

# Enhancement of Soil Carbon Sequestration by Amendment with Fly Ash

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## ABSTRACT

Degraded lands (e.g., mine sites, highway rights of way, eroded lands) typically are depleted of organic-C. Progress in the reclamation of these lands can be measured, in part, by the return of soil organic-C levels to those seen in undisturbed, otherwise similar, soils. Moreover, such reclamation has the added benefit of sequestering significant amounts of C, thus helping buffer the transition to a hydrogen-based energy system. Our research seeks to understand the process by which organic-C is sequestered by soils (i.e., humification) and to develop practical approaches for enhancing the rate at which this sequestration occurs. We have focused on a model humification reaction involving a common phenoloxidase enzyme (tyrosinase) and organic monomers (polyphenols, hydroxybenzoic acids, and amino acids). We have noted a synergetic effect on the reaction rate in aqueous suspensions when highly porous silica or manganese-oxide minerals are present in addition to the enzyme. As the porous silica bears some similarity to the cenospheres commonly found in fly ash, we also 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 the reaction. In aqueous suspensions, 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 reaction rate by several-fold. A more complicated response was observed in an eight-week intermediate-scale experiment involving a calcareous mine soil that was amended with the four fly ashes, contacted one or more times with a monomer/enzyme solution, and then allowed to air dry. Net humification, as determined by resistance of C to aqueous extraction relative to that for the unamended soil, was greatest with the bituminous fly ash, and only marginal with the sub-bituminous ash. A net loss of C was observed with the other two ashes, and was attributed to dissolution of limestone in the soil by the organic monomers and humic polymers. The high specific surface and residual coal content of the bituminous ash were believed responsible for its superior retention of C and minimal dissolution of limestone. The possible contributions of other fly ash properties, such as alkalinity and iron oxide content are discussed.

## INTRODUCTION

One of the approaches to minimizing the possible effects of climate change stemming from the recent and significant increases in atmospheric CO<sub>2</sub> levels involves fixing or storing C as biomass in terrestrial ecosystems. Fixation of C in woody plants (e.g., trees) is well known and offers a quick and environmentally sound way of removing C from the atmosphere. Another, even larger, potential reservoir for C storage is as organic matter in soil (Metting et al., 2001). Currently, this soil organic matter, termed humus, contains about twice as much C as is in the atmosphere (Eswaran et al., 2000).

Historically, many agricultural soils contained as much as 50% more humus before cultivation as they do now. Indeed, before 1970, more C was lost from soils to the atmosphere as a result of land-use changes than was emitted by fossil-fuel combustion (Houghton et al., 1983). In the present context, this inherited soil-C deficit represents a potential reservoir for C, the refilling of which can help buffer the transition to less C-intensive fuels over the next 30-50 years.

Disturbed and degraded lands, such as reclaimed mine lands and highway rights of way, offer the greatest potential for C storage by virtue of having low humus contents in comparison to adjacent undisturbed lands. Many of these mined lands result from coal production, and are located close to power plants where substantial quantities of fly ash and other coal combustion byproducts (CCBs) are created. As these CCBs present a significant disposal problem there is a strong need to find new beneficial uses for them.

Our research has focused on understanding the fundamental process by which humus is created (i.e., humification) and extending this knowledge to find ways of enhancing the rate of humification. The rate-limiting step in the humification process appears to be the oxidation of polyphenol compounds to quinones (Stevenson, 1994). These quinones then react with peptides and amino acids to form large melanin-like polymers that resist further degradation by microorganisms.

Soil fungi produce enzymes such as polyphenol oxidases and laccases that catalyze the oxidation step (Martin and Haider, 1969; 1971). Soil minerals, such as iron and manganese oxides, can also perform this function (Shindo and Huang, 1984; Stone and Morgan, 1984; McBride, 1987). Recently, we observed a significant synergetic effect when a polyphenol oxidase (tyrosinase) and oxide mineral were both present (Amonette et al., 2000). As soil enzyme activity depends on structural conformation, and longevity depends on protection from microbial predation, we are currently examining the nature of enzyme attachment to soil particles and the impact of physical properties such as pore size on activity and longevity.

Because of the ready availability of fly ash coupled with its typical cenospheric particle shape and high metal oxide content, we have been studying the potential use of fly ash as a soil amendment to enhance the humification process (Amonette et al., 2003). In this paper we summarize the results of our work with four alkaline fly ash amendments alone, and when used to amend a mine soil containing large amounts of inorganic C.

