Biochar Reduces Apparent Soil Enzyme Activities

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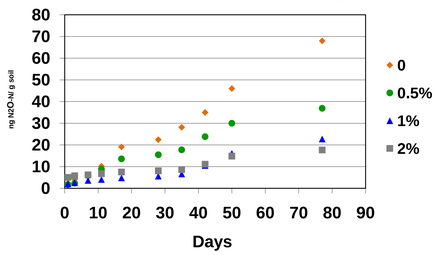
Abstract

Recently, biochar has received attention as a potential agent of enhanced soil carbon sequestration. However, it is a highly bioactive compound with a high capacity for sorption of biomolecules. In preliminary research for an incubation of soil with 1% biochar (wt%), we observed a significant decrease (75%) in the enzyme activity measured in biochar-amended soils when compared with soils free of biochar. In vitro experiments were therefore initiated to determine whether the biochar was inhibiting the enzyme activity or altering the model substrate. Purified β-glucosidase was reacted with biochar then filtered, as was the model substrate *p*-nitrophenyl-β-D-glucoside (PNG). The biochar-exposed enzyme showed no decrease in activity when assayed with fresh substrate. However, the activity of both fresh and biochar-exposed β-glucosidase was significantly diminished when assayed with biochar-exposed PNG, suggesting that the biochar chemically altered or blocked the binding site of the substrate. When biochar was added directly to the tube in which fresh β-glucosidase and fresh PNG were reacting, no decrease in activity was measured, indicating that the binding affinity of this non-biochar exposed enzyme and substrate pair was rapid, and unperturbed by the biochar. Further research is needed into the effects of biochar on other enzyme-substrate pairs in order to determine the implications of decreased apparent soil enzyme activities on the overall health of the soil biochemical system.

Background

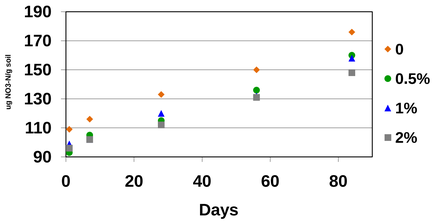
Biochar is being widely recommended as a soil amendment with potentially beneficial effects on soil water retention, CEC, and general condition. As well, the high proportion of recalcitrant C makes it particularly attractive for C sequestration purposes. However, little is known about how this soil amendment will affect the biological function of the soil, or key biochemical traits.

Cumulative N_2O-N in a Soil Amended With a Fast-Pyrolysis Biochar from Switchgrass

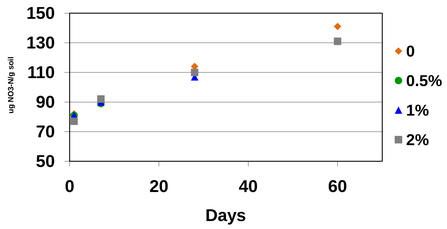


Biochar has been speculated to be highly sorbent, similar to activated C. It has been suggested that biochar sorbs N_2O, reducing estimates of denitrification. Alternatively, the reduction in N_2O emitted could be due to suppression of mineralization causing less nitrification and less N_2O production

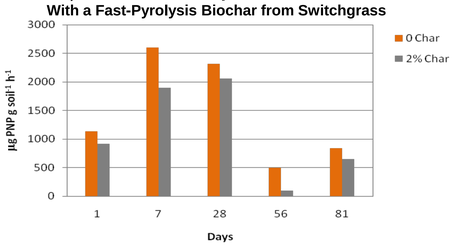
N mineralization Biochar A



N mineralization Biochar B



β-Glucosidase Activity in a Soil Amended With a Fast-Pyrolysis Biochar from Switchgrass



However, we have also noticed that biochar can have a pronounced effect on soil processes such as N mineralization and enzyme assays (β-glucosidase), even at relatively low levels.

Note that biochar A is a fast-pyrolysis biochar, and biochar B is a slow pyrolysis biochar, both made from the same starting stock of switchgrass.

Research Question:

Does biochar impede soil processes such as enzyme activities by blocking active sites on the enzyme or the substrate?

Approach

We conducted a simple *in vitro* experiment in which we separately reacted pure enzyme (β-glucosidase) and pure substrate (*p*-nitrophenyl-β-D-glucoside, PNG) with biochar, then centrifuged and filtered to remove the biochar. The biochar-exposed reactants were used in a series of reactions in an attempt to identify how the biochar was interfering with the overall reaction. The assay conditions were typical of the β-glucosidase standard assay, as outlined in Alef and Nannipieri (1995). The biochar was produced by fast pyrolysis of switchgrass.

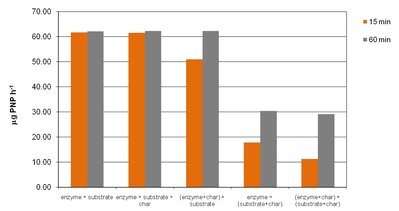
Reactions:

1. Control - Fresh β-glucosidase with fresh PNG.
2. Fresh β-glucosidase, fresh PNG, with char added to the reaction.
3. Biochar-exposed β-glucosidase reacted with fresh PNG.
4. Fresh β-glucosidase reacted with biochar-exposed PNG.
5. Biochar-exposed β-glucosidase reacted with biochar-exposed PNG.

Biochar exposure consisted of adding 0.3 g of biochar to 11 units of β-glucosidase (in 0.5 ml, aq) or equivalent substrate and shaking for 60 minutes. Biochar was removed by centrifuging , then filtering through a 0.2 µm syringe tip membrane.

All reactions were conducted at room temperature, using 11 units of enzyme (0.5 ml, aq), substrate added 1:1 with the enzyme, and when needed, 0.3 g of biochar. The assay was measured at both 60 minutes (typical for soils) and at 15 minutes (pure system).

Results



The only reactions to show diminished activity in response to biochar exposure were the two treatments in which the substrate, PNG, was exposed to biochar. After 60 minutes, the reaction with the biochar-exposed enzyme was not significantly different from the control.

The activity of the biochar-exposed enzyme was slightly diminished at 15 minutes, compared with the control, but by 60 minutes, it had reached the control level.

The addition of biochar to the control reaction (Reaction #2, above) did not affect the activity measured. Presumably the kinetics of the enzyme-substrate binding are so rapid or preferred that the char was unable to interact with the substrate and block the enzyme.

Implications

For β-glucosidase, biochar appears to temporarily block enzyme activity, but mainly perturbs the substrate, *p*-nitrophenyl-β-D-glucoside, reducing the measured enzyme activity by 80%. In a soil, this may suggest that a biochar amendment could severely impede biochemical functionality, thereby affecting the overall soil quality.

Given our earlier observations of biochar altering N_2O emission, this experiment will be repeated using different enzymes from the N-cycle.

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