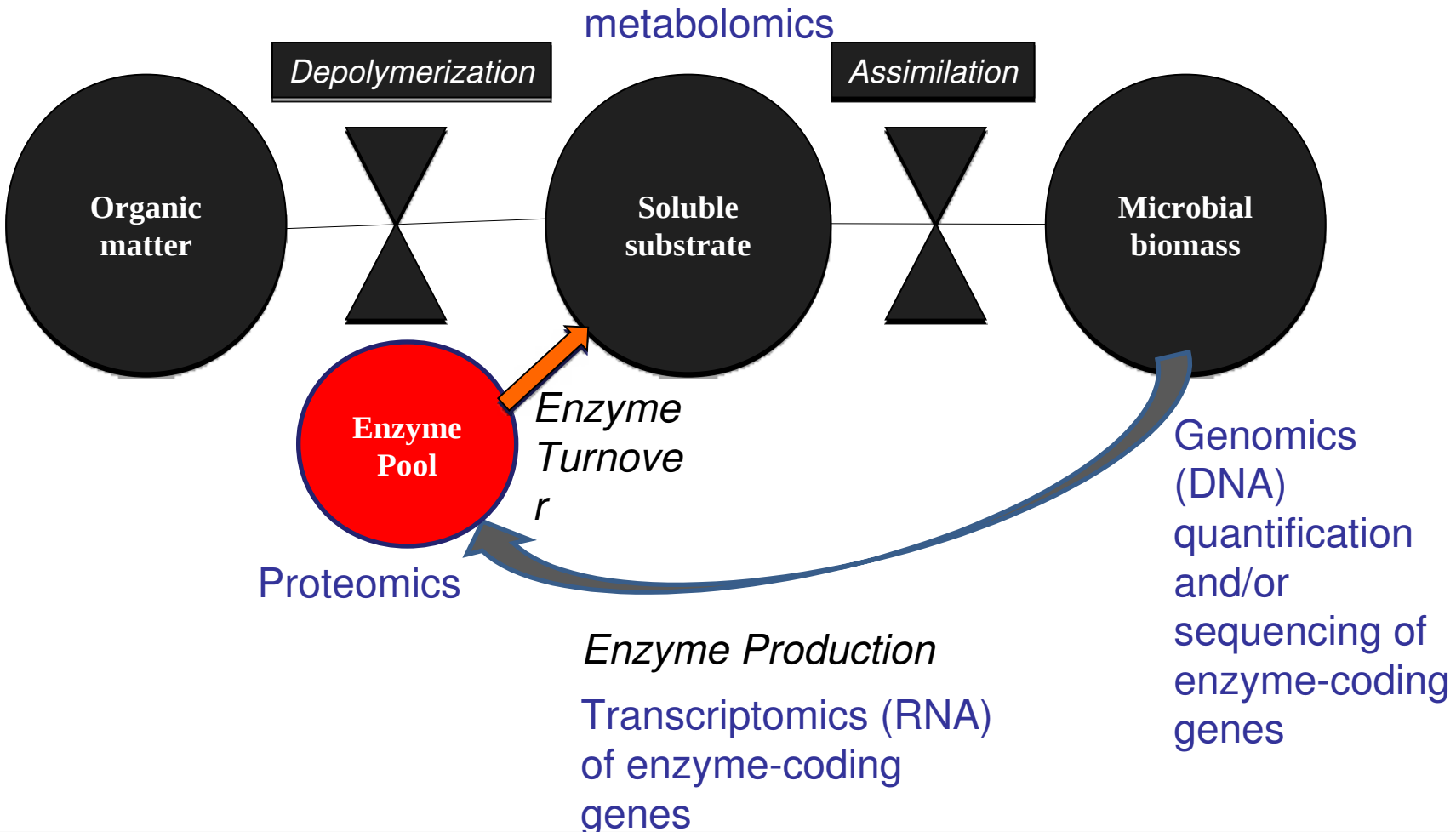
What enzymes are present in an environment?

How do iso-enzymes differ in their biochemistry?

How does stabilization affect enzyme activity?

If only there were some magic tool…

Molecular tools for enzymology



Part 1:
What controls enzyme production?

Enzyme Production: What we think we know

Hypothesis 1: Stoichiometric Economics

Need N? Produce chitinase, protease, etc.

Need C? Produce cellulase, xylosidase, etc.

