

Long-term effects of soil warming on labile carbon availability and microbial community respiration and composition

S. E. Naramore¹

¹Lafayette College, 111 Quad Drive, Easton, PA 18042

Abstract:

In this study warmed soils from the Harvard Forest in Petersham, MA were analyzed as to the long-term effects of warming on the microbial community and soil organic carbon pool. Soils were incubated at various temperatures to account for thermal adaption and seasonality. Samples were analyzed to determine microbial biomass, soil respiration rates, the presence of extracellular enzymes, and the nature of the soil organic carbon pool. Warmed soils showed a decrease in bacterial biomass when compared to non-heated soils as a control comparison. Additionally, it was shown that the soil organic carbon pool was reduced in the heated soils for both labile and recalcitrant components. These results indicate significant long-term changes in soil composition in temperate forests as a result of decade-length warming.

Keywords: climate change, chloroform-fumigation, extracellular enzyme assay, labile carbon, lignin microbial biomass, soil respiration, soil organic carbon, soil warming

Introduction:

In light of the current conditions of our climate and the impact of carbon on global temperatures it is more important than ever that we understand the carbon cycle and the effects of global warming on all carbon pools. Experts estimate that approximately 1550 Pg of carbon is stored in soil, twice the amount than in the atmosphere (Lian *et al.*). If altered this carbon pool could have unforeseen consequences for the global carbon cycle, such as the possibility of a positive feedback loop if this carbon were to enter the atmosphere. Much of the carbon that enters this pool is organic carbon in the form of leaf litter or other decaying organisms. The fate of this carbon and whether forest soil acts as a carbon source, sink, or neutral compartment is highly dependent on the role biota play.

Soil microorganisms play a key role in the decomposition of organic matter in forest ecosystems. How these microbial communities will react to potential soil warming due to climate change is not yet fully understood. It has been hypothesized that under warming conditions initially bacterial decomposition will increase and overtime deplete the labile carbon

store in the soil (Bradford *et al.*). If this were to happen we would assume a decrease in microbial activity in forest ecosystems and change in community composition.

A 2008 paper (Bradford *et al.*) supports this hypothesis, indicating lower levels of bacterial production and biomass in long-term heated soils from the Harvard Forest in central Massachusetts. Soil warming has been going on in large 30x30m plots at the Harvard Forest (Petersham, MA) for the past seven years and in smaller 6x6m plots since 1991 (harvardforest.fas.harvard.edu). The previously mentioned paper only looked at the 6x6m plots. This study is designed to determine what has happened to the warmed soils of the new, larger 30x30m plots of the Slab City tract, in comparison to the soil from the control plots. I looked at what happened to carbon composition in mineral soil, both labile and lignin, the total bacterial biomass, variation in bacterial composition, and how these vary in response to changes in ambient temperature.

Methods:

Site Description

The soil warming plots used in this study are located in the Barre Woods of the Slab City Tract of the Harvard Forest, Petersham, MA. This is a mixed deciduous forest in central Massachusetts dominated by *Acer rubrum* (red maple), *Acer saccharum* (sugar maple), *Betula alleghaniensis* (yellow birch), *Betula lenta* (black birch), *Fraxinus americana* (white ash), *Pinus strobus* (white pine), *Quercus rubra* (red oak), *Quercus velutina* (black oak), and *Tsuga canadensis* (hemlock) (www.harvardforest.fas.harvard.edu). Soils are a sandy loam and moderately well drained. 30x30m plots with 5x5m subplots were established in 2002 with manipulations beginning in 2003. 1x1m areas within several subplots are designated for soil removal. While no plow layer is evident from soil profiles, low stone walls in the area indicate a history of farming. The stand was damaged in the 1938 hurricane and has grown back naturally without human interference.

Soils

Soils were collected from 30m by 30m “megaplots” at the Harvard Forest in Petersham, MA. Both the heated (5°C above ambient) and control plots are divided into 5m by 5m subplots. For each treatment six of these subplots were sampled from at random using a ~5cm soil corer. Organic and mineral soils were separated in the field and the organic layer was brought back to the lab for further analyses. A total of 24 cores were taken, two from each subplot, one designated for respiration data and one for all other procedures. Soil cores were brought back to the lab and incubated at 5°C for approximately one week and similarly at 15°C and 25°C in progressive weeks, also for approximately one week per temperature setting. An adjustment period of 48 hours between each temperature setting was maintained in which samples

remained undisturbed. Soil moisture content was maintained as it was *in situ* by weighing samples and adding water (1ml H₂O equivalent to 1g) to account for any loss. This was done for all 24 samples approximately once in every 24-36 hours including adjustment periods.

