

Fly Ash Amendments Catalyze Soil Carbon Sequestration

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Abstract

We tested the effects of four alkaline fly ashes {Class C (sub-bituminous), Class F (bituminous), Class F [bituminous with flue-gas desulfurization (FGD) products], and Class F (lignitic)} on a reaction that simulates the enzyme-mediated formation of humic materials in soils. The presence of FGD products completely halted the reaction, and the bituminous ash showed no benefit over an ash-free control. The sub-bituminous and lignitic fly ashes, however, increased the amount of polymer formed by several-fold. The strong synergetic effect of these ashes when enzyme is present apparently arises from the combined effects of metal oxide co-oxidation (Fe and Mn oxides), alkaline pH, and physical stabilization of the enzyme (porous silica cenospheres).

Introduction

A significant *enhancement* of C-sequestration by terrestrial ecosystems is needed to help offset the growth in atmospheric CO₂ inputs during the transition from fossil fuels to renewable and alternative-energy sources over the next 50-100 years. While trees and the ocean are important sinks for C, soils can make a large one-time contribution to this effort if ways can be found to return them to pre-agricultural levels of C. Our part of the challenge is to explore ways of enhancing net sequestration of C by soils while minimizing release of other GHGs. In order to shift the equilibrium soil-C to higher levels, either an increase in the formation rate or a decrease in the degradation rate is needed:

$$K_{eq} = k_f/k_r$$

While incompletely understood, the humification process by which soil C is stabilized (Stevenson, 1994) is believed to involve several parallel pathways (Fig. 1). Of these, the polyphenol formation pathway generally dominates. The rate-limiting step for this pathway is believed to be the oxidation of polyphenols to polyquinones, which then polymerize with amino acids to form humic material (Fig. 2). This oxidative polymerization reaction is catalyzed by polyphenol oxidase (PPhO) enzymes such as tyrosinase (Martin and Haider, 1969, 1971; Nelson et al., 1979), but soil minerals such as allophane (Kyuma and Kawaguchi, 1964), Fe and Mn oxides (Shindo and Huang, 1984; Stone and Morgan, 1984; McBride 1987), and smectites (Kumada and Kato, 1970; Thompson and Moll, 1973; Filip et al., 1977; Wang et al., 1978) also promote the reaction.

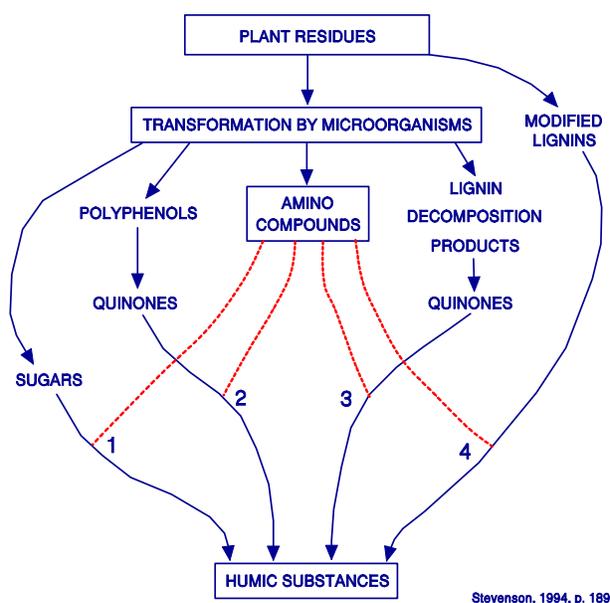
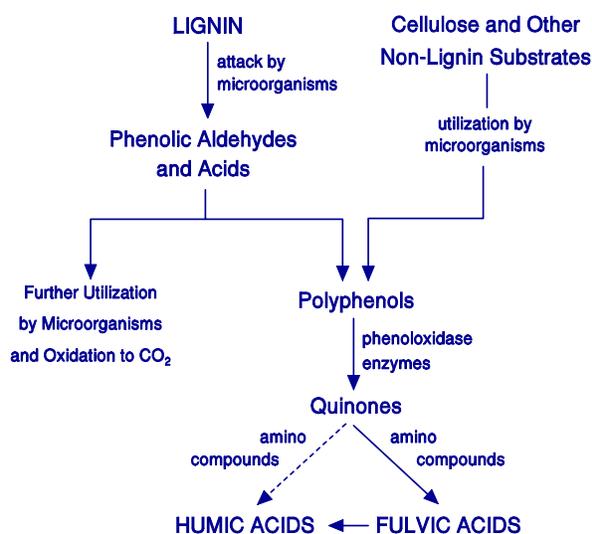