Need P? Produce phosphatase

Hypothesis 2: Resource Allocation

Produce enzymes in response to substrate availability

Enzymes indicate ecosystem development

P

C

N/S

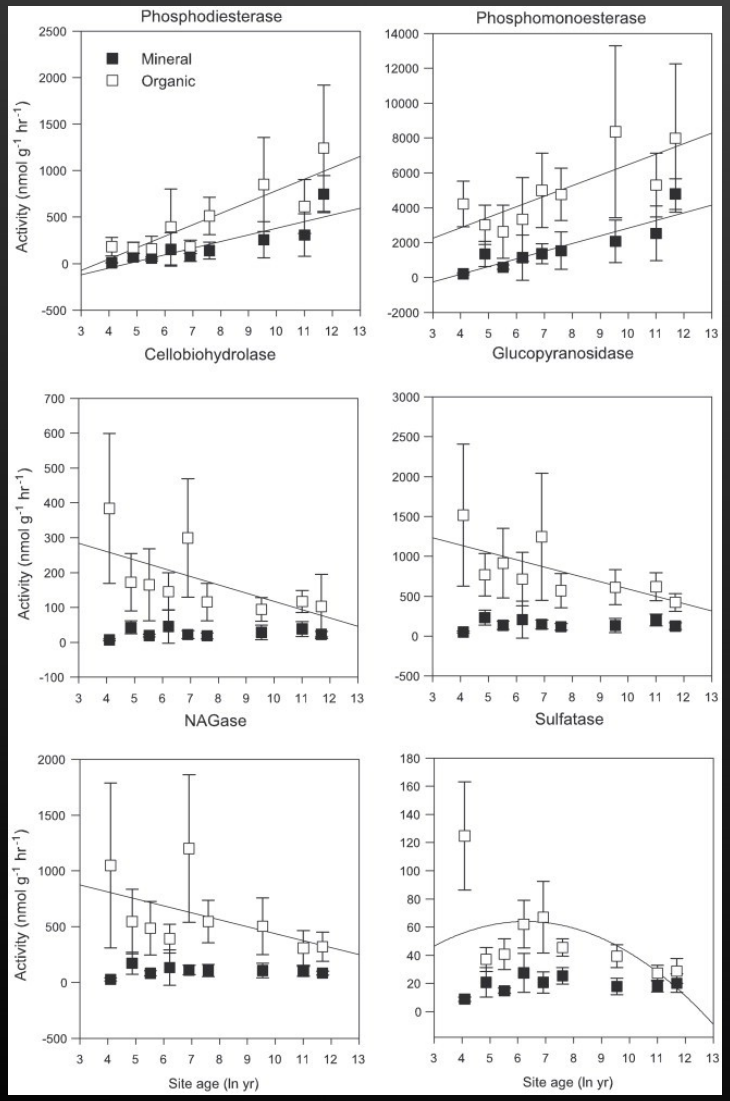


Table 1
Characteristics of mineral and organic soil at each site, determined on composite samples

Soil horizon	Site age (yrs)	C (%)	N (%)	S (%)	P (mg kg^{-1})	C:P
Mineral	60	0.96	0.05	0.006	727	13.18
	130	7.32	0.41	0.042	645	113.43
	280	3.69	0.20	0.021	391	94.37
	530	7.06	0.39	0.040	543	130.02
	1000	6.31	0.41	0.051	666	94.76
	5000	10.85	0.60	0.061	470	230.85
	12 000	8.68	0.39	0.049	326	266.10
	60 000	9.99	0.39	0.045	275	363.27
	120 000	8.58	0.34	0.037	99	866.26
Organic	60	18.55	1.16	0.114	1123	165.18
	130	18.91	0.84	0.106	827	228.66
	280	18.21	0.79	0.103	699	260.52
	530	23.94	1.14	0.137	819	292.31
	1000	23.01	0.98	0.126	958	240.19
	5000	30.31	1.25	0.155	728	416.35
	12 000	23.96	0.97	0.128	554	432.49
	60 000	23.83	0.64	0.091	394	604.82
	120 000	22.36	0.66	0.087	376	594.68

Allison VJ, Condron LM, Peltzer DA, Richardson SJ, Turner BL (2007). *Soil Biology and Biochemistry* **39:** 1770-1781.

Limitations

Enzyme activities reflect enzyme pool size, which results from both production and turnover.

How can we measure enzyme production?

What controls enzyme production?

A. What is the potential for production of enzymes in any given environment?

Quantification of enzyme-coding genes (or metagenomics).

Incubation of environmental samples under optimal conditions (might add protease inhibitor).

Effects of N deposition on phenol oxidase

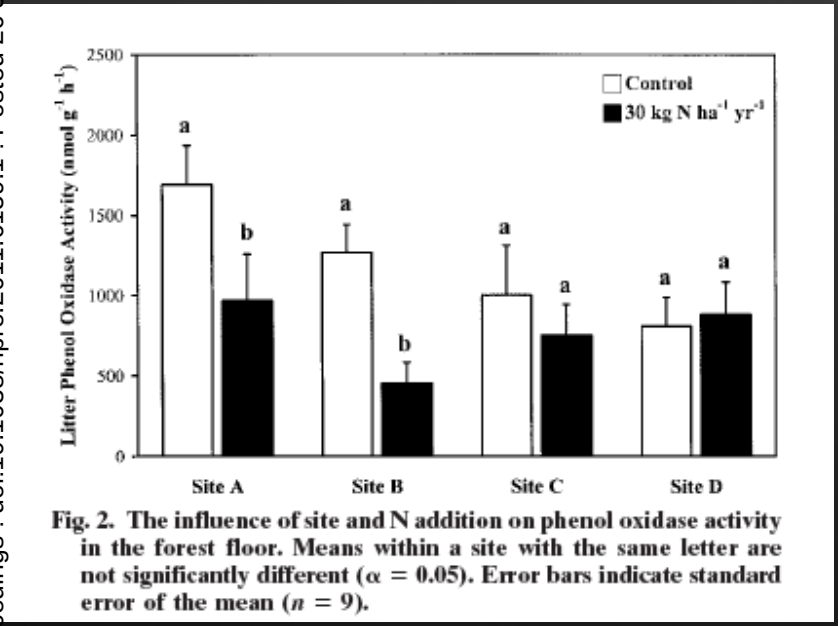
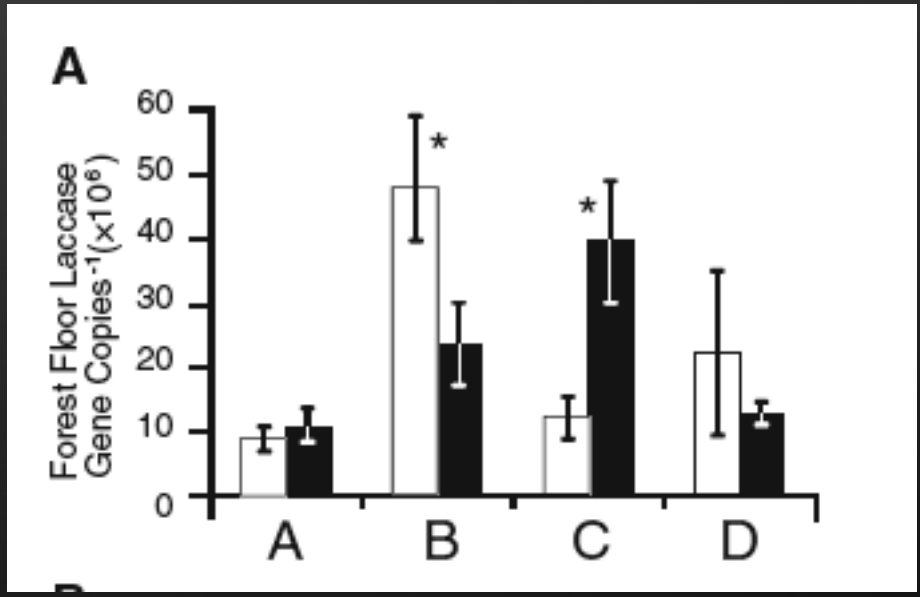


