

The impacts of long term warming on potential soil microbial activity across soil depth

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Abstract

Terrestrial soils store more carbon than exists in the atmosphere and all plant life combined. Thus, small changes in the amount of carbon stored in soil or released back to the atmosphere from soil can have large impacts on atmospheric carbon concentrations and climate change. As climate change progresses, warming temperatures are projected to increase soil microbial activity, leading to shifts in soil carbon accumulation and stabilization. Here, we sampled soils across the soil profile during the decommissioning of a 30-year warming experiment conducted in a subalpine meadow to understand the degree to which warming impacts carbon losses in both surface soils and soils deep within the soil profile.

Introduction

Soil microbes are the organisms responsible for carbon fluxes between the atmosphere and soils (Dutta and Dutta 2016). Since over two thirds of the world's terrestrial carbon is stored in soils, the processes of absorbing carbon dioxide through photosynthesis and ultimately returning that carbon dioxide to the atmosphere through decomposition and respiration result in a delicate balance that dictates global net carbon fluxes (Dutta and Dutta 2016). Warming directly affects microbial processes like respiration since microbes and the chemical reactions that govern microbial metabolism are temperature sensitive (Classen et al. 2015). Likewise, nutrient mineralization is also expected to increase with accelerated microbial activity, but because nutrient losses and microbial immobilization of these resources will also be impacted by warming, the overall impact of climate change on nutrient mineralization remains unclear (Crowther et al. 2016; Classen et al. 2015; Castro et al. 2009). This includes greater rates of respiration as well as increased rates of mineralization (Crowther et al. 2016). However, this also means more carbon will be lost to the atmosphere, which will accelerate climate change in a

phenomenon known as a positive feedback where increased carbon fluxes to the atmosphere amplify the effects of climate change (Crowther et al. 2016; Dutta and Dutta 2016).

Although warming has been shown to increase soil carbon fluxes, it is still unclear how this affects the carbon cycle on a global scale (Crowther et al. 2016). Even though changes in soil carbon can occur on a small, localized scale, they add up when aggregated across all terrestrial ecosystems to produce massive fluxes that contribute to increased concentrations of atmospheric carbon (Classen et al. 2015; Crowther et al. 2016). It is known that microbial communities regulate carbon cycle fluxes; however, the exact mechanisms behind these processes are unknown and need to be further researched (Dutta and Dutta 2016). Although there have been multiple warming experiments, we still have limited knowledge as to how exactly warming affects global soil carbon stocks and inputs (Crowther et al. 2016). High elevation ecosystems contain high amounts of soil carbon (Harte et al. 1995) and studies show that increases in warming have been known to cause losses in soil carbon due to increased microbial activity (Pandall 2018). In the long term, these losses can be moderated by stabilizing feedbacks within the soil system (Harte et al. 1995); however, with unregulated increases in temperature, significant amounts of soil carbon are released into the atmosphere in the form of CO₂ as a result of respiration (Hicks Pries et al. 2017). Soil acts as both a sink and a source of carbon, but with climate change, that capacity of soils as carbon storage reservoirs will likely be diminished (Dutta and Dutta 2016).

Previous studies have analysed the relationship between soil depth and soil carbon content (Dessureault-Rompré et al. 2016; Dutta and Dutta 2016; Iversen et al. 2011); however, not much is known about how warming affects global carbon stocks (Ma et al. 2018), especially at deeper depths (lower than 10cm) because the majority of microbial activity happens in the top 10 centimetres of soil (Crowther et al. 2016). Because little is known concerning the effect of warming on soil carbon and microbial activity at deeper soil depths, current models may provide an inaccurate representation of the future of global carbon stocks, especially concerning deep soil carbon storage (Crowther et al. 2016).

Both soil microbes and plant community composition are closely linked and changes in one can cause drastic changes in the other (Classen et al. 2015). Shifts in plant community composition can lead to small-scale shifts in the carbon cycle as well as mineralization rates that have the potential to scale up to huge carbon fluxes globally (Classen et al. 2015). Scientists are

still uncertain of the effects of climate change and warming on microbial community dynamics and plant-soil interactions as well as how these interactions change with increasing soil depth (Classen et al. 2015). Climate change and increased warming are driving shifts in aboveground ecosystem structure and function which in turn affects belowground dynamics, but the timing of these shifts may be mismatched as soil buffers belowground communities to aboveground abiotic change (Crowther et al. 2016). This mismatch may explain why there are carbon stock losses with warming (Crowther et al. 2016).