Fig. 1. Pathways for the formation of humic materials (after Stevenson, 1994, p. 189).



Stevenson, 1994, p. 190

Fig. 2. The polyphenol pathway of humification (after Stevenson, 1994, p. 190).

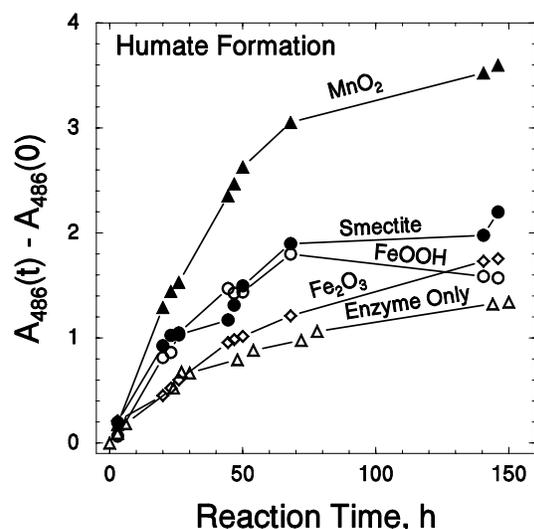


Fig. 3. Rates of enzyme catalyzed humification induced by Fe(III) and Mn(IV) mineral suspensions and by a solution containing no additional mineral oxidant (Amonette et al., 2000).

Amonette et al. (2000) observed a synergetic effect when oxides or smectite minerals were reacted in the presence of tyrosinase (Fig. 3). As phenols are toxic to microorganisms, and the activity of phenol oxidases depends on adequate $O_2(aq)$, the rate of humification as well as that of oxidation is slow in highly anoxic environments such as peat bogs (Freeman et al., 2001). Under highly oxidizing conditions, on the other hand, degradative reactions involving hydrolase enzymes (Hy) dominate and net humification is nil. Under sub-oxic conditions, however, phenol concentrations are high enough to inhibit hydrolase activity while allowing polymerization activity to continue (Fig. 4). We can express these factors controlling humification in a general rate law:

$$d[Hu]/dt = k_0[O_2, H^+] + k_1[PPhO, O_2, H^+] + k_2[MnOx, O_2, H^+] + k_3[FeOx, O_2, H^+] + k_4[Fe\text{-smectite}, O_2, H^+] + k_5[MnOx, PPhO, O_2, H^+] + \dots - k_6[Hy, O_2, H^+] - k_7[Hy, Mineral, O_2, H^+] - \dots$$

where the concentration of humic monomers (i.e., $[Hu]$) is in excess.

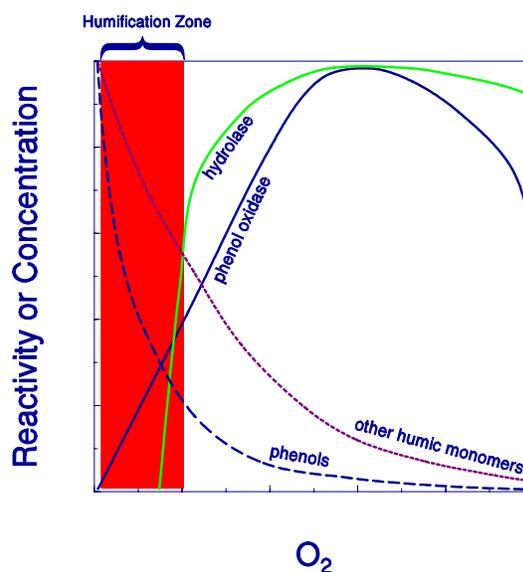


Fig. 4. Humification hypothesis for soil showing relation between the concentrations of oxygen, phenols, and other humic polymers, and the activities of polyphenoloxidase and hydrolase enzymes.