Soil Respiration

Soil respiration was measured approximately twice weekly, or twice per temperature setting. A representative sample from each subplot was designated for this respiration study. Soils were analyzed at ambient temperature, not at incubation temperature. Samples were analyzed at each soil temperature using a LICOR 6200 infrared gas analyzer (IRGA), to measure Carbon Dioxide concentration over a time interval of four minutes. Using the raw rate (ppm/sec) determined by plotting the IRGA values the carbon flux was then calculated using the below equation (Equation 1) where dC/dt is the predetermined raw rate, P is the pressure in Pa, R is the universal gas constant, T is temperature in K, V is the volume of the chamber's headspace in m³, and A is the area of the soil in m². Flux was then adjusted to show carbon as a function of grams soil in the container.

Determination of Available Carbon

Carbon availability was determined using two methods, a Potassium Sulfate extraction for labile carbon and lignin extraction. Carbon extracted from soil using K₂SO₄ can be assumed to be labile, readily available carbon. These extractions are part of the Chloroform-fumigation procedure described below and following those procedures described in Vance, with some modification. Slurries of 10g dry weight equivalent of soil and 50mL of 0.5M K₂SO₄ were agitated for one hour and then gravity-filtered through Waitman 1 filter paper. Filtrates were then run through a DOC (dissolved organic carbon) analyzer. Lignin was determined using a modified version of the litter extraction method described in Ryan *et al.* 20g dry weight of six soil samples were extracted with 72% H₂SO₄ and filtered through a crucible to find the combined mass of lignin and ash. Crucibles were then ashed to eliminate the remaining lignin.

Bacterial Biomass

As no one method is ideal for bacterial biomass will be ascertained using two techniques supported by the literature. Substrate-induced respiration (SIR) and chloroform-fumigation were both used in the 2008 Bradford study conducted at the Harvard Forest and were repeated for this study. SIR measures biomass as a function of the maximum CO₂ production of the soil-substrate mixture. Methods employed will be those used in Fierer (2003) with the Bradford (2008) modifications regarding time of incubations, incubations during the assay were conducted at room temperature. Autolyzed yeast extract at a concentration of 12 g/L, was added to 10g dry weight equivalent of soil for each sample and agitated for four hours. Respiration rates were measured at T₀, T₂ hours, and T₄ hours. The chloroform-fumigation

method uses the difference in flushed DOC between fumigated and non-fumigated extractions to determine relative biomass based on carbon measurements (Vance 1987). Samples were fumigated for three days in a vacuum desiccator kept in the dark. DOC measurements from both the fumigated and non-fumigated samples were used to calculate the estimated microbial biomass (Equation 2) where C microbial biomass, EC is the difference in carbon between the fumigated and non-fumigated samples, and kEC is a constant estimated to be 0.45.

Enzyme Activity

An indication of community composition can be ascertained by determining the presence and activity rate of extracellular enzymes. In this study we expect the difference in community to be directly related to the quality and quantity of labile carbon in soil organic matter. As other studies have previously shown long-term warmed soil has less labile carbon than control (naturally warmed) soils (Bradford 2008). Therefore we expect that bacteria in these warmed soils would have to adapt to break down recalcitrant carbon compounds such as lignin. This degradation is an oxidative process which can be aided by peroxidase and phenol oxidase, extracellular enzymes produced by certain bacteria (Sinsabaugh et al). Activity for these enzymes was determined using the method described by Sinsabaugh. A soil slurry of 2g bulk soil to 10ml sodium acetate buffer (pH 5) was mixed. To this we added 2mL of L-3,4-dihydroxyphthalanine (L-DOPA) to 12 samples (one from each subplot) and 2ml L-DOPA and 0.2mL 0.3% hydrogen peroxide to a second set of 12 samples. All samples were incubated for one hour, then read on a spectrophotometer at 460nm. Raw absorbencies were used to determine using the below equations (Equation 3, Equation 4, and Equation 5) with the parameters specified.

Results:

Soil respiration over the course of the study was variable. Respiration rates were highest just after a temperature change, but thereafter decreased. Overall there was no net increase in the carbon flux in response to temperature that could be discerned. Additionally with calculated error (as standard error) there is no statistically significant difference between samples from the control plots and those from the heated plots (Figure 1), however the actual data points for the control soils were above those from the heated plots.

The carbon quality of the soils is shown to differ between the control and heated soils. The amount of lignin per gram of dry soil is greater in the control soils than in the heated soils per gram of dry soil (Figure 2). Similarly the amount of labile carbon as extracted by K_2SO_4 proved to be greater in control soils than in heated soils with increased temperature resulting in a greater difference in carbon at the end of the study than at the beginning (Figure 3). The rate of decrease in labile carbon means appears to be greater in the samples from heated plots

resulting in lower levels of labile carbon in the 15 and 25°C soils than the 5°C samples, in which labile carbon is similar in both the control and heated plots.

