ESF-ESCHANGE GRANT – FINAL REPORT
MOLTER PROGRAMME

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Project title	Effect of pyrogenic carbon in the forest soil on microbial community structure and functionalities
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1. Introduction

Pyrogenic carbon (PyC), incomplete combustion product of vegetation and fossil fuels (Goldberg, 1985), were regarded as chemically and biologically inert (Forbes *et al.*, 2006; Seiler & Crutzen, 1980). Short-term laboratory incubations with soil (Baldock & Smernik, 2002; Bruun *et al.*, 2008; Hamer *et al.*, 2004; Kuzyakov *et al.*, 2009) indicated that PyC can also eventually degrade and it is now widely accepted that a significant quantity of these resistant fraction of soil organic matter (SOM) must have undergone degradation in terrestrial environments (Kim *et al.*, 2004). In addition, interactive priming effect on SOM due to PyC addition in soil has been reported in recent literatures (Hamer *et al.*, 2004; Wardle *et al.*, 2008). However, it is unknown whether the PyC affect the community structure of microbes and thus leading to priming effect on the resistant SOM fraction.

My PhD project is looking into the dynamics of PyC degradation and their implication on soil properties and carbon cycle. The experimental set up involved installation of the cylindrical mesocosms (~ 15cm) in the soil with 3 plot replicates and two types of highly labeled substrate added at 2 cm depth from the surface, (1) $^{13}C/^{15}N$ labeled wood, *Pinus ponderosa* (^{13}C: 800 ‰, ^{15}N: 4.2 atom %), (2) $^{13}C/^{15}N$ labeled PyC (same wood pyrolysed at 450 °C under N_2 atmosphere) and (3) a no- litter control, either treated with nitrogen or not, resulting in a two-factor, two-treatment experiment. In our experiment we were able to observe decomposition of 0.05 µg CO_2 s^{-1} m^{-2} for PyC and 2.08 µg CO_2 s^{-1} m^{-2} for wood (calculated after 10 months). In addition, we observed 31 % of priming effect in PyC treated mesocosms.

2. Purpose of the stay

The purpose of my stay at the Lawrence Berkeley National laboratory (LBL), Berkeley US was to analyse the effect of PyC addition in the forest soil on the microbial community structure. Within the projected work, I proposed to test if microarray-based gene analysis of the microbial communities allows insight into the influence of PyC addition to the microbial community processes in the terrestrial system. The second objective was to test the change in the activities of soil enzymes after 10 months of the treatment of soil with PyC.

3. Methods and Material

After sampling, soil samples from each mesocosm (n =18) were divided into 3 depth profiles (0-5cm, 5-10cm and 10-15 cm). Each soil sample was homogenized and roots and bigger plant parts were manually removed. The soil were frozen at -80ºC and shipped to LBL.

3.1 DNA extraction from soil

2g (wet weight) soil were placed in a 50 mL Falcon centrifuge tube, containing 2 ml modified CTAB (hexadecyltrimethylammonium bromide) extraction buffer (1 part 10% CTAB in 1M NaCl and 1 part 0.5M phosphate buffer, pH 7.5-8.0) and 200µl 0.1 M aluminium ammonium sulfate. To the mixture 2ml phenol:chloroform:isoamyl alcohol (25:24:1) was added and samples were beaten at 5.5 m/s for 30 s in Fastprep instrument. The samples were centrifuged at 16k *g* for 5 minutes at 4ºC. The aqueous supernatants from lysing tubes were transferred in 15ml Falcon tubes containing 2 ml chloroform and centrifuged at 16000 *g* at 4ºC for 5 minutes. The aqueous phase is collected in 15ml Falcon tubes and 1µl linear acrylamide and 2 volumes of PEG solution (30%polyethylene glycol in 1.6 M NaCl) were added and incubated at room temperature overnight. The PEG solution mixture was later centrifuged at 16k *g* and PEG solution was removed leaving behind the DNA pellet. The pellet DNA is washed with 800µl ice-cold 70% ethanol and centrifuged to later remove ethanol. 50µl DEPC water is added to resuspend pellet and purified using Allprep.