## MATERIALS AND METHODS

### Materials

Four alkaline fly ashes were obtained from facilities in Texas and Colorado. These fly ashes included three Class F ashes derived from bituminous and lignitic coal, and one Class C ash from sub-bituminous coal. One of the bituminous Class F ashes also contained significant amounts of flue-gas-desulfurization (FGD) product. In addition to the four fly ashes, we studied two model silica compounds obtained from Aldrich Chemical Company: 1) porous silica (Davisil, 15-nm mean pore diameter), and 2) nonporous quartz sand. The porous silica was used as a model compound to simulate the physical properties of fly ash. Unreclaimed soil from a mine site in Virginia was also used in the study. Selected physical and chemical 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. 1).

**Table 1. Selected physical and chemical properties of fly ash, silica, and soil materials.**

Fly Ash and Related Materials	Surface Area (m <sup>2</sup> /g)	Pore Volume (cc/g)	Mean Particle Size (μm)	pH <sup>a</sup>	Total C (wt%)	Extractable Fe <sup>b</sup> (μg/g)
Bituminous (Class F)	26	0.043	18	7.8	10.10	2060
Bituminous + FGD (Class F)	9	0.035	13	7.2	2.63	1310
Sub-bituminous (Class C)	1.3	0.006	15	10.5	0.05	1050
Lignitic (Class F)	1.2	0.005	11	7.8	0.09	5100
Porous Silica (Davisil)	308	1.15	250-500	— <sup>c</sup>	—	—
Quartz Sand	0.54	0.001	212-300	—	—	—
Virginia Mine Soil	—	—	—	—	4.83	900

<sup>a</sup>0.5 g in 5 mL 0.1 M phosphate buffer (pH 6.5) after 30 days

<sup>b</sup>Extracted by NH<sub>2</sub>OH using method of Chao and Zhou (1983)

<sup>c</sup>Not determined

Our experiments involved a model humification reaction based on that of Nelson et al. (1979) in which organic monomers including polyphenols, hydroxybenzoic acids, and amino acids are reacted with a polyphenol oxidase enzyme (tyrosinase) to yield humic polymers. Organic monomers for this 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. One unit of enzyme activity corresponds to the ability to increase the

absorbance of L-dihydroxyphenylalanine (L-DOPA) at a wavelength of 280 nm by 0.001 absorbance units per minute at 25°C at pH 6.5.

#### 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  $\mu$ L aliquots analyzed for tyrosinase activity using the rate of the reaction with L-DOPA as determined by a change in absorbance at 478 nm.

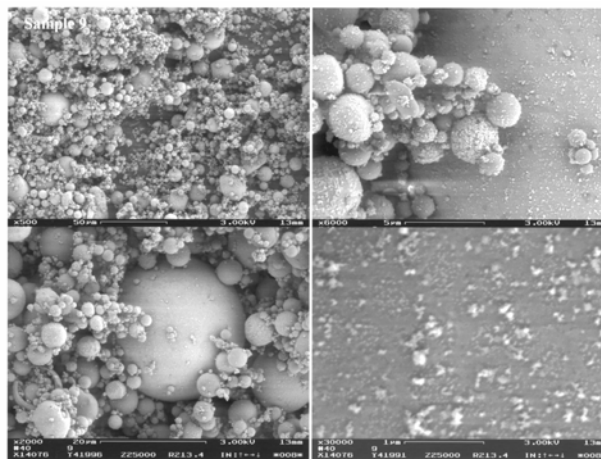


Fig. 1. Scanning electron micrographs of sub-bituminous fly ash sample.

*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. 2), 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 the 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.

Similar experiments were conducted using the Virginia mine soil either unamended or amended to levels of 10 or 25 wt% with fly ash. The soil/fly ash studies were conducted under oxic conditions. Monomer/tyrosinase was added with the same amount of monomer split between one, two, or four additions, and in single additions with two times or four times as much monomer as in the split additions. With split additions, the system was allowed to air dry for a minimum of two weeks before adding more monomer.



Fig. 2. 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 with silica, average C retention values (14 reps per solid phase across all environmental conditions) show that the Davisil particles (D/MnOx, D/MgCly in Fig. 3) 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.