Using the chloroform-fumigation method, of which the above mentioned Potassium Sulfate extraction is a part, I was able to show that there is significant difference in microbial biomass between the control and warmed soils. At all three temperature settings the mean control biomass was statistically greater than that of the corresponding heated mean (Figure 4). This biomass appears to be relatively stable with temperature variation throughout the study. I was unable to calculate SIR estimated biomass due to issues with the raw data presented. Respiration rates almost universally decreased or did not change with the addition of yeast extract (Figure 5). If the assay had worked properly respiration rates would have increased overtime and reached a maximum rate from which I could have calculated the estimated biomass using the difference. Therefore the further calculations needed to estimate biomass were not applicable and I am unable to conclusively estimate biomass in this manner.

Extracellular enzymes varied between the heated and control soils as well as the general biomass. Of the lignin-degrading enzymes tested for the highest amount of activity was associated with phenol oxidase (Figure 6). If looked at cumulatively the greatest amount of enzyme activity is associated with soils from the heated soil plots. Overall the greatest amount of enzymatic activity was found in the heated soils and at low temperatures. Over time as the temperature of the soils was increased all enzyme activity slowed to the point at which there was no statistically significant difference between treatments or enzyme tested for.

Discussion:

The lack of any significant respiration increase didn't agree with my pre-study expectations. Past practice seems to have it that bacterial growth and respiration should increase with increased temperature. This concept is supported by the short-term results of soil warming experiments (Melillo *et al*). I had surmised that my own temperature alterations, conducted on a weekly basis, would have resembled these short-term effects. The above results show, however, that there is rapid temperature adjustment in these soils in a laboratory setting. There is also a certain amount of experimental error with respect to temperature as samples were removed from the incubator and respiration measurement taken at room temperature (~20-25°C). This being so, I would still have assumed that biomass would have differed with samples and thus respiration differences would be due to the number of microbes rather than the temperature.

These stagnant respiration rates correspond, however, to relatively little change in bacterial biomass. Using the chloroform-fumigation method there was little change in the mean estimated biomass relative to temperature change. Carbon flux rates indicate this difference, though there is no statistical difference based on standard error, mean respiration was higher

in the control then the heated soils, possibly due to the difference in biomass between heated and control soils.

While temperature appeared to have little effect on either biomass or respiration in the short term, there was a significant difference in biomass between the heated and control soils. Soils from heated plots consistently showed lower microbial biomass numbers than the control soils. This may suggest that the reduction in respiration rates in heated soils back to pre-treatment, or in this case control, rates after long-term warming may be in part due to the loss of microbial biomass from heated soils (Bradford *et al*). This concept is further supported by evidence from other soil warming studies which show a loss of biomass accompanying a return to original respiration rates (Bradford *et al*).

The effect of soil warming on soil carbon pools is likely very closely tied to the microbial community living in those soils and their responses to soil warming (Bradford *et al*). As bacteria and other microorganisms adjust to a warming regime so too does the decomposition cycle and thus the carbon cycle accordingly. The initial increases in respiration, observed at the beginning of a soil-warming experiment (Melillo *et al*), are hypothesized to deplete soil organic carbon as a result of an increase in microbial biomass due to artificial warming (Melillo *et al*). Overtime as we have observed respiration rates fall back to those of pre-treatment, represented by the control plot. However, changes in the available carbon pool remain even after respiration falls back to pre-manipulation levels either as legacy of the original depletion or as part of an alternative steady-state.

Labile carbon, as extracted by K_2SO_4 , is depleted with respect to temperature change in the soils from the heated plot despite the fact that all soils were incubated at the same temperature in the laboratory. This may indicate a difference in the microbial community in the heated plot compared to the control plot, further work would be necessary to confirm this. The total starting amount of labile carbon is, however, effectively the same in both the heated and control soils which would indicate similar labile carbon availability *in situ*. If this were the case then the differences in biomass, enzymatic activity, labile carbon consumption rate, and lignin content of soils would be independent of labile carbon availability. The chance of this situation being true seems unlikely in light of the literature (eg. Melillo *et al* and Bradford *et al*). I would infer that the relatively high labile carbon content of the heated soils is in part due to the time of the year in which samples were collected (autumn). Due to an influx of available labile carbon from leaf litter during this season it seems reasonable that at the onset samples would have similar labile carbon content as well as peak carbon levels for the year. The fact that it is more quickly depleted in the heated soils supports the concept that these heated soils are effectively "labile-carbon starved" most of the time and thus the microbial community is suited to such conditions.