3.2 PCR

Polymerase chain reaction (PCR) is an enzymatic process that rapidly amplifies specific DNA sequences (Saiki et al., 1985). The bacterial 16S rRNA gene was amplified by PCR from soil DNA using universal bacterial primers 907R and 515F and Takara Bio Inc. Hot start Ex-Taq polymerase.The PCR thermocycler was set as 98ºC for 30 s for denaturation, amplification (25 cycles) at 98ºC for 30 s, 66ºC for 30 s and 72ºC for 1 minute followed by extension cycle at 72ºC for 10 minutes.After the PCR, the replicates were combined and proceeded to concentration and purification step.

3.3 Purification and concentration of PCR product

SPRI beads (Agencourt AMPure XP, Beckman genomics, Cat A63880) were added to combined PCR products and mixed thoroughly by pipette mixing. The mixed samples were incubated for 5 minutes for maximum recovery. This step binds PCR product 150bp and larger to the magnetic beads. The tubes are placed in magnetic plate for 2 minutes to

separate beads from the solution. The supernatant is removed and 120µl of fresh 75% ethanol is added and incubated for 1 minute. Etanol is pipette off and excess ethanol is allowed to evaporate. The tubes are removed from the plate and 30µl 0f elution buffer is added. The tubes are placed back on the plate for 1 minute to separate the beads from the solution. The eluant is transferred to new tube which contains the purified PCR products. The amplicon pools were quantified using Bioanalyzer and 50ng of each sample were pooled in a 1.5ml tube. The next step goal is to concentrate the amplicon pools. For this, 1/10 volume of sodium acetate and 0.8-1 volume isopropanol were added and mixed by pipetting. The mixture was incubated at -80C for 15 minutes and centrifuged at 4C for 20 minutes. The supernatant was removed and pellet was washed with 1 volume of 70% ethanol. The pellet was air dried and resuspended in 20 µl TE buffer.

3.4 Soil enzyme assay

Five enzymes were selected for this study: β-1,4 glucosidase (BG), β-N-acetylglucosaminidase (NAG), , β-cellobiohydrolase (CBH), Phenol Oxidase and peroxidases. Fluorescence-based soil assays for BG, NAG and CBH were based on protocols using the following respective substrates: 4-methylumbelliferyl β-d-glucopyranoside (Sigma, M3633), 4-methylumbelliferyl-N-acetyl-β-d-glucosaminide (Sigma, M2133) and 4-Methylumbelliferyl β-D-cellobioside (Sigma, M6018). Colorimetric soil assays were conducted for Phenol oxidase and Peroxidase using the substrates 3,4-Dihydroxy-L-phenylalanine (Sigma, D9628) and 3,4-Dihydroxy-L-phenylalanine with hydrogen peroxide respectively. For the fluorescence-based assays, standard curves were developed and the enzyme activities were calculated against the appropriate curve.

4. Results

4.1 Microarray analysis

The pyrotag sequencing was sent to Genomic center (LBL) and results were sent in second week of May. The results are still under processing.

4.2 Enzyme assay

After 10 months of PyC treatment at 2cm depth in soil mesocosms, PyC had variable effects on enzymes studied. BG, NAG and CBH did not show any significant difference ($p<0.05$) between different treatments. Also, there is a high variability in ambient nitrogen (-N) treatment for all the enzymes. NAG showed the lowest activity among the three tested enzymes.

The calorimetric enzymes are still under test and hence not presented here.