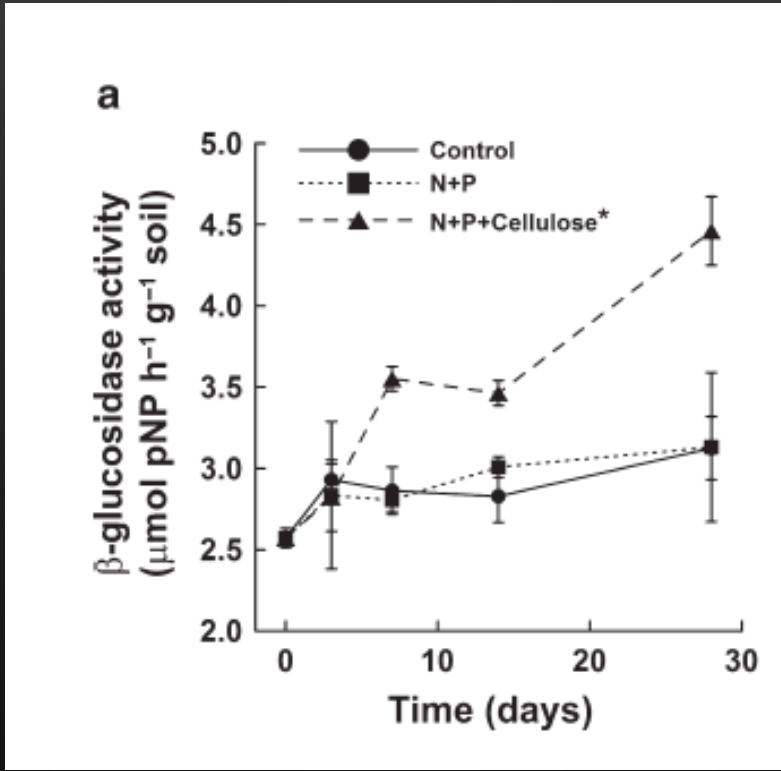
Fig. 2. The influence of site and N addition on phenol oxidase activity in the forest floor. Means within a site with the same letter are not significantly different ($\alpha = 0.05$). Error bars indicate standard error of the mean ($n = 9$).



DeForest, J. et al (2004). SOIL SCIENCE SOCIETY OF AMERICA JOURNAL, 68(1), 132–138.

Hassett, J. E., et al. (2008). Microbial Ecology, 57(4), 728–739.

Substrate Availability Affects Enzyme Production



Allison et al 2010

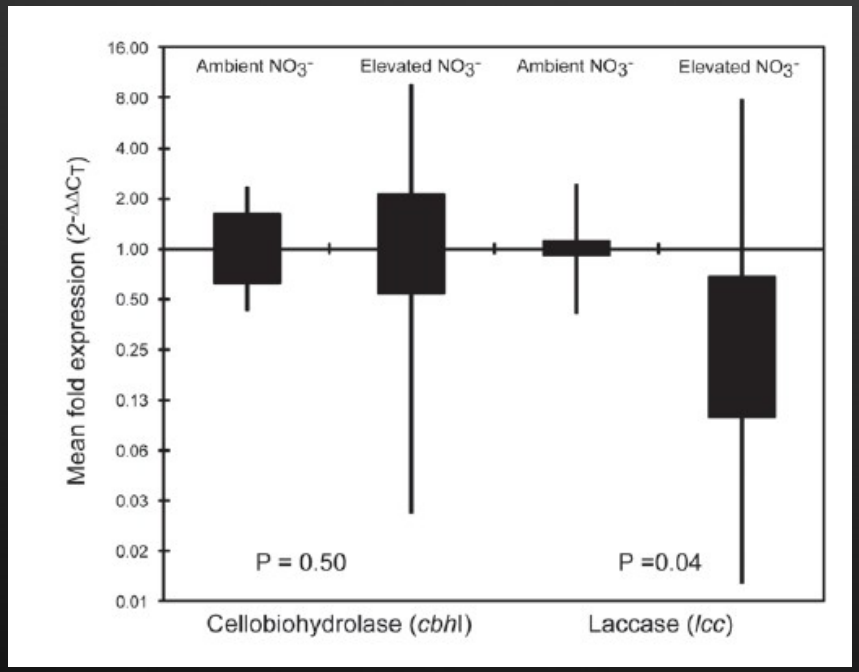
What controls enzyme production?

B. How does enzyme production vary with abiotic conditions?

Quantification of mRNA transcripts for enzyme-coding genes.

Incubation of environmental samples under a range of conditions (might add protease inhibitor).

Quantification of mRNA transcripts



Cellobiohydrolase enzyme activity 22% lower under simulated N deposition

No consistent effect of N deposition on laccase activity.

Edwards et al PLoS One 2011.

What controls enzyme production?

C. Which taxa produce enzymes under different conditions?

Sequencing of mRNA transcripts (or metatranscriptomics).

Direct detection of iso-enzymes via proteomics.

Incubation of pure cultures under different conditions, followed by measurement of activity.

