Humification Chemistry

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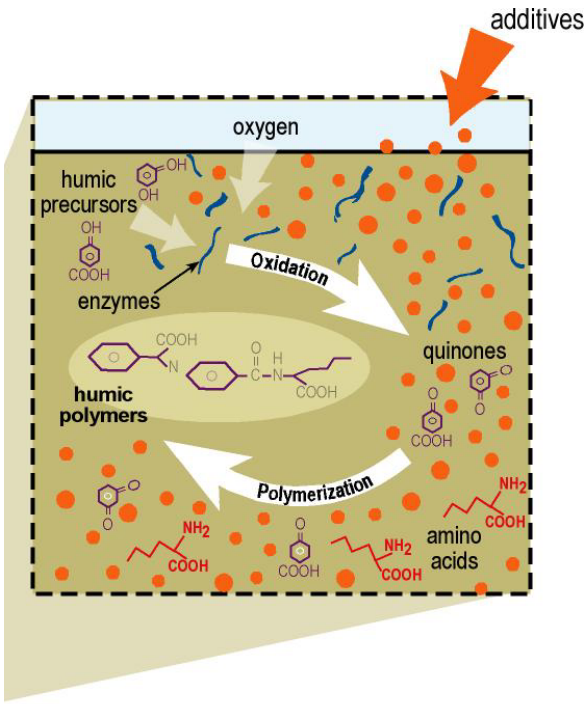
Research Objectives

- Develop fundamental understanding of humification process from a chemical perspective
- Investigate ways of manipulating enzyme chemistry to promote net humification
  - Impact of oxidizing soil minerals
  - Management of moisture/redox regimes
  - Enzyme stabilization
  - Amendment strategies



Approach

- Laboratory & Microcosm Studies (PNNL)
  - Enzyme stability
  - Wetting/drying, redox cycles
  - Mn oxide (Rice)
  - Fly ash

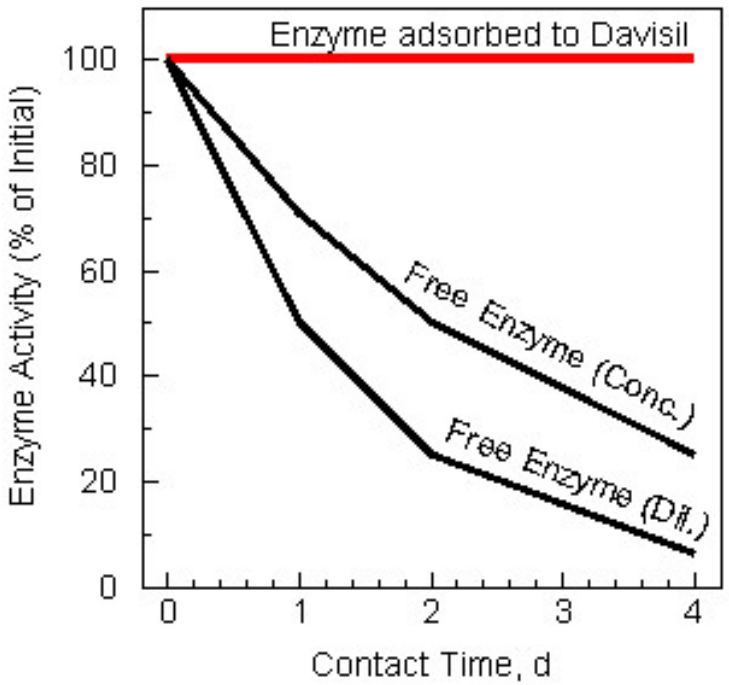
- Field "Minicosm" Studies (Santee Exp. Forest)
  - Wetting/drying cycles
  - Fly ash and lime
  - Initial soil C content
  - ^{13}C tracer

