Microbial Respiration of Soil Organic Matter across Varying Temperatures in the Harvard
Forest Soil Warming Experiment
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Abstract

Human practices such as our use of fossil fuels have caused the accumulation of carbon dioxide into the atmosphere. Surplus carbon in the atmosphere directly influences the climate and biogeochemical cycles. Understanding how changes in climate will alter carbon budgets in terrestrial systems served as the foundation for this experiment. Soil samples collected from the Harvard Forest Soil Warming experiment in Petersham, Massachusetts were incubated in triplicates at a range of temperatures between 15°C and 30°C. Carbon flux rates were then compared between the two plots (heated and control) to examine the impact previous warming had on soil respiration. Soil samples from the heated plots had significantly higher CO₂ fluxes than the control plots when incubated at 20°C. At the time of field sampling, the heated plots had higher concentrations of ammonium (NH₄ = 2.23μM/ g dry soil) and nitrate (NO₃ = 0.58 μM/ g dry soil).

Keywords: Soil respiration, soil organic matter, climate change, soil warming, carbon cycle, Harvard Forest, Petersham, MA
1. Introduction

The Intergovernmental Panel on Climate Change (IPCC, 2014) anticipates an increase in temperature, ranging from 1°C - 4°C over this century. This warming has the potential to increase soil respiration rates (Melillo et al., 2002). Soil respiration is comprised of root respiration and microbial decomposition of soil organic matter (SOM), and its rate depends on temperature and moisture conditions (Savage and Davidson 2001). Warming induces carbon loss from soil by altering rates of microbial decomposition (Frey et al., 2013). Compared to plant biomass, soil stores four times as much carbon (Stocker et al., 2013) and a positive feedback in soil microbial communities to an increase in temperature may cause the lost soil carbon to move to the atmosphere, where some accumulates and causes changes in the Earth’s climate.

The affect of warming on the flux of carbon from the soil to the atmosphere is being studied in the field to further understand the impact of increasing temperatures on soil respiration. Jerry Melillo et al. studied carbon cycle feedbacks using soil-warming plots in the Harvard Forest, located in Petersham, Massachusetts. Melillo et al. estimated that the decay of soil organic matter was the greatest factor in soil respiration (74% in the control plots and 82% in the heated). Over the 25 years that the soil warming experiment has been running Melillo measured soil CO$_2$ evolution rates monthly between April and November using a static chamber technique (Melillo et al., 2002). Over the life of the experiment, the respiration response has exhibited a three-phase pattern. An ephemeral soil respiration response to warming occurred over the experiment’s first decade, with soil respiration greater in the warmed plots than in the controls (Phase I). In the next phase of the response, soil respiration rates were equal in the control and heated plots (Phase II). During the third phase of the study (years 18 - 25), Melillo et
al. (unpublished) observed another trend reversal, with soil respiration once again higher in heated plots relative to controls (Phase III).

In the heated plots, scientist also discovered an increase in net nitrogen mineralization due to warming, which may have increased the amount of nitrogen stored in woody tissue (Melillo et al., 2002). This may have caused an increase in vegetation carbon storage, to compensate for some of the carbon lost from the soil due to warming.

To better understand the effects of warming on microbial respiration rates after 25 years of treatment, I measured respiration rates of soil collected from the heated and control plots at the soil-warming experiment. I incubated triplicate soil samples from each treatment under varying temperatures. I hypothesized that microbial respiration rates of soil from the heated plots would be greater than microbial respiration rates of control soils incubated at the same temperature.

2. Site Description

In 1988, a Long-Term Ecological Research (LTER) site was established at the Harvard Forest in Pertersham, Massachutes. In this area, the climate is cool, temperate and humid, with weekly air temperatures that vary. In July, temperatures may reach a high of 20°C and in January a low of -6°C is possible (Melillo et al., 2002). An annual average of precipitation throughout the year is around 108cm (Melillo et al., 2002). Harvard Forest soil is primarily of the Canton series (coarse-loamy or sandy or sandy-skeletal, mixed mesic Typic Dystrochrepts), with a surface pH of 4.85 (Melillo et al., 2002). Dominant tree species include paper birch (Betula papyrifera), red maple (Acer rubrum), and black oak (Quercus velutina) (Melillo et al., 2002). January of 1991, scientist installed a soil warming experiment in the Forest’s Prospect
Hill tract, to study soil response to a 5°C increase in temperature. Prospect Hill is populated by red oak (*Quercus rubra*) and red maple (*Acer rubrum*) (Savage and Davidson 2001). At this site, soil characteristics, trace gas emissions and climate conditions are monitored. A 5°C difference between the heated and control plot is maintained by an automated thermistor network, with heating accomplished by providing power to electrical resistance cables buried 10cm below surface and 20cm apart. In total, there are six replicate (6x6m) plots per treatment. For this experiment, soil was collected from three heated plots and three (non-heated) control plots.