Sequencing of mRNA transcripts

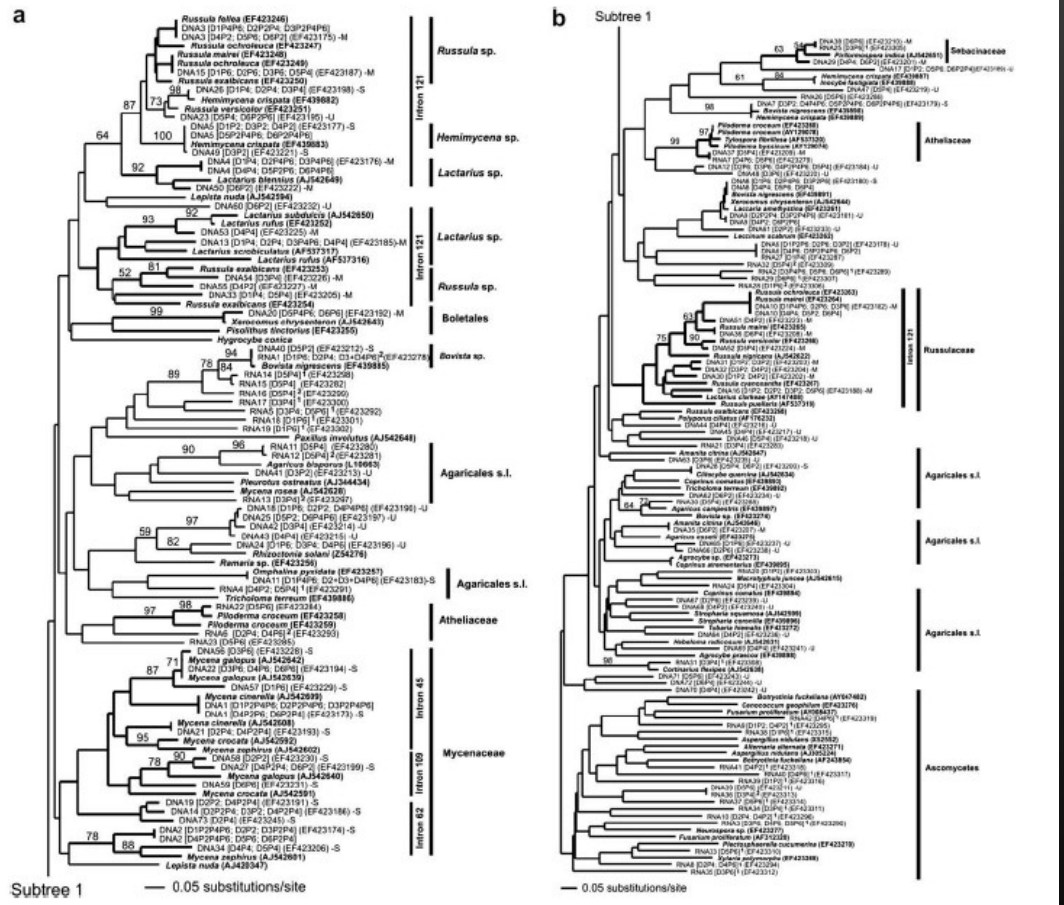


Fig. 2. Neighbor-joining tree calculated from the coding region of fungal laccase genes obtained from soil samples as well as fungal references using K2P-distances. Branch support was assessed by 2000 bootstrap replicates, which are given above branches. Occurrence of introns supporting fungal taxa is indicated. Major monophyletic fungal groups are indicated with bold branches, their trophic status is indicated (m, mycorrhizal; s, saprotrophic; u, unknown). The occurrence of each environmental laccase gene sequence is given in brackets (D1–D6 indicating sampling dates March 2004–April 2005; sampling plots: P2, P4, P6) with their accordingly GenBank accession number. Expressed laccase genes, high ranked "1" refers to a sequence obtained with primer combination Cu1Af/Cu2r, whereas "2" indicates the amplification with both primer sets and without number means the finding only with primer set Cu1f/Cu2r.

Kellner, H., P. Luis, B. Schlitt, and F. Buscot. 2009. Temporal changes in diversity and expression patterns of fungal laccase genes within the organic horizon of a brown forest soil. Soil Biology and Biochemistry 41:1380-1389.

Be wary…

- A transcript is only a transcript.
- Transcripts must be translated into proteins before we actually have an enzyme

Part 2: What controls in-situ enzyme activity

a. The amount of enzymes present (controlled by production – turnover)
b. The kinetic properties of the enzymes present (differs with iso-enzyme production)
c. Interactions with the physical and chemical matrix

What enzymes are present in an environment?

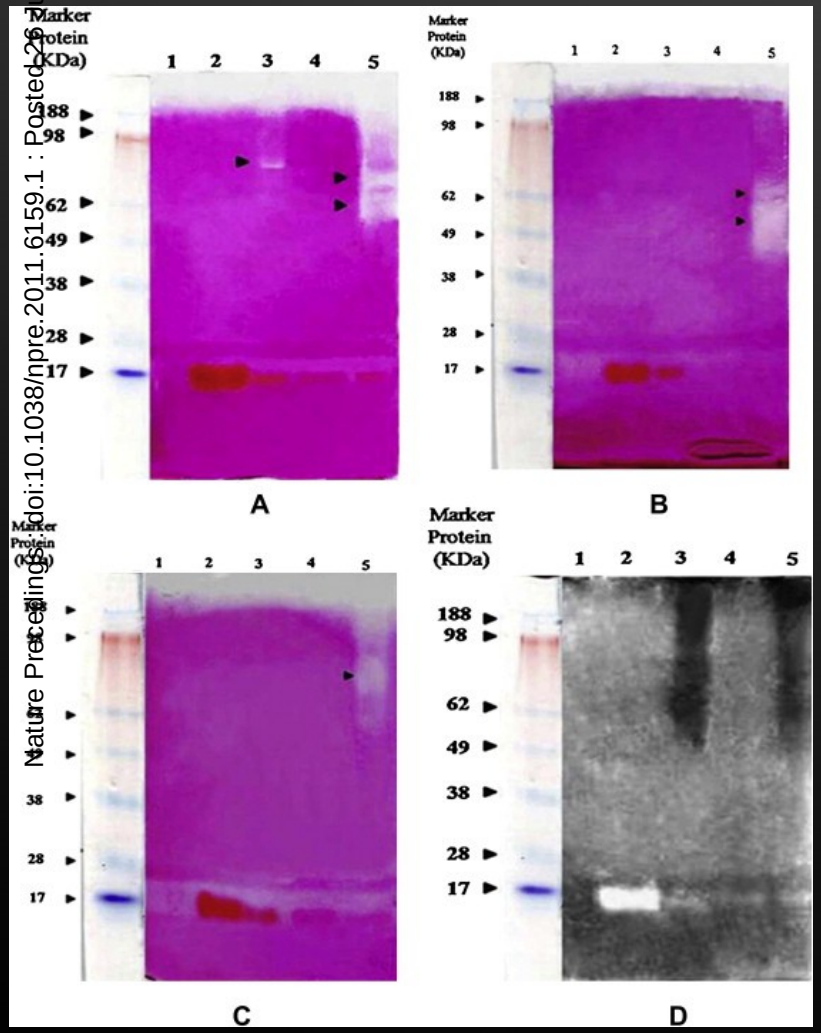
Direct detection of enzymes using proteomic techniques.