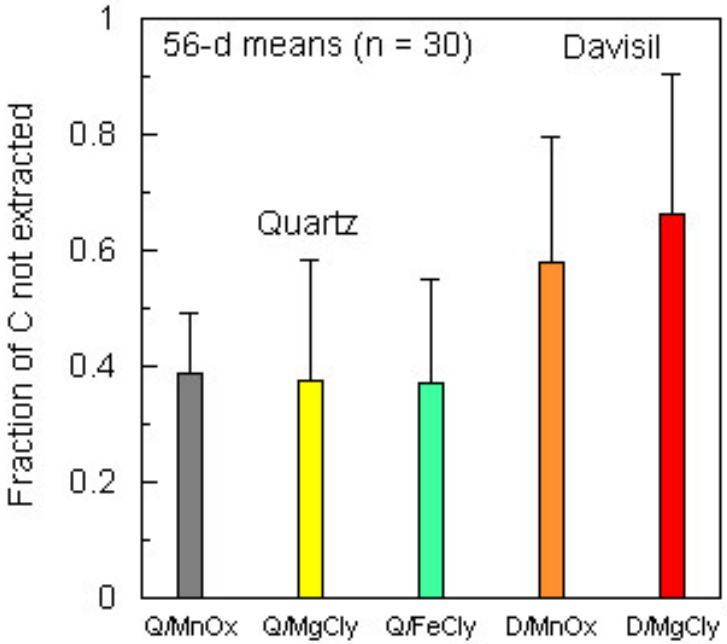
Laboratory Studies

- Use model humification reaction (Nelson et al., 1979) involving polyphenol (orcinol, resorcinol), hydroxybenzoic acid (p-hydroxybenzoic acid, vanillic acid), and amino acid (L-glycine, and L-serine) monomers (2 mM each) and tyrosinase as the polyphenol oxidase
  - Homogeneous systems:
    - pH 6.5 100 mM H_2PO_4 buffer primarily
    - Additional experiments at pH 5, 7.5, and 9
  - Heterogeneous systems
    - Porous silica
    - Fe(III)/Mn(IV) oxide minerals
    - Alkaline fly ashes (as received and after neutralization)
    - Calcareous soil alone and amended with fly ash
    - pH 6.5 initially
    - Some experiments under controlled moisture/atmosphere
- Follow progress by UV-Vis spectroscopy (Kumada et al., 1967; Shindo and Huang, 1984) to measure humification and, separately, enzyme activity
- Measure total and extractable C in microcosm studies

Enzyme Stabilization*

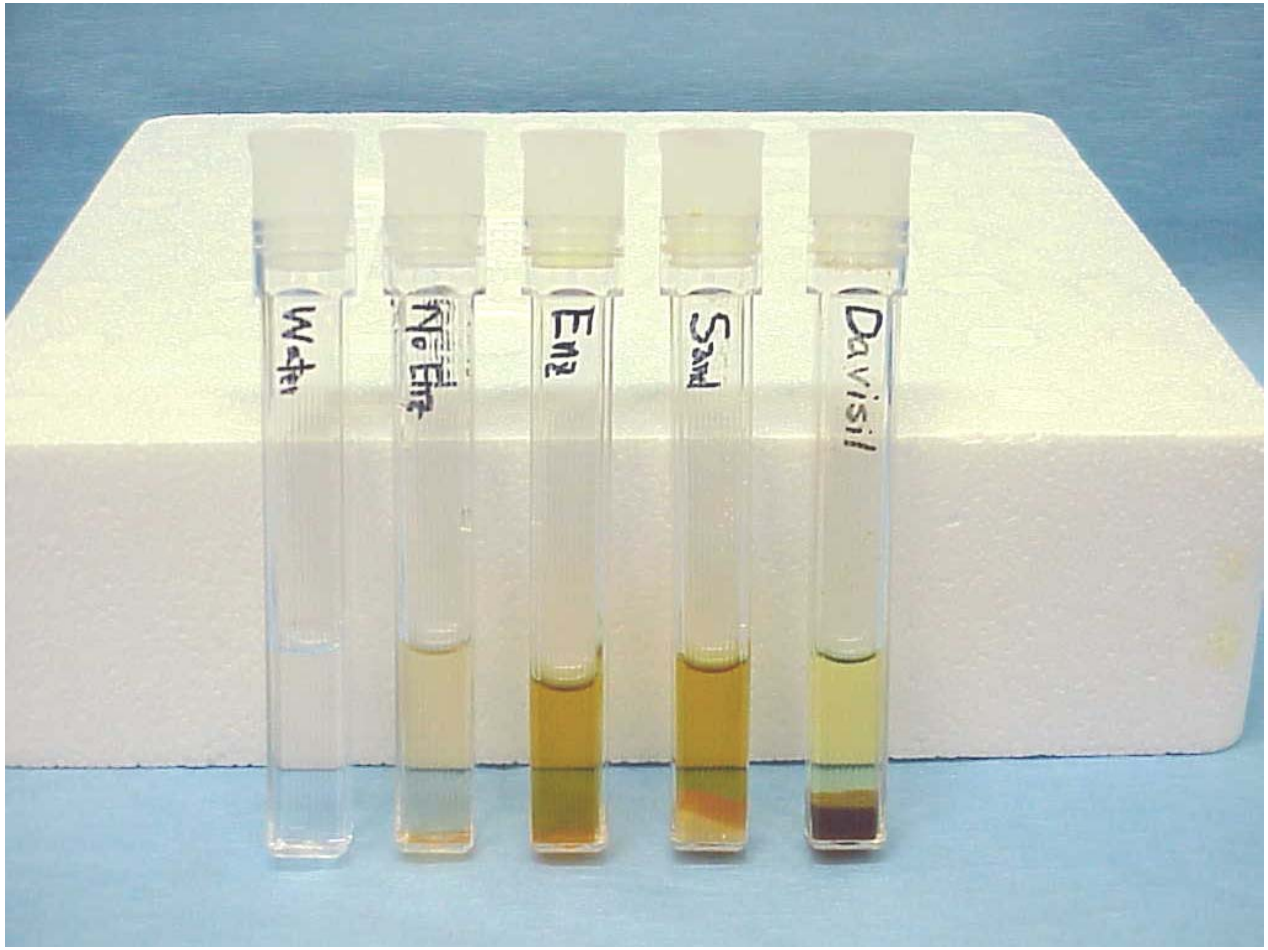
- Porous silica (Davisil) stabilizes phenol oxidase in aqueous solution and significantly increases net humification in synthetic soil experiments
- Stabilization dominates chemical factors