Because fly ash has physical properties that promote sub-oxic microenvironments, often contains high quantities of oxide minerals, and is readily available as an inexpensive soil amendment, we examined its ability to promote the humification reaction.

Experimental

Materials

In addition to the four fly ashes, we studied two model silica compounds: 1) porous silica (Davisil, 15-nm mean pore diameter), and 2) nonporous quartz sand. Selected physical properties of these materials are given in Table 1. Scanning electron micrographs of the fly ashes revealed a dense aggregate of cenospheres coated with fine particles (Fig. 5).

Organic monomers for the model humification reaction included orcinol, resorcinol, p-hydroxybenzoic acid, L-glycine, L-serine, and vanillic acid (Amonette et al., 2000). Tyrosinase with an initial activity of about 2400 units/mg was obtained from Sigma Chemical.

Table 1. Selected physical properties of fly ash and silica materials.

Fly Ash and Related Materials	Surface Area (m ² /g)	Pore Volume (cc/g)	Avg Particle Size (μm)
Bituminous (Class F)	26	0.043	18
Bituminous + FGD (Class F)	9	0.035	13
Sub-bituminous (Class C)	1.3	0.006	15
Lignitic (Class F)	1.2	0.005	11
Porous Silica (Davisil)	308	1.15	250-500
Quartz Sand	0.54	0.001	212-300

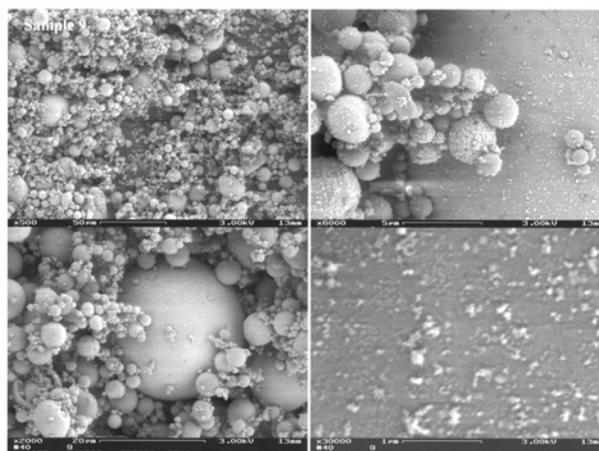


Fig. 5. Scanning electron micrographs of sub-bituminous fly ash sample.

Batch Experiments

Enzyme Stability--With no organic monomers present, various amounts of fly ash or porous silica were reacted with tyrosinase (1 mg/mL) in a 25-30 mL phosphate-buffered (0.1 M, pH 6.5) solution. Samples were shaken at 150 rpm. After periods ranging from 1 to 18 days, the suspensions were centrifuged, and 10 μL aliquots analyzed for tyrosinase activity using the rate of the reaction with L-DOPA as determined by change in absorbance at 478 nm.

Humification--We prepared a mixture of the organic monomers at 2 mM concentration in a 100 mM phosphate solution buffered at pH 6.5. A separate 1 mg/ml tyrosinase solution was also prepared. To a series of 7.5-ml polystyrene 1-cm-pathlength cuvettes, we added fly ash, quartz, or Davisil, 1 ml buffer, 3.5 ml of the buffered organic monomer solution, and 0.5 ml of the tyrosinase solution. Each cuvette was then capped and incubated at 22°C. Preliminary experiments showed no effects of room lighting on the reaction nor any need to continuously supply oxygen to the cuvettes. At selected intervals after mixing, an aliquot of the mixture in the cuvette was centrifuged and an absorbance spectrum collected.