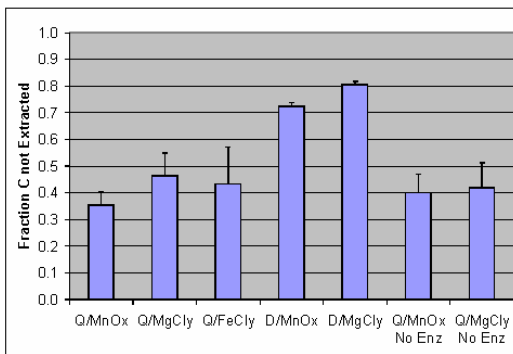


Fig. 3. Fraction of C not extracted after eight-week humification experiment involving variety of environmental conditions. Values are means over all treatments for each material (Q = Quartz, MnOx = Mn oxide, MgCly = Mg-saturated smectite, FeCly = Fe-saturated smectite, D = Davisil, No Enz = enzyme-free controls)



Fig. 4. 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.

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. 4), whereas much is suspended in the treatments with quartz particles (second from right, Fig. 4), and in the absence of solid particles (center, Fig. 4). Davisil offers a high surface area for sorption and retention of humic polymers.

## Fly Ash

**Enzyme Stability**—When reacted in aqueous suspensions, 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. 5). 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. 6), where the sub-bituminous and lignitic ashes offered significant stabilization of the enzyme relative to the “free enzyme” condition with no ash present. The bituminous ash, however, maintained the enzyme activity at the same level as the free enzyme.

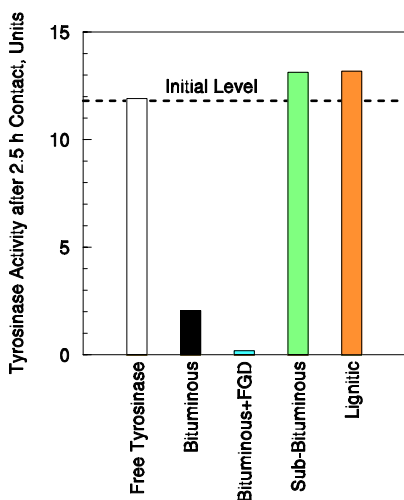


Fig. 5. Absolute activity of soluble and adsorbed tyrosinase after two hours of contact with buffered solution in presence and absence of fly ashes.

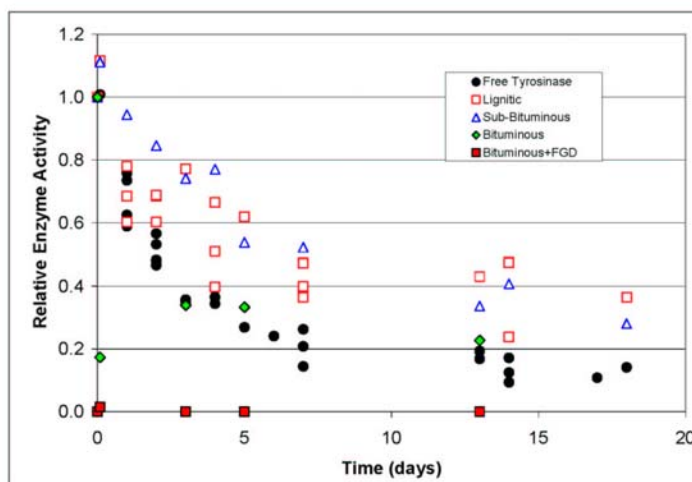


Fig. 6. Relative activity of tyrosinase after various periods of contact with buffered solution in the presence and absence of fly ashes.

**Batch 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. 7). No reaction was seen in the absence of enzyme (Fig. 7, 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. 8) and spectroscopically by the development of an absorption peak at 485 nm (Fig. 9).

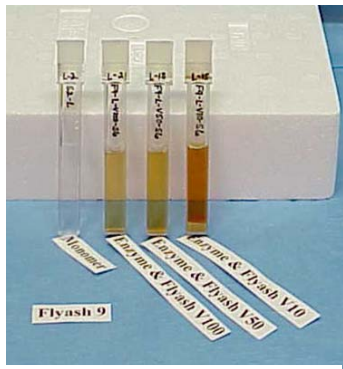


Fig. 7. Batch humification experiments with three concentrations of sub-bituminous fly ash after three days of contact. Enzyme-free control is at left

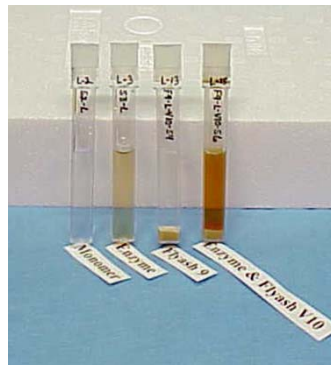


Fig. 8. Batch humification experiments after three days. Monomer solutions with (l to r) nothing, enzyme, flyash, and flyash plus enzyme.