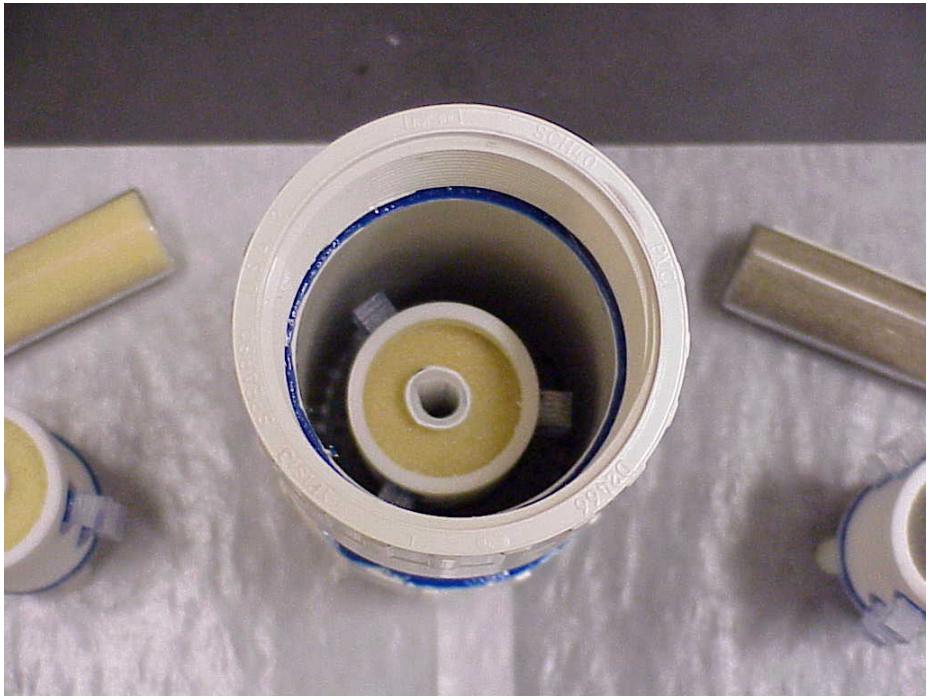


*Work funded jointly by CSiTE and NETL

Humification: Physical Stabilization in Porous Silica



Microcosm Studies

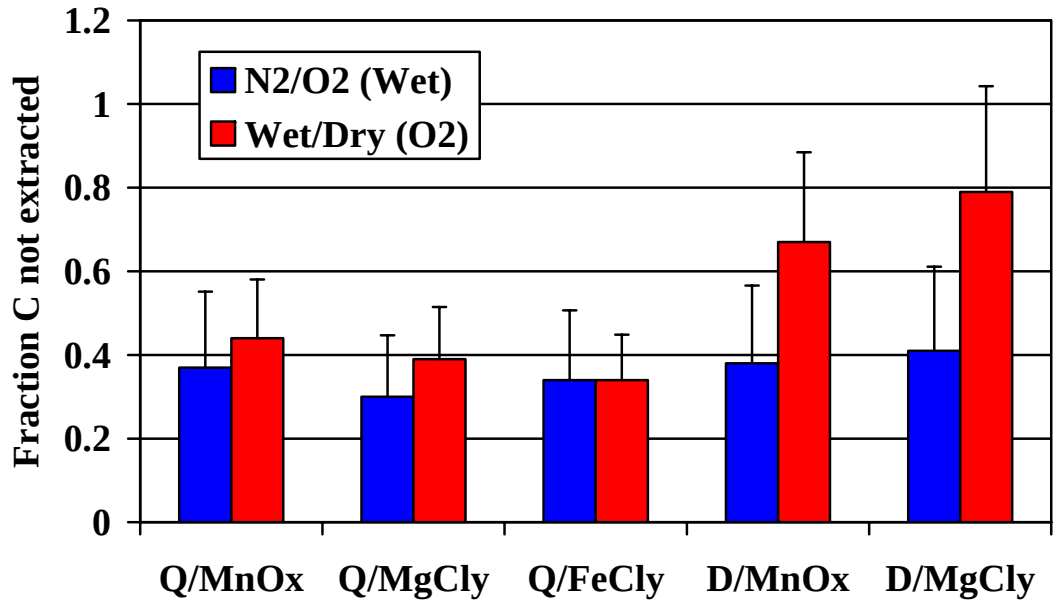


Microcosm inside “chimney”