Intermediate-Scale Humification Experiments

Davisil and quartz sand particles were coated with Mn-oxide or smectite, packed into reactors with water-impermeable, gas-porous walls (Fig. 6), inoculated with a monomer/tyrosinase solution, and then incubated under a variety of environmental conditions including cycling between wetting and drying and between oxic and anoxic conditions. After 8 weeks, samples were extracted with water and amount of C released was determined. From this the amount of C retained (i.e., humified) in the solid phase was estimated based on the original amount of C added.



Fig. 6. Experimental apparatus for intermediate-scale humification experiments consisting of porous-walled reactor filled with smectite coated sand (left) and mounted on gas manifold (right) for control of moisture and redox conditions.

Results

Porous Silica

In the intermediate-scale humification experiments, average C retention values (14 reps per solid phase across all environmental conditions) show that the Davisil particles (D/MnOx, D/MgCly in Fig. 7) are clearly superior to the quartz particles. As the chemistry of Davisil and quartz is similar, this result suggests that physical parameters such as microporosity are very important. Based solely on its physical properties, then, fly ash would be expected to have a large beneficial impact on humification.

The importance of microporosity is clearly evident in the batch humification experiments. All the humified material is concentrated in the Davisil particles (far right, Fig. 8), whereas much is suspended in the treatments with quartz particles (second from right, Fig. 8), and in the absence of solid particles (center, Fig. 8). Davisil offers a high surface area for sorption and retention of humic polymers.

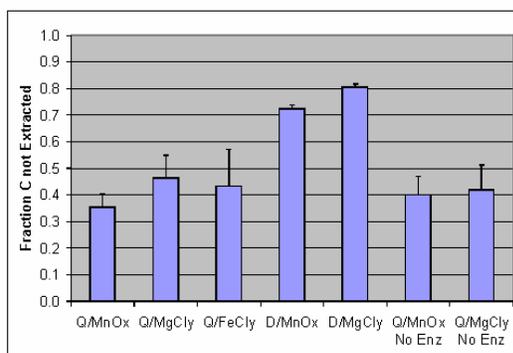


Fig. 7. Fraction of C not extracted after eight-week humification experiment involving variety of environmental conditions. Values are means over all treatments for each material (Q = Quartz, MnOx = Mn oxide, MgCly = Mg-saturated smectite, FeCly = Fe-saturated smectite, D = Davisil, No Enz = enzyme-free controls)

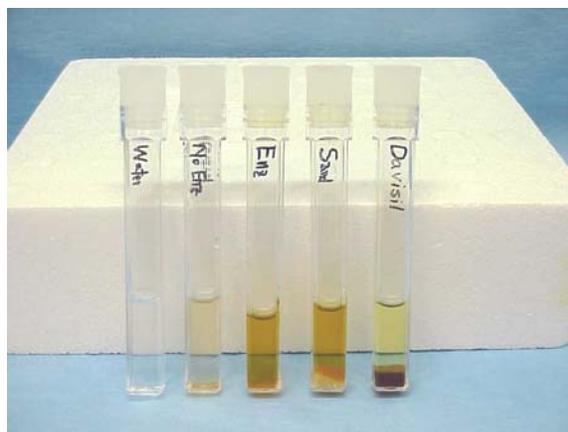


Fig. 8. Batch humification experiments in 7.5-mL cuvettes after three days of contact. Treatments (l to r) water control, monomer with no enzyme, monomer with enzyme, quartz sand in monomer with enzyme, and Davisil in monomer with enzyme.