3. Methodology

3.1 Soil Respiration

Three plots were selected from each treatment and two soil cores (10cm in diameter and 10cm in length) were collected from the designated sampling area of each plot. Soil horizons were homogenized; roots, insects and debris were separated from soil samples and sieved using a stainless steel (7” in diameter with a 5.5 mm opening) sieve. Specimen cups were designated to a treatment (heated or control) and a temperature (15°C, 20°C, 25°C or 30°C). Each was filled with 120ml of soil collected from the field. Subsamples were also collected for later nutrient analysis. Samples were then stored in their respective incubators (Figure 1). Every other day soil respiration was measured inside a small chamber made from polyvinylchloride (PVC). The total system volume was approximately 500ml including 175ml of tubing. Specimen cups filled with soil were placed inside the chamber and carbon concentration was measured using the Licor LI 6250 CO₂ analyzer. Temperature (kelvin) for each sample was measured using a Hanna instruments thermometer. Pressure was measured each sampling day with a barometer pressure reader and the values were converted from Inches Hg to atmospheric pressure. For each sample, CO₂ concentration was measured for four minutes using the LI 6250. The linear portion of each
Flux (µmol CO2 / g soil C / day) = (slope * atmospheric barometric pressure * system volume L) / (gas constant * air temperature (°K) * gC/ g dry soil) * (60 seconds * 60 minutes * 24 hours)

Flux measurements were made every other day for fourteen days. The first week of data collected were used in calculating mean respiration values across the studied temperatures.

3.2 Nutrients Analysis

Soil subsamples were dried at 60°C overnight and reweighed for wet weight/dry weight corrections. Subsamples were ground using a mortar and pestle. Approximately 20 mg of dried soil samples were packed for CHN analysis and 10g for KCl extractions. Ten grams were removed from the soil subsamples and stored in specimen cups followed by the addition of a 2 molar KCl solution. Two KCl blanks were also made. KCl mixtures were placed on the shaker table for 1 hour and a half at 200 rpm. Samples were then stored at 4°C overnight to allow soil to settle to the bottom. Samples were filtered using a GF/F filter and 40 ml of each sample were used to measure the concentration of attainable ammonium (NO₄) and nitrate (NO₃). This was accomplished using a Shimadzu 1601 spectrophotometer and Lachat Flow Injection Analyzer, respectively, following lab protocol (Strickland and Parsons, 1972; LaMotte Hach Method, SES, 2015).
CHN analyses were completed using the Thermo Scientific FLASH 2000 NC Analyzer. From the data collected during the CHN analysis, the percent of carbon per gram of dry soil was used to normalize carbon flux.

3.3 Statistics

A two-way analysis of variance was performed with balanced data discussed by Snedecor and Cochran (1967, Table 12.5.1, page 347) using excel. Carbon flux data taken over the first week of this study were used for the two-way ANOVA. Flux values were compared between the two treatments (heated and control) at the intersecting temperatures (20°C and 25°C) (Figure 3).

4. Results

4.1 Soil Carbon and Nitrogen Stocks

The data collected from the CHN analysis indicated that the control plot (6 % C/ g dry soil) had slightly less carbon than the heated plot (8 % C/ g dry soil) (Figure 4). The concentration of N present for the heated plot (0.3 % N/ g dry soil) was less than the control (0.4 % N/ g dry soil) (Figure 4). Carbon to Nitrogen ratios differed between the two plots. The control plot had a 15:1 C:N ratio which is nearly half of the C:N ratio of the heated plot (27:1) (Figure 4).

4.2 Carbon Fluxes

Soil from the heated and control plots were incubated at two common temperatures – 20°C and 25°C. I found that over the first week of incubation, the mean respiration rates from heated soil samples incubated at 20°C were significantly higher than those of soils from the control plots incubated at this temperature (Figure 7). However, I observed no difference between the mean soil respiration rates in soils from the heated and control plots when they were
incubated at 25°C for this time period (Figure 7). This result for the incubations at 20°C is qualitatively the same as the field observations of CO₂ fluxes from the heated and the control plots (Figure 8).

Over the first week of the study, the carbon flux from heated plot soil samples incubated at 20°C remained constantly higher than the other measured points both in the heated and the control (Figure 6a, Figure 6b). Whereas, the daily flux values from the control plot showed less variation in response to temperature (Figure 6b). Heated soil samples incubated at 25°C started off as the highest respiration point followed by a steep decline (Figure 6a). During the sampling period heated soil samples incubating at 30°C radically changed from the lowest flux measurement to the highest towards the end (Figure 6a). The flux of carbon released from soil in the heated plots were highest at the two overlapping temperatures, 20°C and 25°C (Figure 7); these values are significantly different when compared using a two way ANOVA (Figure 3).