Stay tuned for the following talk!

Diversity of soil cellulase isoenzymes is associated with soil cellulase kinetic and thermodynamic parameters

Banafshe Khalili [a], Farshid Nourbakhsh [a,*], Nafiseh Nili [b], Hossein Khademi [a], Bahram Sharifnabi [b]

Soil Biology & Biochemistry, 2011.



Activity and kinetic parameters of cellulase in soils as affected by land use.

Soil	Activity[b]	Lineweaver-Burk plot ($1/V$ vs $1/S$)		Hanes-Woolf plot (S/V vs S)	
		K_m[a]	V_{max}[b]	K_m	V_{max}
Forest					
Native	828 a	17.0 b	1561a	18.4 b	1650 a
Cultivated	170 b	28.6 a	454b	22.2 a	375 b
Rangeland					
Native	291 a	11.5 b	464 a	11.0 b	453 a
Cultivated	155 b	13.7 a	251 b	18.0 a	302 b
Saline					
Native	542 a	9.3 b	890 a	6.1 b	726 a
Reclaimed	146 b	13.6 a	227 b	20.0 a	295 b

Different letters within each study area indicate statistically different values (LSD, $P < 0.05$, $n = 3$).

[a] K_m are reported as CMC ($g\,l^{-1}$).

[b] Cellulase activity and V_{max} are reported as μg glucose g^{-1} soil $24\,h^{-1}$.

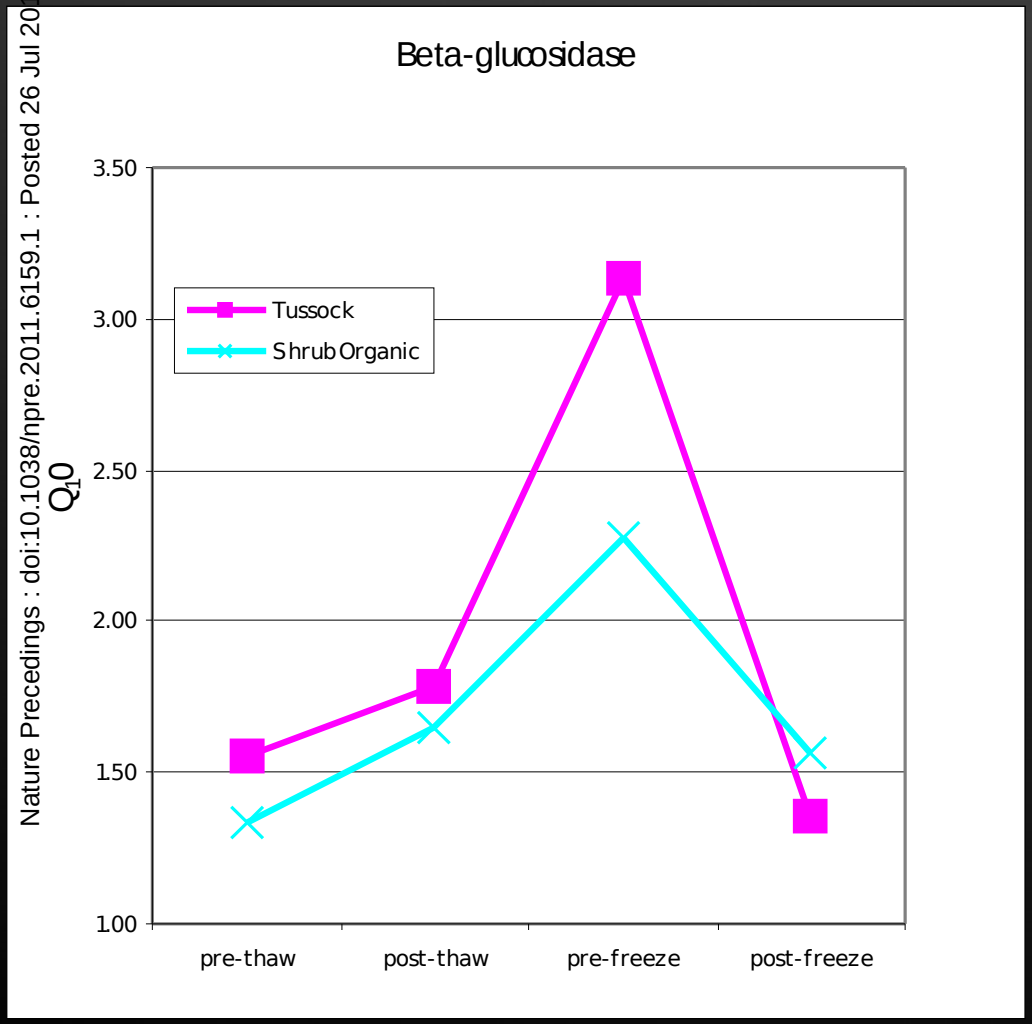
Controls on in-situ activity

- Temperature
- Moisture and soil structure (diffusion)
- pH
- Stabilization and enzyme conformation