Fly Ash

Enzyme Stability—The fly ashes differed significantly in their ability to stabilize the tyrosinase enzyme. After only two hours of contact, the activity of the enzyme was reduced by more than 80% with the bituminous ash, and by more than 98% with the bituminous ash that contained FGD products (Fig. 9). In contrast, the sub-bituminous and lignitic ashes actually enhanced the measured enzyme activity. These initial observations were confirmed in several long-term studies (Fig. 10), where the sub-bituminous and lignitic ashes offered significant stabilization of the enzyme relative to the “free enzyme” condition with no ash present.

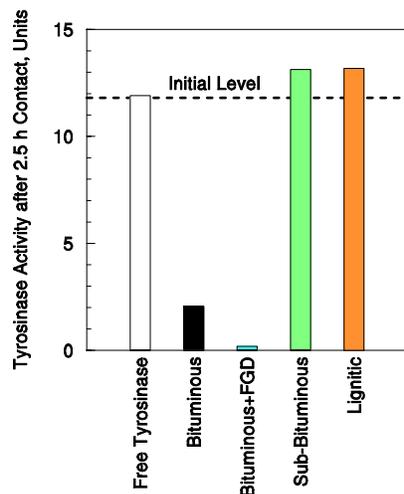


Fig. 9. Absolute activity of soluble and adsorbed tyrosinase after two hours of contact with buffered solution in presence and absence of fly ashes.

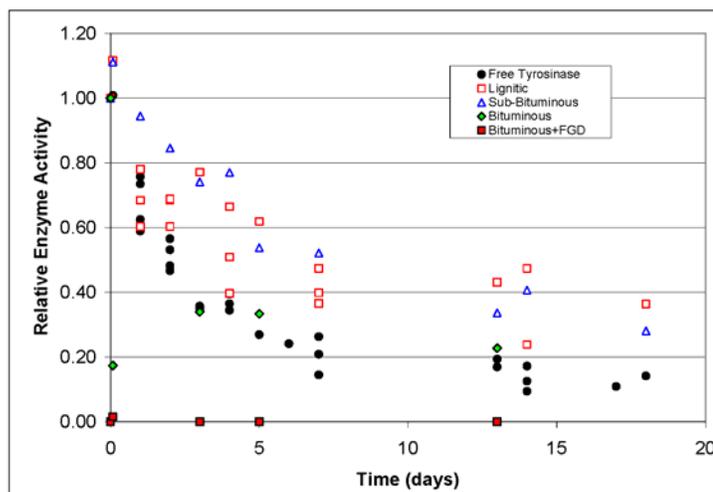


Fig. 10. Relative activity of tyrosinase after various periods of contact with buffered solution in the presence and absence of fly ashes.

Humification—The beneficial effects of the sub-bituminous and lignitic ashes extended to the actual humification reaction. As the amount of fly ash increased (enzyme and monomer concentrations constant), a darker solution was obtained, indicating more humification had occurred in three days of contact (Fig. 11). No reaction was seen in the absence of enzyme (Fig. 11, left). Perhaps more importantly, a strong synergetic effect was seen when both the enzyme and fly ash were present. The absence of either substance yielded much lower degrees of humification after three days than when both were present. This is shown visually (Fig. 12) and spectroscopically by the development of an absorption peak at 485 nm (Fig. 13).

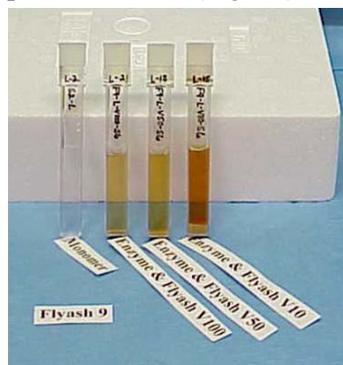


Fig. 11. Batch humification experiments with three concentrations of sub-bituminous fly ash after three days of contact. Enzyme-free control is at left

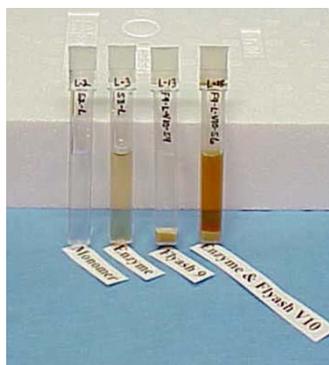


Fig. 12. Batch humification experiments after three days. Monomer solutions with (l to r) nothing, enzyme, flyash, and flyash plus enzyme.