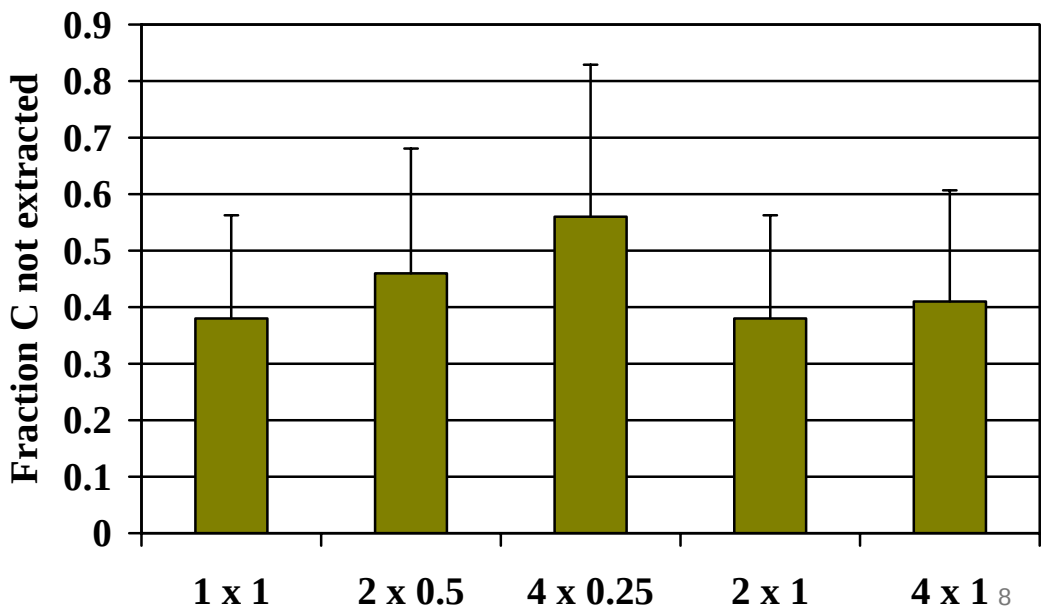
Chimneys mounted on gas manifold

Cycles

- Wetting/drying in presence of air promotes humification when porous silica (Davisil) present

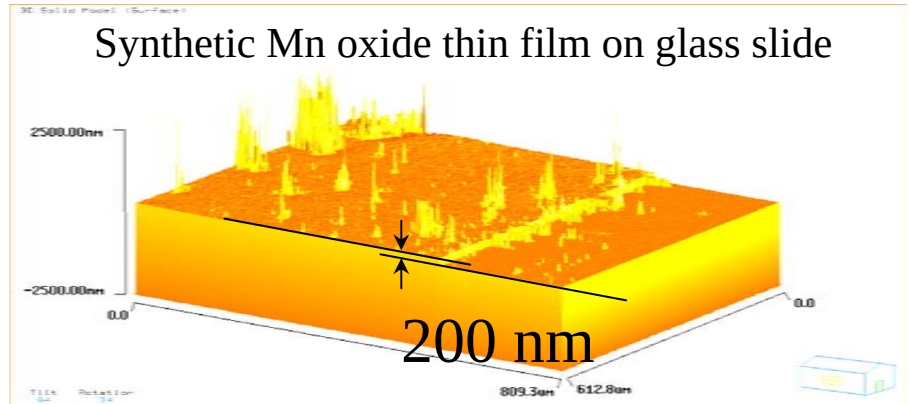


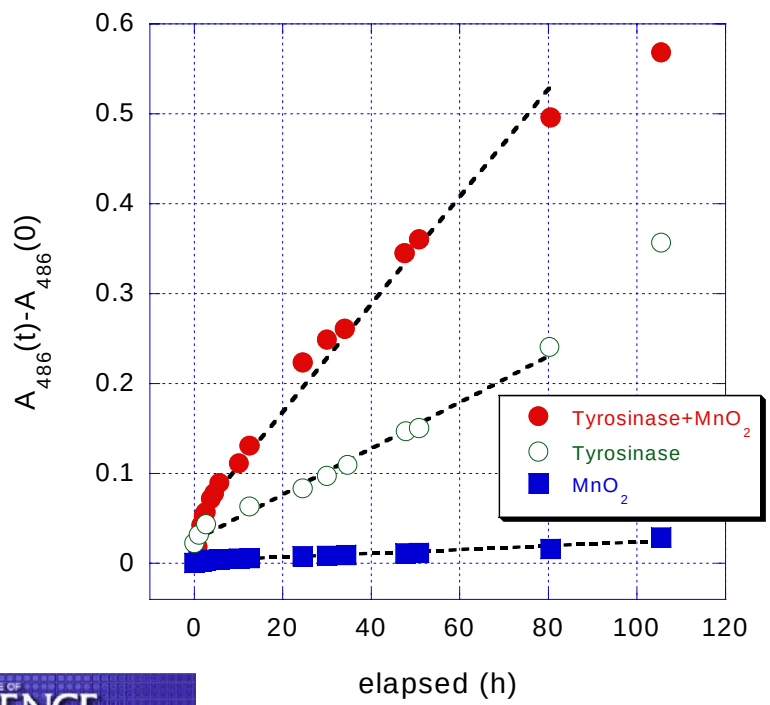
- Repetitive cycles with small monomer additions more effective per unit of monomer added