Lignin content in soils was lower in the heated than in the control soils. Assuming that lignin inputs to the soil would be the same in both locations we can assume that the reduction

of lignin in the heated soils is due to microbial decomposition of those compounds. As lignin is a more recalcitrant form of organic carbon than those extracted with K_2SO_4 described above we would assume that lignin would be decomposed after the labile carbon stock was depleted.

Again this doesn't appear to be the case as the highest occurrence of lignase (phenol oxidase and peroxidase) activity occurred early in the study when temperatures were lowest and the labile carbon pool was highest. When the temperature was adjusted to 15°C there appeared to be a reverse as labile carbon was depleted and enzymatic activity was similarly reduced. The reason for this is unclear. It is possible that under lower temperature conditions lignin-degrading microbes were better able to compete with those adapted to use labile carbons. Temperature inhibition of such microbes may also explain why the labile carbon had not been depleted before soils were collected in the first place as the *in situ* soil temperatures at the time of collection would have most resembled that of the first temperature setting.

Another possibility is the effects of the laboratory setting. In addition to the amount of enzyme being greatest at 5°C, the samples were freshest at this time. It is possible that the enzymes themselves were affected by the laboratory environment and the temperature change. Enzymes may have denatured with temperature change or degraded by the microbial community in response to disturbance.

Notwithstanding the presence of lignin-degrading extracellular enzymes in the soils and the concentrations of said enzymes being higher in those soils with lower lignin concentrations is indicative of the biotic influence on organic carbon decomposition. That these samples also happen to be those from the heated plots supports the idea that *in situ* labile carbon is depleted more quickly in heated soils and thus the microbial community must shift to use more recalcitrant forms of carbon such as lignin.

It would be useful as well if we were to analyze the lignin content of the litter and lignin and quantity of fine root material to better determine the role of soil microbes in forming soil lignin pools. Were further studies to be done on this subject I would highly recommend the addition of litter carbon analysis. In that manner the effects of warming on microbial communities could be put in context with the plant community in reference to their influence on soil carbon pools; thus, providing a better representation of the controls on amount of available carbon. We can presume, however, that based on literature trends increasing respiration is associated with a decline in fine root material in soils thus reducing the lignin and ultimately total organic carbon pool of these soils (Burton, Melillo, & Frey). As higher respiration rates are associated with higher temperatures *in situ* it may be that this difference in fine roots has attributed to the lower amounts of lignin in the sampled heated soils.

Conclusions:

This study has shown that there are long-term effects of *in situ* soil warming on the microbial community and thus the organic carbon pool of forest ecosystems. While over time as shown in previous studies (Melillo *et al*) respiration rates vary little from what is found in control soils; there are significant differences in the microbial community and soil organic carbon pools. An alteration such as this if it were to occur on a global scale as a result of climate change could have unforeseen effects on the carbon cycle and the role of forests as carbon sinks. The effects of a reduced soil organic carbon pool on both the biotic portion of the forest ecosystem and carbon cycling warrant further investigation.

Acknowledgments:

I would like to thank Jim Tang for his invaluable advice and support throughout this process, as well as Jerry Melillo for pointing me in the right direction when looking for a project idea. Additionally, I am very thankful for the help and lab support from Richard McHorney, William Daniels, and Stefanie Strebel. From the Harvard Forest I would like to thank Rose Smith for taking me out to the warming plots and allowing me to use her extracellular enzyme procedure (from the Sinsabaugh paper). Finally, I would like to extend my gratitude to the Ecosystems Center at the Marine Biological Laboratory for the use of their facilities and a wonderful Semester in Environmental Science.

Literature Cited:

- Bradford M.A., Davies C.A., Frey S.D., Maddox T.R., Melillo J.M., Mohan J.E., *et al.* (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, 11:1-12
- Burton A.J., Melillo J. M., & Frey S.D., (2008) Adjustment of Forest Ecosystem Root Respiration as Temperature warms. *J. Integr. Plant Biol.* 50(11):1467-1483
- Fierer N., Schimel J.P., & Holden P.A. (2003) Variation in microbial community composition through two soil depth profiles. *Soil Biol. & Biochem.*, 35:167-176
- Harvard Forest. Harvard University, www.harvardforest.fas.harvard.edu (2008)
- Liang N., Hirano T., Zheng Z.-M., Tang J., & Fujinuma Y., (2010) Soil CO₂ efflux of a larch forest in northern Japan. *Biogeosciences*, 7:3447-3457
- Melillo J.M., Steudler P.A., Aber J.D., Newkirk K., Lux H., *et al* (2002) Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, 298:2173-2176
- Plassart P., Vincelas M.A., Gangneux C., Mercier A., Barray S., & Laval K., (2008) Molecular and functional responses of soil microbial communities under grassland restoration. *Agrucul., Ecosys. And Enviro.*, 127:286-293
- Ryan M.G., Melillo J.M., & Ricca A (1989) A comparison of methods for determining proximate carbon fractions of forest litter.
- Sinsabaugh R.L. *et al* (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, 11: 1252-1264
- Sturluson M., Neilsen T.G., & Wassmann P., (2008) Bacterial abundance, biomass and production during spring blooms in the northern Barents Sea. *Deep-Sea Research II* 55:2186-2198
- Vance E.D., Brookes P.C., & Jenkinson D.S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol. & Biochem.*, 19:6, 703-707