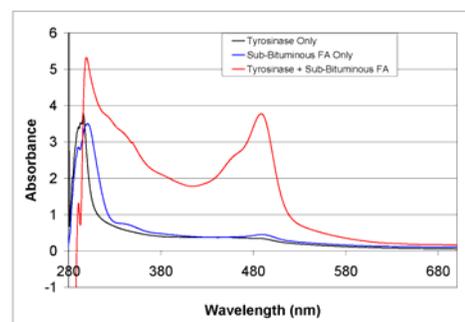


Fig. 13. UV-Vis absorption spectra for supernatants in batch humification experiment after 5 days contact with enzyme alone, sub-bituminous fly ash alone, and the combination of enzyme with fly ash.

Discussion

While the gross beneficial effects of the sub-bituminous and lignitic fly ashes on stabilization of tyrosinase and humification are evident, the particular properties responsible for these effects remain elusive. The experiments with porous silica indicate at least some of the effect may be purely due to physical stabilization of the enzyme inside nm-sized pores.

Chemical analysis of acid extracts from the fly ash (data not shown) indicate that these two ashes have two to three times as much Fe in them, and up to five times as much Cu as is in the bituminous ashes. The Fe is probably present at least in part, as an oxide, and from the earlier work with these minerals, may therefore help to account for the enhanced humification. The Cu is expected to be important as it is the central metal ion in the tyrosinase structure. However, unlike Fe oxide, separate batch studies in which different amounts of soluble Cu were added to the enzyme/monomer mixture had no effect on the humification rate (data not shown).

The ashes are alkaline, and it is possible that high pH may play a significant role in the enhanced activity. Equilibration of 1:10 suspensions of ash in 0.1-M, pH-6.5 phosphate buffer, yielded a pH of 8.9 for the sub-bituminous ash and 7.1 for the lignitic ash after three days, indicating substantial solid-phase alkalinity in the sub-bituminous ash. While this alkalinity seemed to have little bearing on the stabilization of the enzyme (i.e., the two ashes yielded similar results), it did have a strong effect on the humification results. The high-alkalinity sub-bituminous ash treatments yielded nearly four times greater humification in three days than the moderate-alkalinity lignitic ash treatments (data not shown).

As they exhibit both significant alkalinity and high specific surface, the depressive effects of the bituminous ashes on tyrosinase activity likely stem from chemical effects. Humification requires a supply of oxygen to react with the polyphenols to generate quinones. Because FGD products are strongly reducing in nature (typically containing calcium sulfite) they would suppress humification by removing oxygen from the system. The oxidizing/reducing nature of the bituminous ash is unknown, but from the low Fe content, it is likely to be less oxidizing than the sub-bituminous and lignitic ashes that were tested.

Conclusions

The sub-bituminous and lignitic alkaline fly ashes that we tested both stabilized the tyrosinase enzyme and enhanced humification. In contrast, the bituminous fly ashes suppressed these reactions substantially. The presence of FGD products in one of the bituminous ashes completely inhibited tyrosinase activity after only a few hours of contact. Nanometer-sized pores, high alkalinity, and presence of Fe oxides in the sub-bituminous and lignitic ashes are believed to be responsible for their beneficial effects. Where feasible from a net C-accounting standpoint, amendments of degraded soils with ashes similar to these two is expected to yield significant increases in soil C sequestration. Soil management techniques that promote Fe oxide formation and moderate to alkaline pHs might be applied in other situations to achieve comparable results.

Acknowledgments

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