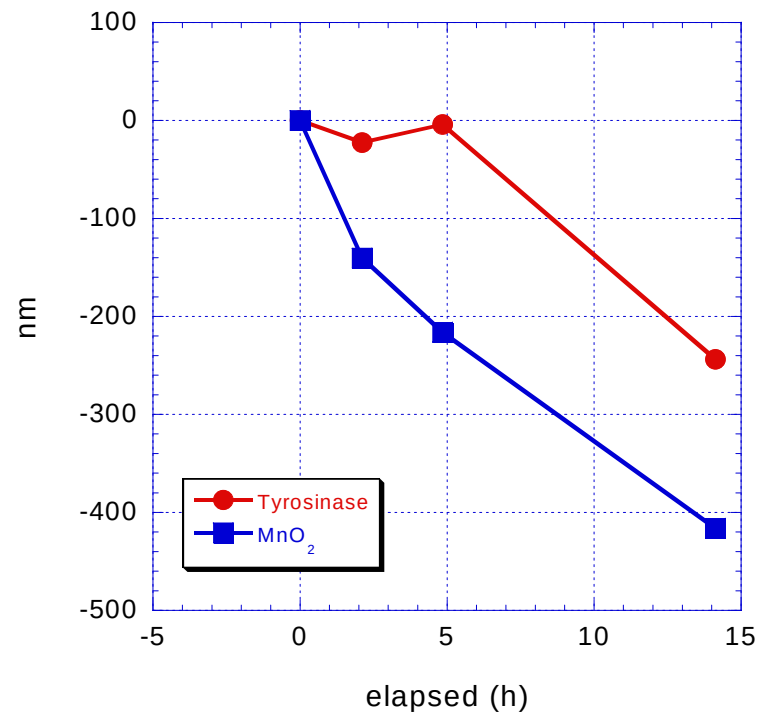


Catalytic Synergy

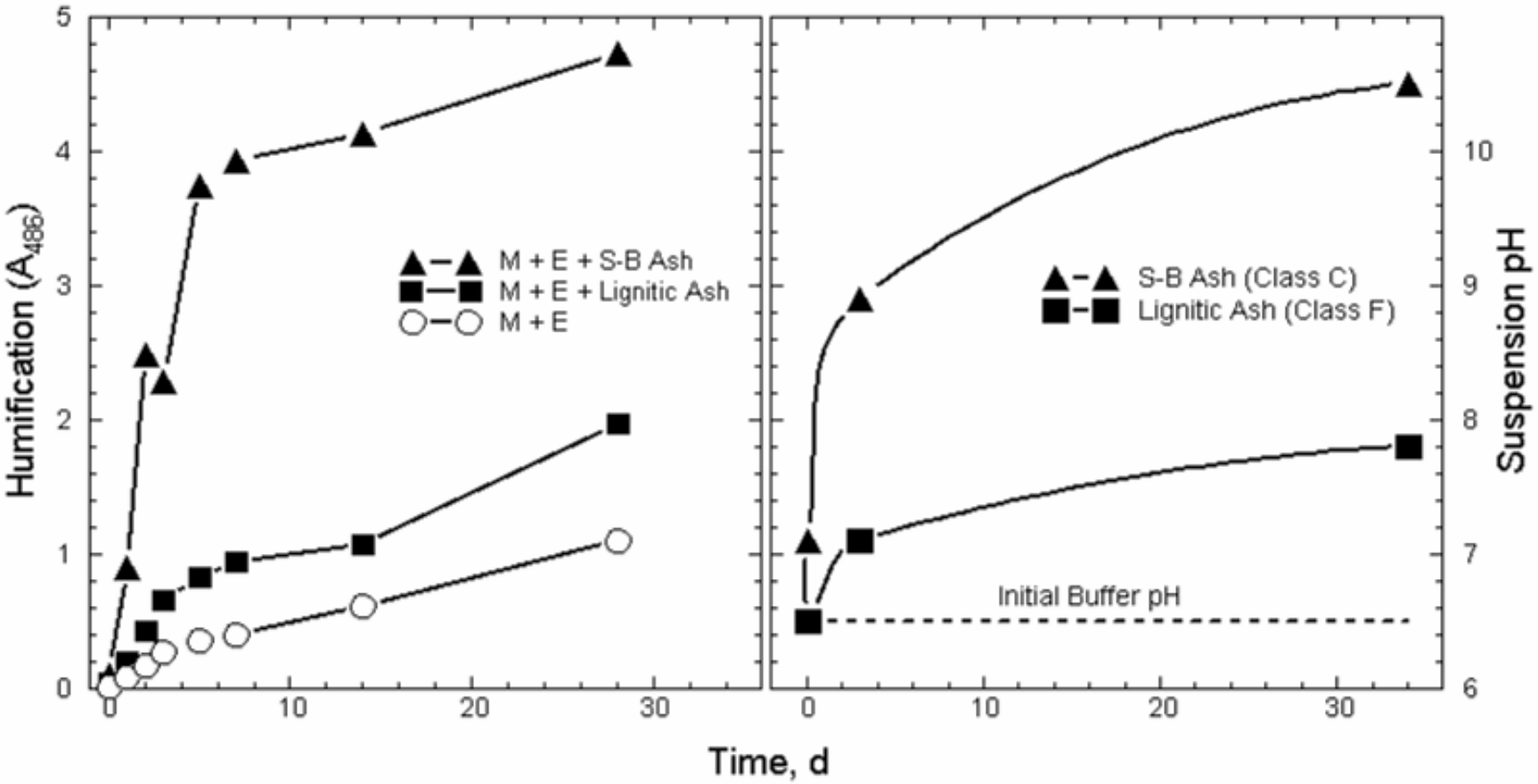
- Phenol oxidase is at least *twice* as effective when Mn oxide is present