Figures and Equations:

Equation 1 carbon flux calculation

$$F = \frac{dC_v}{dt} \frac{P}{RTA} V$$

Equation 2 bacterial biomass

$$C = \frac{EC}{kEC}$$

Equation 3 Phenol Oxidase OD

$$OD = \text{SampleABS} - \text{ControlABS}$$

Equation 4 Peroxidase OD

$$OD = \text{SampleABS} - \text{ControlABS} - OD_{\text{Phenol Oxidase}}$$

Equation 5 Enzyme activity

$$\text{Activity} = \frac{OD}{1.66 \mu\text{mol}^{-1} * \text{incubation time h} * \frac{\text{g sample}}{\text{ml sample homogenate}}}$$

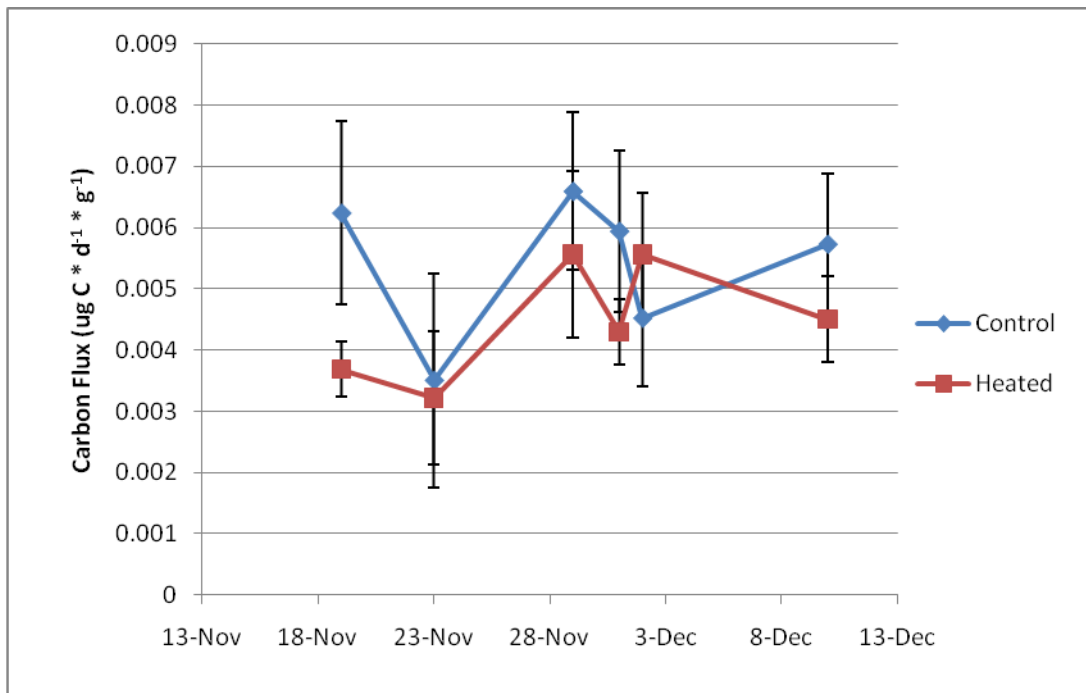


Figure 1 carbon flux of Harvard Forest soils, both control and heated, over time and temperature variations from 5-25 degrees Celsius. Error bars indicate standard error.