Limitations to current approach to measuring enzyme activity

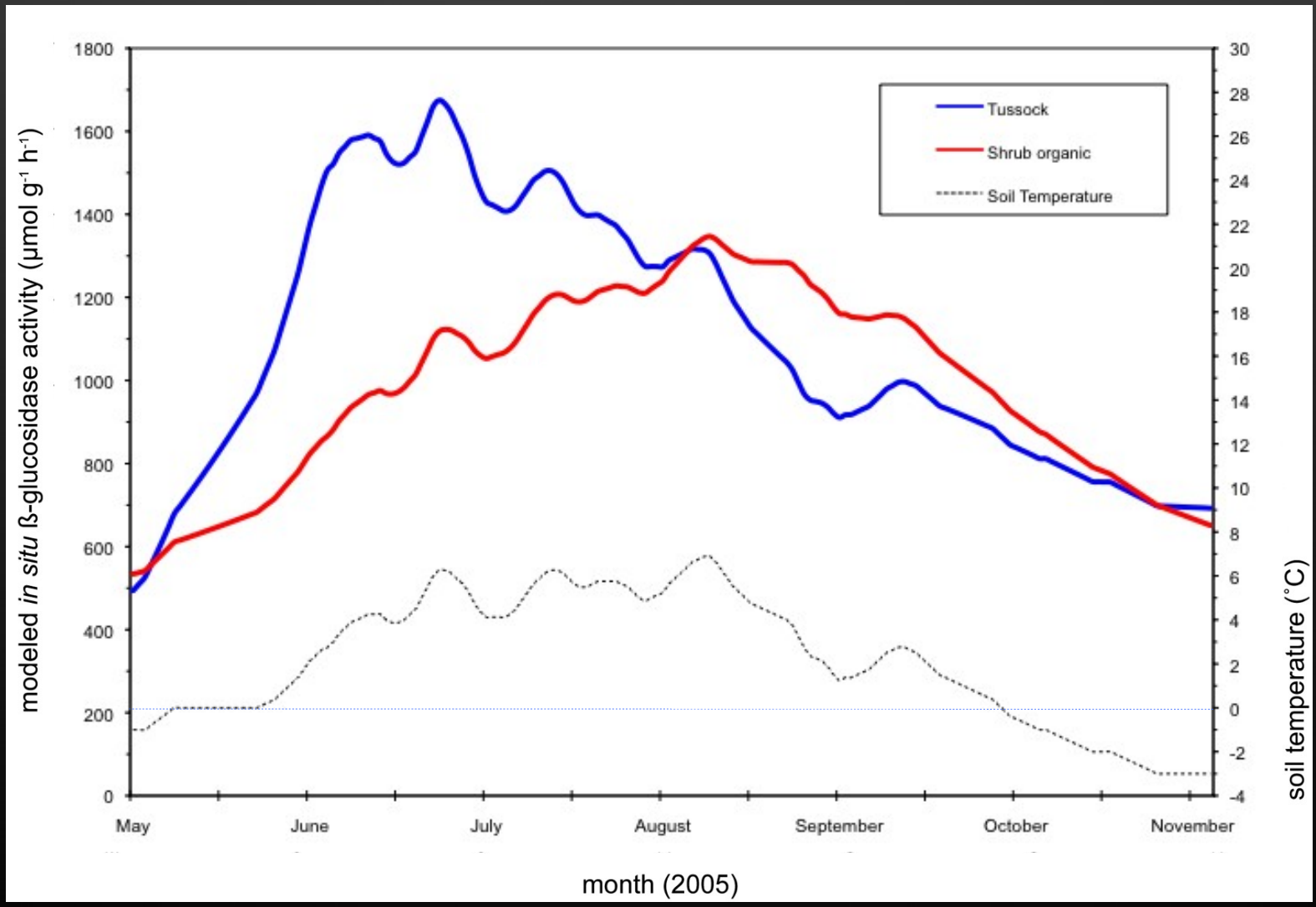
- Substrate must be added
- Usually only measured under a single condition (temperature, pH)
- Soil structure is destroyed in slurries