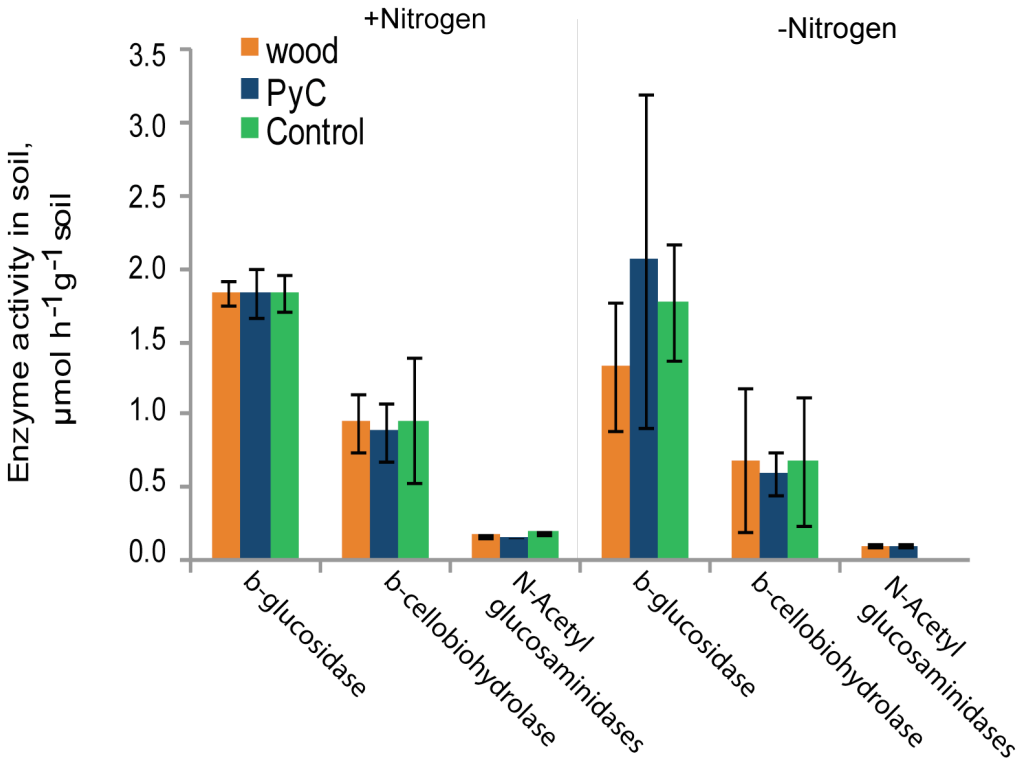

Figure 1: Enzyme activity after 10 months with +N and -N.
NAG was multiplied 10 times in order to reflect the effect and comparison to other sets of enzyme. The enzyme activity among treatment plots was not significantly different. The measure enzyme activity is low with added N for BG while reverse is true for CBH and NAG. Thus PyC shows variable effect on different enzymes and different treatments (added N and ambient N).

5. Outcomes

The result for enzyme activity was presented (Poster Session BG1.6/OS3.7/SSS4.6) at European geosciences union (EGU) meeting 2011, Vienna.

6. Forthcoming actions

The results from the study made during the stay at Berkeley would lead to possible publication in peer review journal and manuscript is under preparation.

References

Baldock, J.A., Smernik, R.J., (2002) Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry*, 33(9), 1093-1109.

Bruun, S., Jensen, E.S., Jensen, L.S., (2008) Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Organic Geochemistry*, 39(7), 839-845.

Forbes, M.S., Raison, R.J., Skjemstad, J.O., (2006) Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. *Science of the Total Environment*, 370(1), 190-206.

Goldberg, E.D., (1985) *Black carbon in the environment: properties and distribution*.

Hamer, U., Marschner, B., Brodowski, S., Amelung, W., (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry*, 35(7), 823-830.

Kim, S.W., Kaplan, L.A., Benner, R., Hatcher, P.G., (2004) Hydrogen-deficient molecules in natural riverine water samples - evidence for the existence of black carbon in DOM. In: *Symposium on New Approaches in Marine Organic Biogeochemistry held in Honor of the Life and Science of John I Hedges*, pp. 225-234, San Juan Isl, WA.

Kuzyakov, Y., Subbotina, I., Chen, H.Q., Bogomolova, I., Xu, X.L., (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology & Biochemistry*, 41(2), 210-219.

Seiler, W., Crutzen, P.J., (1980) Estimates of Gross and Net Fluxes of Carbon between the Biosphere and the Atmosphere from Biomass Burning. *Climatic Change*, 2(3), 207-247.

Wardle, D.A., Nilsson, M.C., Zackrisson, O., (2008) Fire-derived charcoal causes loss of forest humus. *Science*, 320(5876), 629-629.