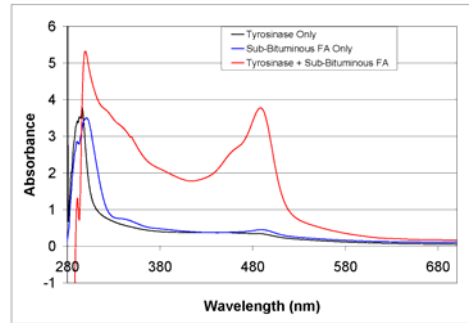


Fig. 9. UV-Vis absorption spectra for supernatants in batch humification experiment after 5 days contact with enzyme alone, sub-bituminous fly ash alone, and the combination of enzyme with fly ash.

*Intermediate-Scale Humification*—With respect to the manner of monomer addition, the unamended soil showed more C retained when split additions of monomers were made (Fig. 10). Smaller fractions of added C were retained when the initial monomer concentration was doubled or quadrupled. A net loss of C was observed at the highest initial monomer concentration, suggesting that the monomer was reacting with the carbonate minerals present in the soil to release more inorganic C than was precipitated as humic material.

The same general trends with monomer addition strategies were seen for the soil amended with the three Class F ashes (bituminous, bituminous+FGD, and lignitic) although the degree of carbonate dissolution varied. For the soils amended with lignitic or bituminous+FGD ash, even the primary monomer addition yielded a net loss of C from the system (Fig. 11).

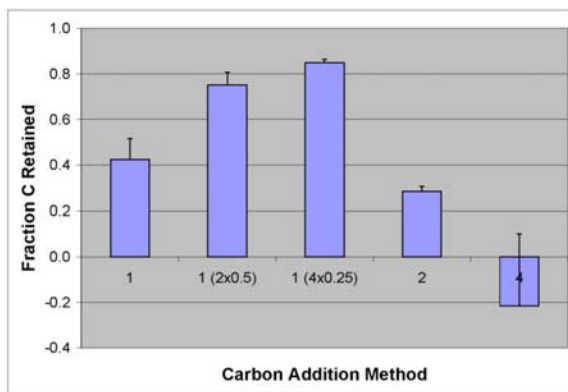


Fig. 10. Net fraction of C added as monomer that was retained by the unamended mine soil with split additions of monomer or doubling and quadrupling of monomer amounts.

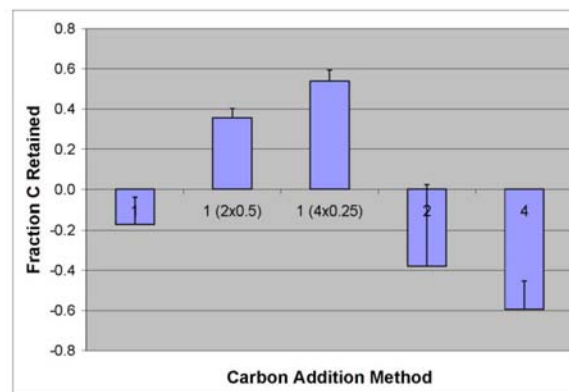


Fig. 11. Net fraction of C added as monomer that was retained by the mine soil amended with 25% lignitic fly ash. Monomer additions same as Fig. 10.

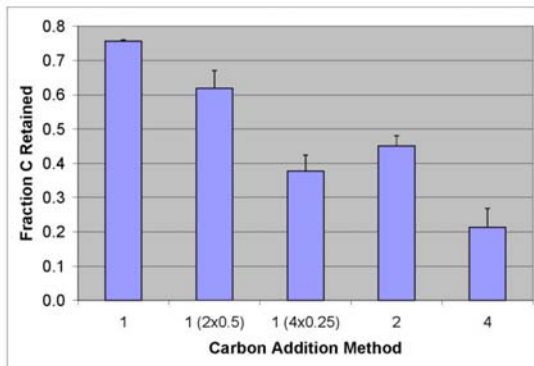


Fig. 12. Net fraction of C added as monomer that was retained by the mine soil amended with 25% sub-bituminous fly ash. Monomer additions same as Fig. 10.