4.3 Inorganic Nitrogen Concentrations

The concentration of NO₃ in the heated plot soil extractions (0.58 µM/ g dry soil) is nearly double of the amount of nitrate in the control plot soil extractions (0.22 µM/ g dry soil) (Figure 5). NH₄ was also present in higher amounts in the heated plots (2.23 µM/ g dry soil) whereas in the control (0.4 µM/ g dry soil) less was measured (Figure 5).

5. Discussion

In this study, when incubated at the same temperature, soil extracted from the heated plots of the warming experiment had higher rates of respiration than soil from the control. It is possible that soil from the heated plots continued to have higher respiration values relative to the control due to a shift in the structure of soil microbe communities (DeAngelis et al., 2015; Frey
et al., 2008 and Allison et al., 2008). DeAngelis et al. found that after 20 years of warming community structure differed between the heated and (non-heated) control plots at the Harvard Forest. They also found the heated plot to be more diverse, perhaps due to a decline in previously dominant taxa. Microbial community structure may have shifted as a response to temperature (Frey et al., 2008), this change may have enhanced microbial communities ability to degrade recalcitrant carbon compounds (Pold et al., 2015). After 25 years of warming, the amount of labile carbon decreased (Bradford et al., 2008) and soil microbes had to adapt in order to increase efficiency (Frey et al., 2013). Frey et al. found that substrate quality and temperature influence efficiency, and that at higher temperatures heated soil microbes were able to efficiently utilize recalcitrant compounds. Similarly, DeAngelis et al. suggested that long-term warming might have also lead to a decrease in substrate availability, which favor living conditions of oligotrophic taxa. The structure of microbial communities is influenced by substrate quality and availability, which changes as a response to warming. Warming experiments are important for both ecology and our understanding of global change but also to genomics and the understanding of microbial feedbacks in response to changes in climate.

5.1 Conclusions

The data collected partially supports my original hypothesis. Soil collected from the heated plots, when incubated at 20°C, had higher respiration rates than soils from the control plots incubated at that temperature. The reason for this difference in responses at 20°C is unclear. There is this possibility that soil from the heated plots differ in species composition due to an adaption driven by decades of warming. Understanding the influence previously heated soil has on terrestrial communities, in response to subsequent changes in temperature, may one-day further explain the processes microbial communities undergo in response to temperature.
Although this study was short-term, additional data points could have possibly displayed a more in-depth understanding on the impact increasing temperatures have on previously heated soil microbes. Overall, this study displayed trends in CO$_2$ flux, extractable nutrients and (although small) the percent of carbon per gram of dry soil. These trends are distinct between the two treatments and support previous studies, which suggest a difference in microbial communities caused by long-term heating.
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Literature Cited


GW Snedecor, WG Cochran; Statistical Methods. (6th edn.) Iowa State University Press, Ames, IO (1967) chapter 10


Figure 1. Experimental set up. The first box is a replicate of field conditions and the heated (marked with the letter H) represents soil stored at 5°C higher than the control. The next box to the right displays control soil samples incubated at 20°C and heated soil samples incubated at a 5°C increase. The third box displays a 10°C increase in temperature relative to field conditions, following the same 5°C increase in heated soil samples as previously mentioned.
Figure 2. Example of the rate of carbon flux measured over time. Linear portion of graph were used to calculate the rate of carbon released over time. Flux rates were measured for each soil sample.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Plot</td>
<td>&lt;0.1</td>
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Figure 3. Two way ANOVA test were used to calculate P value. The difference between the heated and control plot at 20°C and 25°C are statistically significant.
Heated Samples | Control Samples
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Average Percent Carbon (% / g dry soil) | 8 | 6
Average Percent Nitrogen (% / g dry soil) | 0.3 | 0.4
C:N Ratio | 27:1 | 15:1

Figure 4. The data from the CHN analysis was used to calculate the C:N ratio, average percent of carbon and nitrogen per gram of dry soil.
Figure 5. Concentration of NO$_3$ and NH$_4$ available in the soil solution, this chart displays that ammonium and nitrate appear to be higher in the heated plot compared to the control.
Figure 6a. Raw data displaying the flux released from the heated soil samples over a seven-day period. RH20 is indicative of respiration values from the heated plot at 20°C; RH25 indicates respiration values of the heated plot at 25°C followed by RH30 which displays respiration values at 30°C.
Figure 6b. Raw data displaying the flux released from the control soil samples over a seven-day period. RC15 is indicative of respiration values from the control plot at 15°C; RC20 indicates respiration values of the control plot at 20°C followed by RC25 which displays respiration values at 25°C.
Figure 7. This chart displays the mean CO₂ flux at each temperature in regards to treatment. Heated soil samples respire higher than control samples, this trend is noticeable at 20°C and 25°C.
Figure 8. The difference of CO$_2$ flux between the heated – control plots of the Harvard Soil Warming experiment. Soil flux is displayed in this chart starting in 1991 and ending in 2014. (Jerry Melillo granted permission to use this graph).