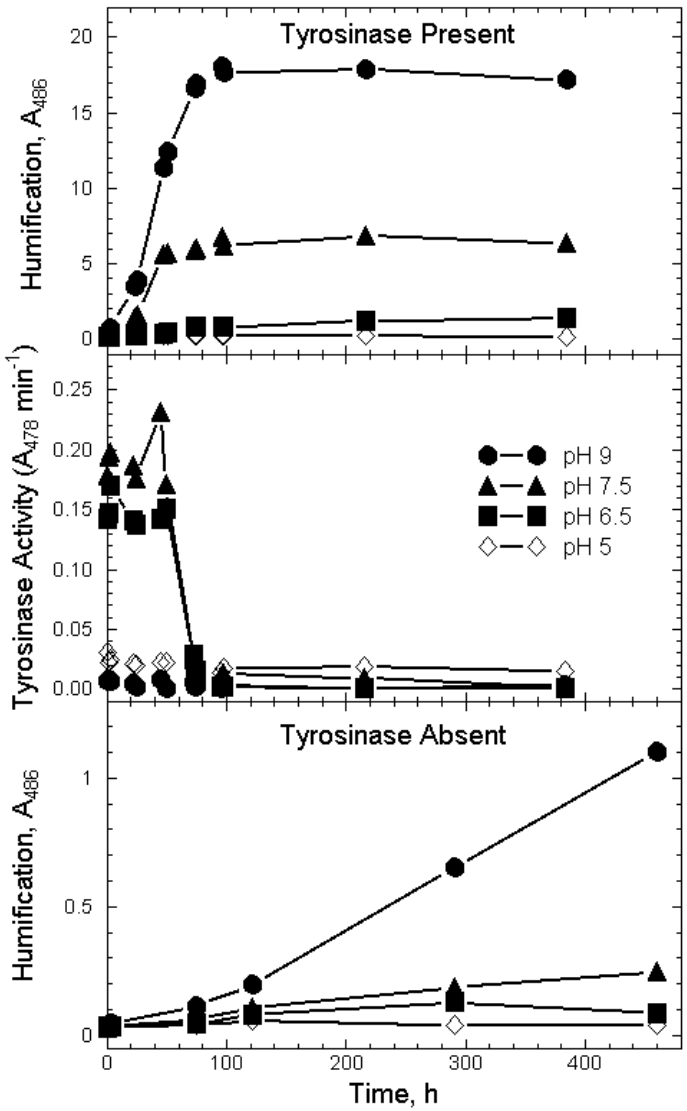


Fly Ash and pH

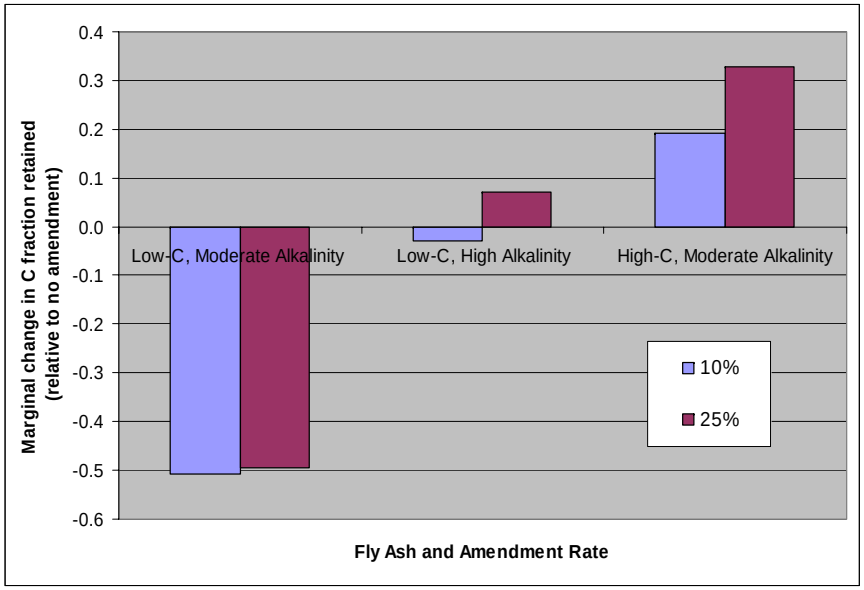


Effect of Alkaline Fly Ash

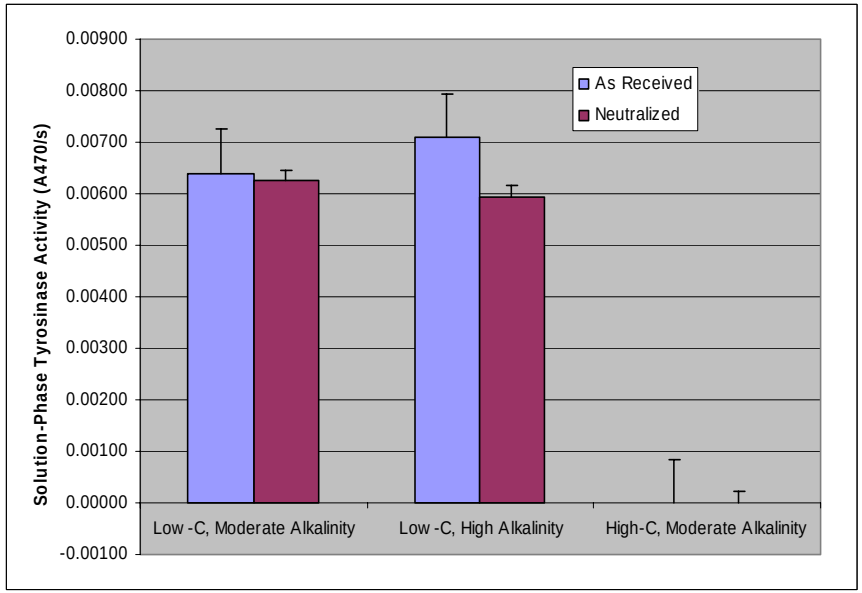
- Three mechanisms involved in humification:
  - Physical stabilization
  - Direct Oxidation
  - Promotion of Oxidation and Condensation by Alkalinity
- Enzyme-mediated oxidation optimal at pH ~7
- Large pH dependence of condensation and nonenzymatic path drives optimum to higher pH
- Liming of soils key to enhancing net soil C sequestration
- C costs of lime/fly ash transportation need to be considered



Soil Amendments and Impact of Fly Ash Properties



Marginal Change in Soil C Fraction Retained (Relative to No Amendment)



Charcoal Content on Solution-Phase Enzyme Activity

Fly Ash and Humification in Soils

- Carbonate content of soil must be considered!
  - If no carbonate, then lignitic and sub-bituminous ashes probably better
  - If carbonate present, then need high-C ash to minimize reaction of organic acids to release inorganic carbon
- For soils too distant from source of ash to make net sequestration feasible, management to maximize *wetting/drying cycles*, promote *moderate to alkaline pH*, and form *Fe and Mn oxides* is advised

Field "Minicosm" Study

- Two soils (ca. 60 kg/tank)
  - A (2.7%C) horizon (Lenoir Series, Aeric Paleaquult)