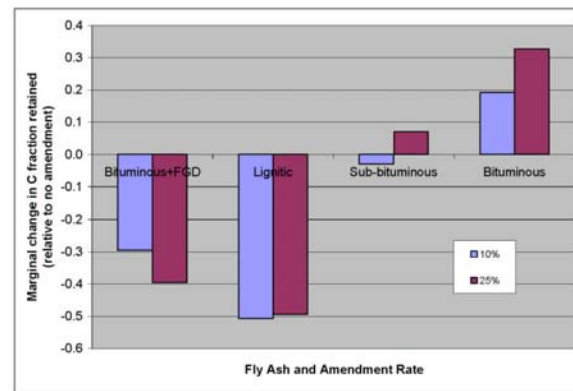


Fig. 13. Marginal change in net fraction of added C retained by the mine soil amended with fly ashes at a 10% or 25% level.

When the sub-bituminous Class C ash was used to amend the soil, a different profile was seen (Fig. 12). Positive net C retention by the system was seen, but both split additions and larger additions of monomer caused decreases. The high alkalinity of this ash (Table 1) likely minimized the dissolution of carbonate, although it is unclear why split additions also resulted in decreases in net C retention.

As the ultimate test of the fly ash amendments we compared the marginal change in net C fraction retained (i.e., the net C fraction retained by the fly ash amended system minus the net C fraction retained by the unamended soil). The results of this comparison (Fig. 13) show decreases in the C retained on the order of 30-50% of the C added as monomer when the bituminous+FGD or lignitic fly ashes were used as amendments. The situation improved to essentially no net change when the sub-bituminous fly ash was added, and to a net gain of 20-30% with amendment by the bituminous ash.

## DISCUSSION

While the gross beneficial effects of the sub-bituminous and lignitic fly ashes on stabilization of tyrosinase and humification in aqueous suspensions 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 (Land et al., 2003). 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 (Table 1), and it is possible that high pH may play a significant role in the enhanced activity. The sub-bituminous ash, in particular, carries substantial solid-phase alkalinity. While this alkalinity seemed to have little bearing on the stabilization of the enzyme (i.e., the sub-bituminous and lignitic ashes yielded similar results), it did have a strong effect on the humification results, both in aqueous suspension and in the intermediate-scale experiments with soil. In aqueous suspension, 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 it exhibits high specific surface, the depressive effect of the bituminous ash on tyrosinase activity likely stems from surface chemical effects. The substantial coal content in the bituminous ash likely provided a large hydrophobic surface that may have sorbed the monomers and humic polymers, thus preventing them from attacking the carbonates in the soil. Such sorption would also apply to tyrosinase itself and may have lowered its activity, thus accounting for the results in Fig. 5. Although enzyme activity with the bituminous ash was lower than for the sub-bituminous and lignitic ash, its ability to minimize dissolution of the carbonate resulted in the bituminous ash being the best amendment for this calcareous mine soil.

The presence of FGD products with bituminous ash, however, clearly shut down the humification reaction, which 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 itself is unknown, but from the low Fe content and high C content, it is likely to be less oxidizing than the sub-bituminous and lignitic ashes that were tested.

Lastly, we note that aqueous extraction studies on all the fly ashes and the soil in the absence of monomers produced negligible amounts of C. This result reinforces the argument that it is the degree to which the monomers react with the fly ash that determines the net C sequestration that can be achieved with a calcareous soil.

## **CONCLUSIONS**

In aqueous suspensions, 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. Sorption and partial deactivation of the enzyme by the coal present in the bituminous ash likely decreased its reactivity towards humification. This sorption also minimized contact between the humic monomers/polymers with carbonate present in the soil, thus yielding the largest net C sequestration of any ash

amendment. 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 non-calcareous degraded soils with ashes similar to these two is expected to yield significant increases in soil C sequestration. Such increases, however, will be severely impaired, if not reversed, when ashes with minimal hydrophobic sorption capacity are added to soils that already contain substantial carbonate. For these soils, addition of a high-C bituminous fly ash will yield better C sequestration results. Clearly, the properties of both the fly ash and of the soil must be considered before amendment recommendations for optimum C sequestration can be made. For soils too distant from a source of fly ash, adoption of soil management techniques that promote frequent wetting and drying cycles, Fe oxide formation, and moderate to alkaline pHs is expected to yield comparable results to fly ash addition.

## ACKNOWLEDGMENTS

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