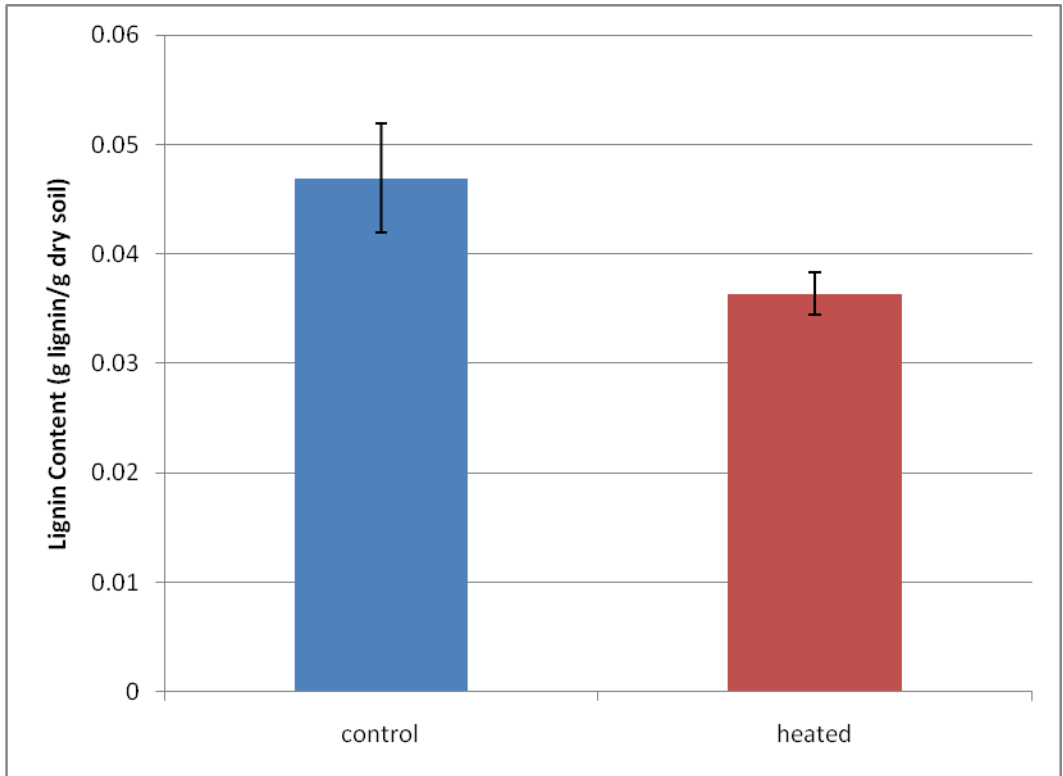


Figure 2 mass of lignin per gram of soil as measured at the end of incubations. The blue bar indicates control samples and the red bar indicates samples from heated plots. Error bars indicate standard error.

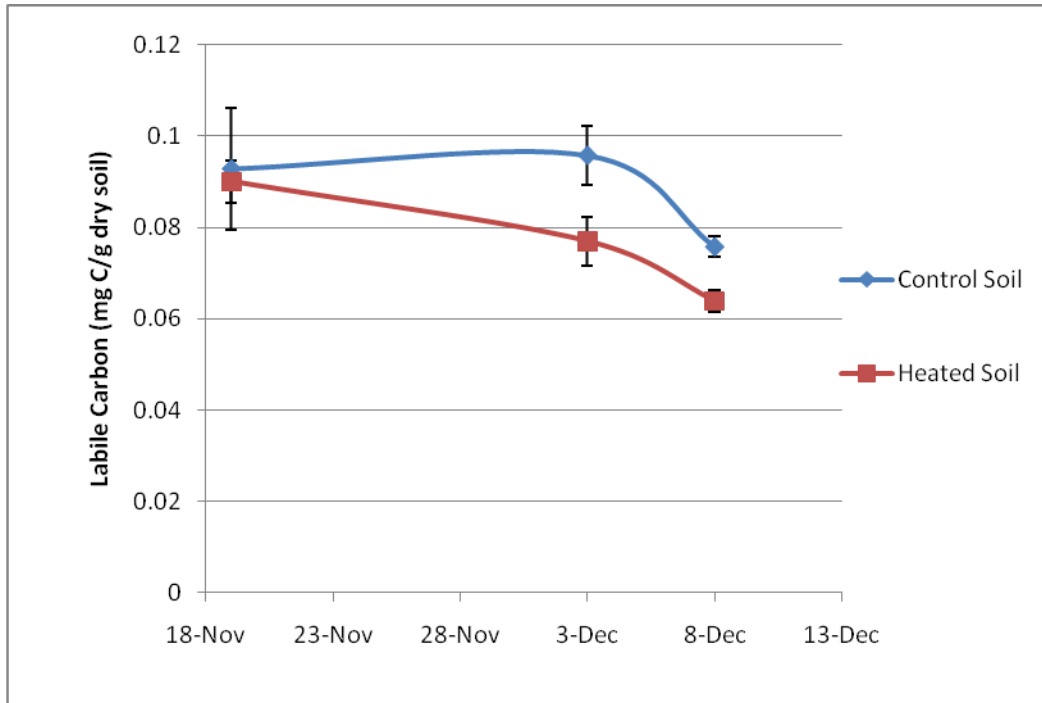


Figure 3 labile carbon extracted from soils over time and temperature change shown as mg labile carbon per gram of dry soil. Error bars indicate standard error.