  - E (0.5%C) horizon (Goldsboro Series, Aquic Paleudult)
- Three pH treatments
  - 4.1 (native), 6.5 (lime), 6.5 (alkaline fly ash)
- Four hydrologic treatments
  - Saturated; dry to field capacity (ca. 0.1 bars), then maintained
  - Saturated; dry to ca. 1 bar, then maintained
  - Saturated; dry to ca. 3 bars (controls saturation cycle length)
  - Maintained at ca. 3 bars
- Three replicates (total of 72 experimental units)
- Simpler model humification reaction
  - Three monomers (resorcinol, p-hydroxybenzoic acid, and L-glycine)
  - E soils receive ^{13}C-enriched glycine
  - ca. 800 g C/tank added in four 200-g aliquots (total of four hydrologic cycles)
  - enzymes as provided by soil
- Started May 2004, first cycle length ca. 10 weeks

Measurements

- Monitoring
  - Moisture @ 10 cm
  - Temperature
  - Redox potential (Pt electrode)

- Sampling
  - Leachates: DOC, dissolved oxygen, total phenols, $\delta^{13}C$
  - Gas emissions: CO_2 (Licor static chamber); N_2O?
  - Soil cores: POM, MOM, total C and N, $\delta^{13}C$

Santee Soil Humification – Crust Properties

Higher C levels and less ^{13}C in crusts formed on ash treated soils

Effect of soil amendment			
Mean	Ash	Control	Limed
[C] %	2.6	1.4	1.9
$\delta^{13}C$ ‰	+64.4	+85.3	+90.1

Lower C levels and less ^{13}C in crusts formed under most heavily watered soil moisture regime (leaching effect?)