Seasonal changes in enzyme temperature sensitivity



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

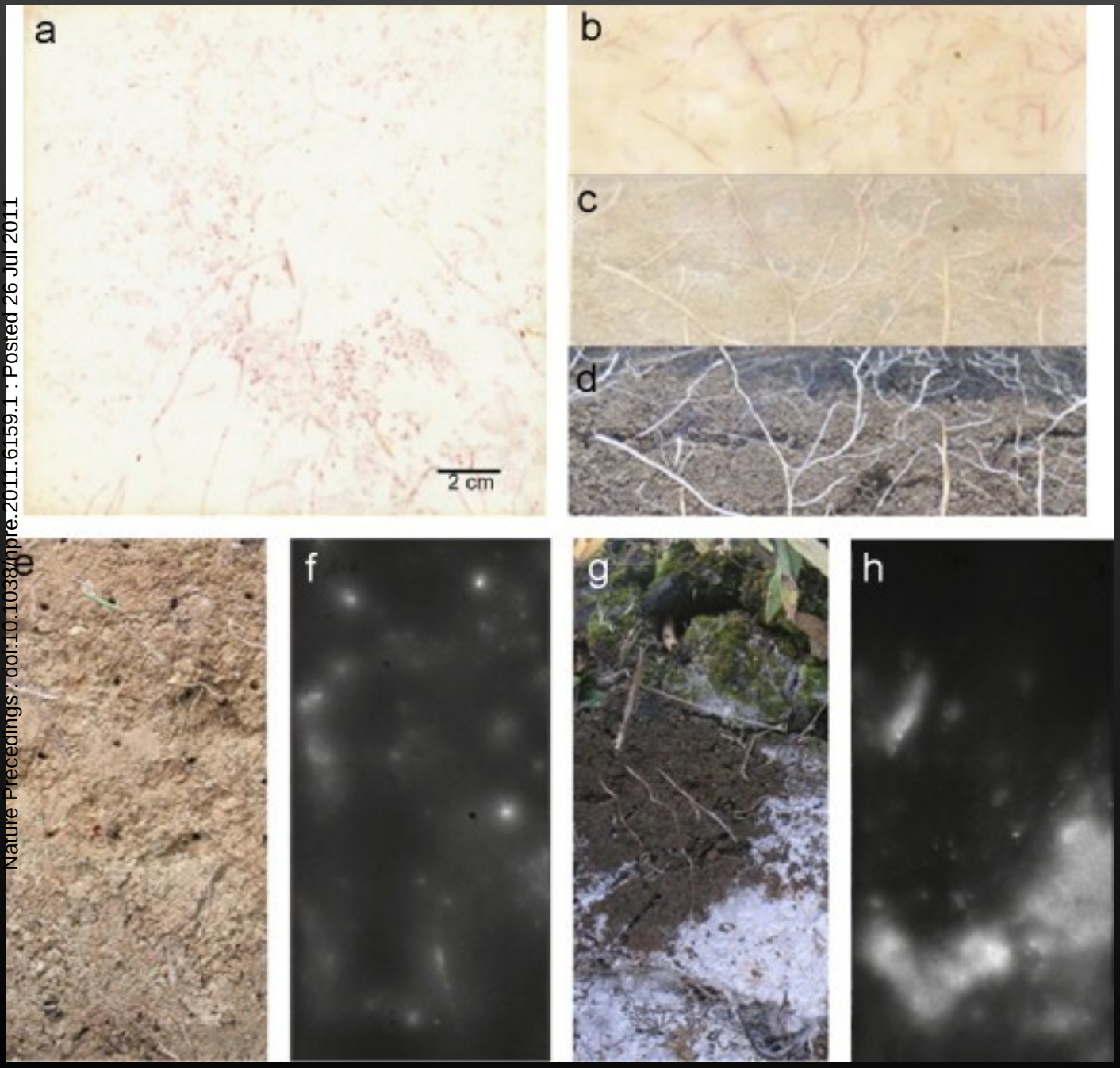
Modeled in-situ enzyme activities



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

What controls in-situ enzyme activity?

- in-situ detection of enzyme activity
- Detection of enzyme product formation by metabolomics (mass spectrometry)



Dong et al 2007, SBB.

Direct detection of enzyme degradtion products with NimZymes

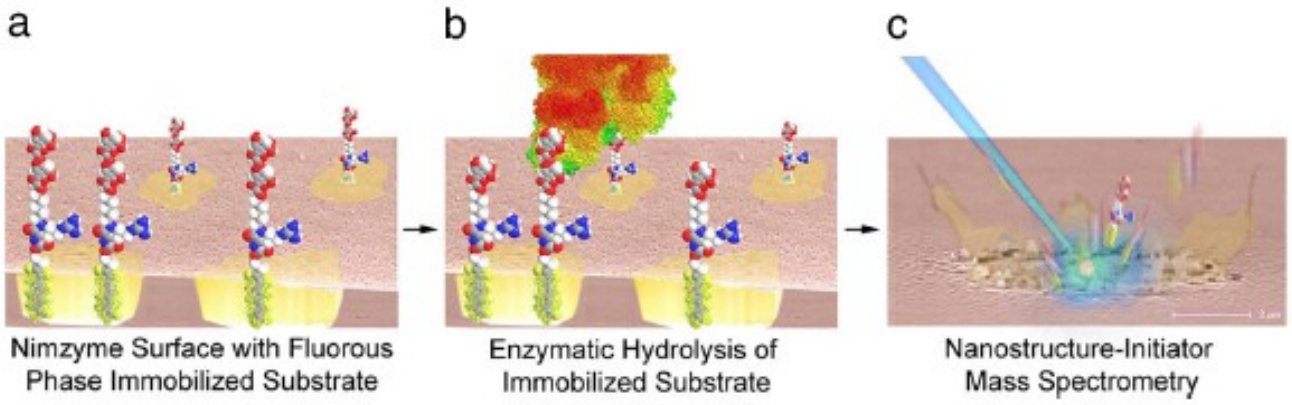


Fig. 1. Illustration of the Nimzyme assay. (*a*) Immobilization of metabolites in the fluorous "clathrate" phase of the NIMS surface. (*b*) Incubation of the surface with the sample to screen for enzymatic activity. (*c*) Laser irradiation, resulting in vaporization of the fluorous phase, efficiently transferring the immobilized substrate and products into the gas phase.

Northen, T. et al. 2008. A nanostructure-initiator mass spectrometry-based enzyme activity assay. PNAS.