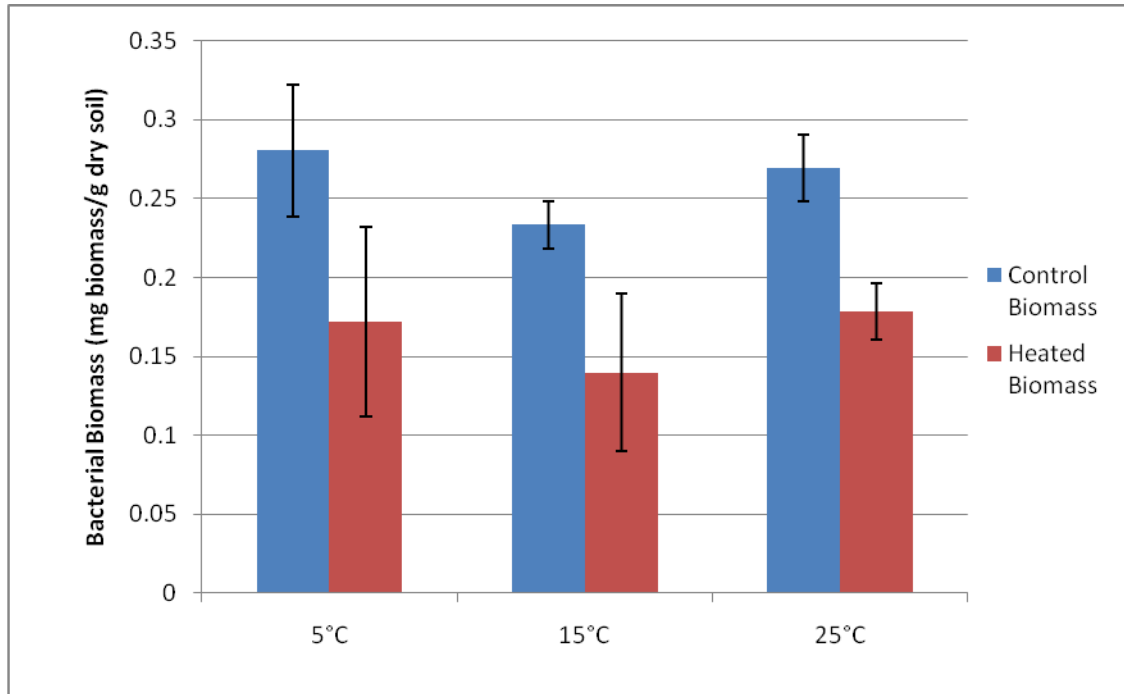


Figure 4 bacterial biomass estimated by the chloroform-fumigation method at each temperature interval. Blue bars indicate the mean biomass of control samples and the red of heated samples. Error bars indicated standard error.