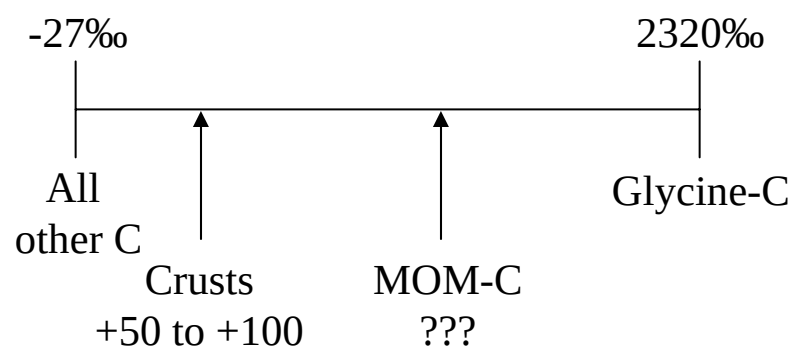
Effect of watering regime				
Mean	T1 (driest)	T2	T3	T4 (wettest)
[C] %	3.2	1.8	2.1	0.9
$\delta^{13}C$ ‰	+94.8	+86.7	+85.4	+52.8

Crust in tanks w/o ^{13}C-labeled glycine was -24.2 ‰

Santee Soil Humification – Partitioning of Soil C Stocks

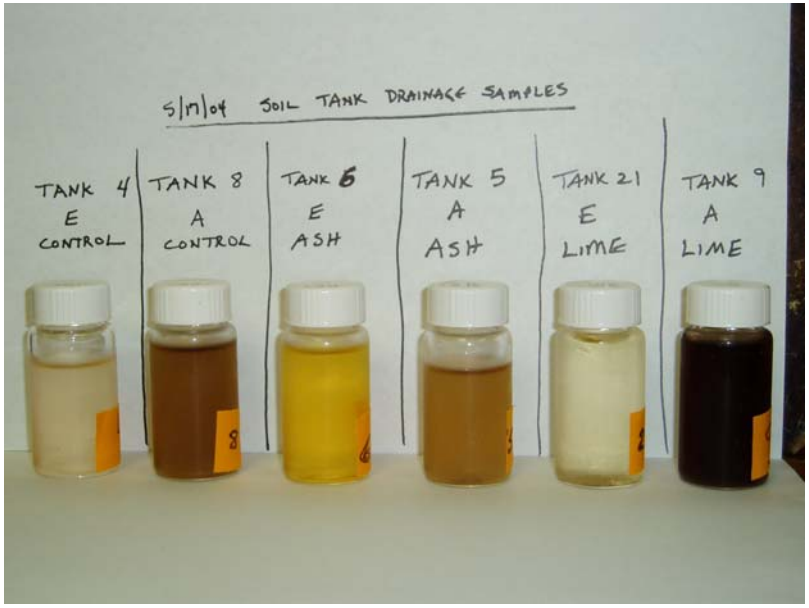
- Proportion of soil C in particulate organic matter and mineral-associated organic matter is not different between A- and E-horizons
- Humification products should associate with MOM
- ^{13}C analysis of POM and MOM soil fractions is planned
- Signature of ^{13}C-glycine in monomer solution was 2320 ‰
- 1.6 to 6.4% of C in soil crusts was derived from glycine monomer

Partitioning of soil C between POM and MOM in A- and E-soils		
Variable	A-horizon	E-horizon
POM-C (mg g^{-1} soil)	7.5 (0.06)	1.3 (0.01)
MOM-C (mg g^{-1} soil)	20 (0.04)	3.5 (0.06)
Fraction POM-C	0.28 (0.08)	0.28 (0.05)
Coefficient of variation in parenthesis		



Santee Soil Humification – ^{13}C in Leachates

- Leachate samples were evaporated (~45 °C) and redissolved in deionized water
- Volume of leachate adjusted to yield ~ 800 µg C for IRMS
- Concentrated samples were placed on chromosorb and analyzed for $^{13}C/^{12}C$ ratios
- Preliminary analysis of 9 leachate samples indicated $\delta^{13}C$-values range from -1.8 to 144 ‰



- Mixing model calculations suggest that 1 to 7% of the DOC in these leachates originated from ^{13}C-labeled glycine in monomer solution

Significance & Summary

- Laboratory research suggests that humification can be enhanced by:
  - Physical stabilization of enzyme and products in microporous materials (silica, charcoal)
  - Maintenance of high pH (liming, alkaline fly ash addition)
  - Increasing frequency of wetting/drying cycles
  - Practices that maintain optimum levels of soil oxide phases [Mn(IV), Fe(III)]
- Field research to verify these observations is planned or already in progress