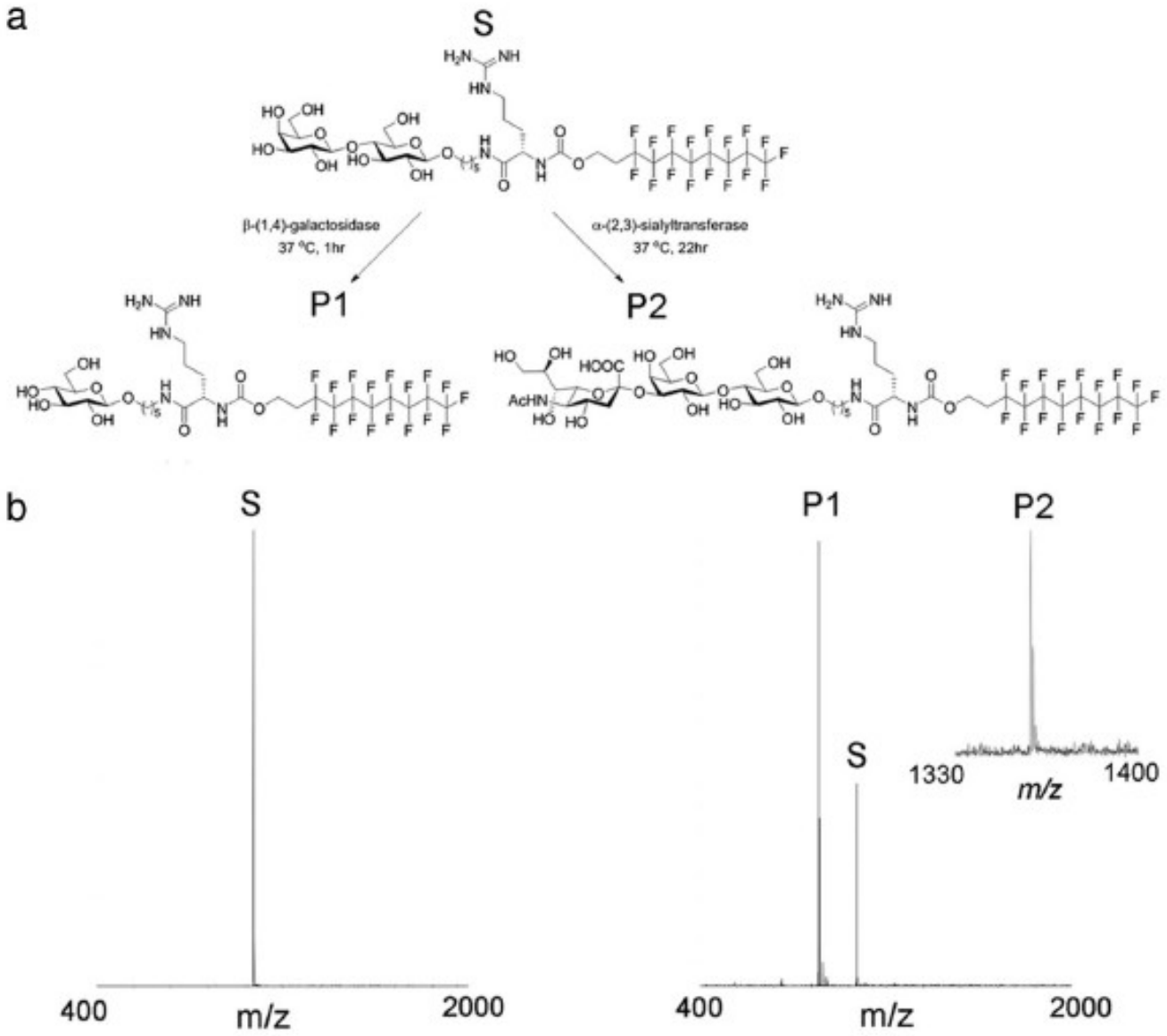
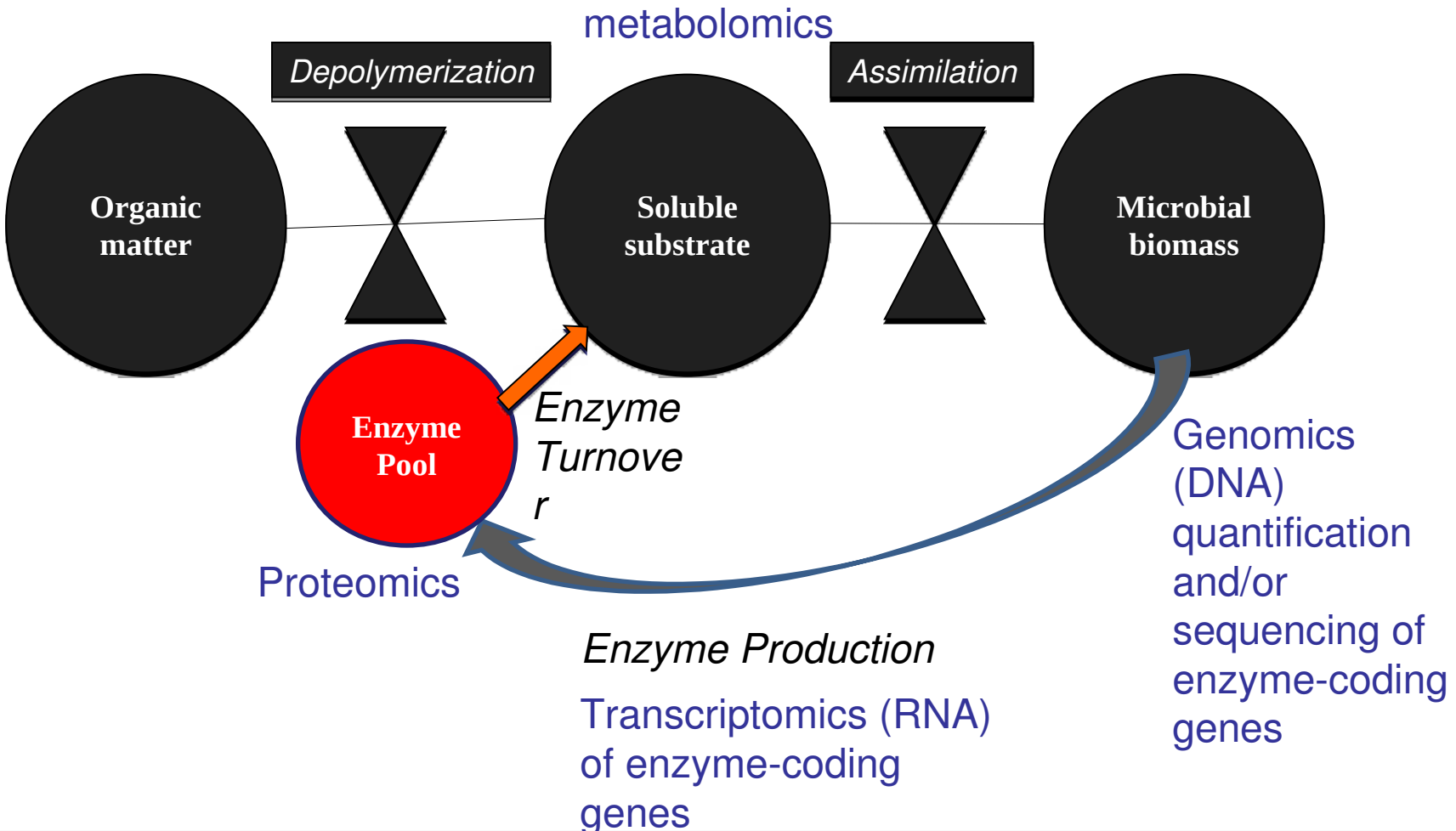


Fig. 2. On-chip NIMS enzymatic activity assay (Nimzyme assay). (*a*) Substrate (*S*, MH+ *m/z* 1,074.30) structure and the products of β-1,4-galactosidase (P1, MH+ *m/z* 911.24) and α-2,3-sialyltransferase (P2, MH+ *m/z* 1,365.40). (*b*) Mass spectra of the substrate (*Left*) and resulting products (*Right*). β-galactosidase (40 pg) reaction performed in pH 6 ammonium phosphate buffer and sialyltransferase (100 microunits) by diluting 1:5 in freshly prepared reaction buffer; pH 7.5 Tris, $MgCl_2$, with 1 mM CMP-sialic acid.

Some progress, lots of hope…



The next frontier or fool's gold?

http://enzymes.nrel.colostate.edu