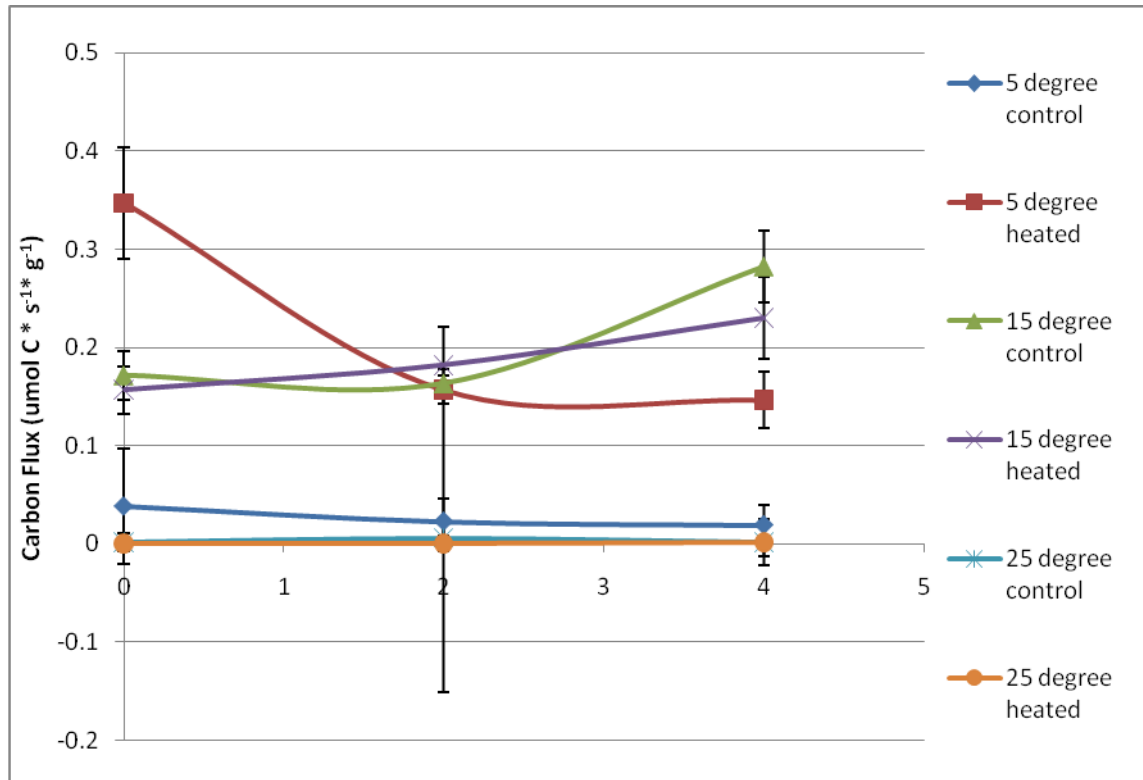


Figure 5 Mean respiration rates for substrate induced respiration assay (SIR). Preliminary results were inconclusive and the decrease in respiration between the initial time and two hour intervals at which measurements were made resulted in an inability to further calculate and estimate a change in biomass over time. Error bars indicate standard error

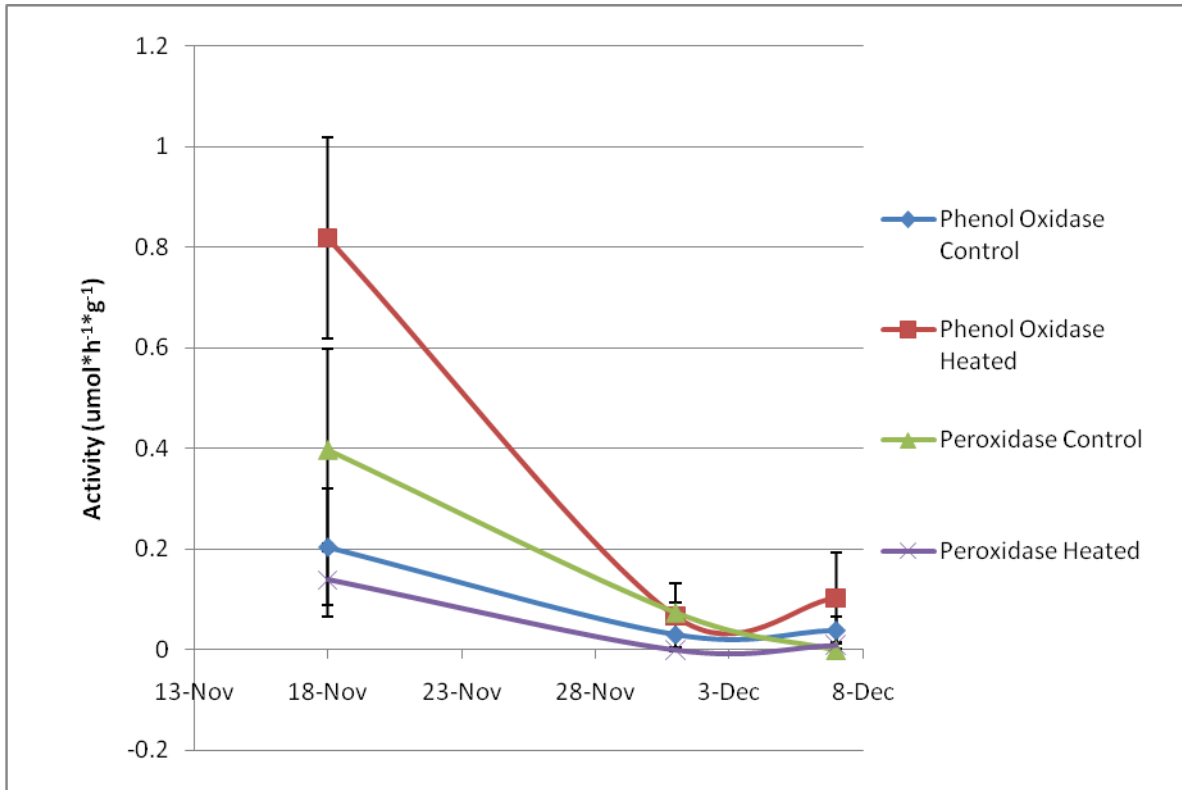


Figure 6 enzymatic activity of phenol oxidase and peroxidase over time and temperature variation. Each line reflects the mean of either control or heated samples of either the phenol oxidase or peroxidase assay. The blue line was the phenol oxidase control, the red was the phenol oxidase heated, the green the peroxidase control, and the purple the peroxidase heated. Error bars indicate standard error.