Although we know that soil microbial activity is affected by temperature and moisture (Dutta and Dutta 2016; Harte and Shaw 1995), we know very little about how localized soil carbon fluxes respond to warming with increasing soil depth, let alone how this response scales to affect soil carbon stocks and fluxes globally (Crowther et al. 2016). Here, I will take advantage of one of the longest running warming experiments in a sub-alpine meadow to explore how warming impacts microbial activity with soil depth. We hypothesize that microbial activity will decline with soil depth in response to declining soil carbon with depth; microbial activity will increase with warming; and that warming will stimulate microbial activity at greater depths due to the documented increase in above-and below-ground biomass in response to warming. The results from this warming experiment can enable us to have a more holistic understanding of the capacity of entire soil profiles to store carbon in a warming world.

Study Design

For the last 30 years, the Gothic Research Meadow in Gothic, Colorado has been warmed (up to 3 °C) using passive warming through electric heaters (Harte et al. 1995) (see Map 1). This experiment is set up with ten 3x10 m plots with the long axis of the plots spanning lower (L), middle (M), and upper (U) ecotone zones; however, we only sampled in the upper zone (Harte et al. 1995). The plots alternate between warming and control plots with the even numbered plots designated as warming plots and odd numbered plots designated as control plots (Harte et al. 1995).

Methods

During the plot harvest in July, we collected soils from each of the warmed and control plots using a backhoe and a trowel. A backhoe dug ten trenches a meter deep at the front of each of the plots in the upper zone. From inside the trench, we took a total of three soil cores per trench in the upper zone of each of the plots using a trowel, because the soils were too rocky to use an oakfield sampler as originally planned. The soil cores were taken in ten-centimetre horizons (0-10cm, 40-50cm, and 80-90cm) horizontally into the plots from inside the trenches. Soil samples were kept cool in the field and stored in the refrigerator in the lab.

Once we returned to the lab, we sieved the soils to 2 mm and measured initial soil gravimetric water content (GWC) to determine percent moisture content, which allowed us to know how much water to add to each of the soil samples for the incubation. We measured GWC by heating a subsample of each of the soil samples in an oven at 105 °C for 48 hours. We weighed the soils both before and after drying them in the oven to determine the moisture content of each individual sample (Iversen et al. 2011). We placed a 15g subset for each plot in Falcon tubes and raised them to 15% soil moisture based on GWC measurements for ideal soil moisture conditions. These tubes, in addition to two controls, were then placed in mason jars fitted with a rubber septum for a 28-day incubation. The jars were filled with 10mL of water to maintain soil moisture conditions and prevent evaporation. We sampled the airspace in the mason jars using a syringe (Iversen et al. 2012) on days 1, 2, 4, 8, 17 and 28 and analysed the gas samples through direct injection using a LiCOR 6400. We plotted these measurements using Sigma Plot and calculated the area under the curve for each sample. Using R, we converted the area under the curve for each sample into a concentration of CO₂ (μmol CO₂) and then into a carbon mineralization rate for that sampling day (μmol CO₂ g⁻¹ h⁻¹). These CO₂ concentrations for the direct injection were fitted to a standard curve using known samples of gas from a tank. Some soil was collected to measure nitrogen mineralization, soil carbon content, pH, and microbial biomass at a later date. Additional variables that were taken into account are plant community composition and net ecosystem exchange (NEE).

Results

We ran an ANOVA and found a significant depth effect in the carbon mineralization rates for each of the individual sampling days (Figs. 1, 2, 3, 4, and 5) as well as a significant depth effect in the average carbon mineralization rate over the total incubation period (Fig. 7).

We ran another ANOVA to analyse any differences in gravimetric water content and also found a significant depth effect (Fig. 6). This depth effect in these analyses is only seen between the first horizon sampled (0-10cm) and the other two depths, indicating that as soil depth increases, carbon mineralization rates decrease. For all of the analyses we ran, we found no significant differences in average carbon mineralization rates or total carbon mineralization rates for individual sampling days between warming and control plots or the interaction between warming treatment and depth (see all figures). In addition, we ran a Brown-Forsythe Levene-type test and found no significant differences in variation in the median carbon mineralization rates across depth, warming treatment, or in the interaction between depth and warming treatment (see Tables 1 and 2). All analyses were conducted using the ggplot2, dplyr, plyr, gridExtra, and lawstat packages in R and R Studio. Additionally, there were changes in plant community composition between control and warmed plots, but they did not seem to alter below ground activity since there is no treatment effect with depth.

Discussion

Our findings of a significant depth effect reflect our hypothesis that potential soil microbial activity will decline with depth. However, the depth effect may have yielded different results if the soils had been uniformly warmed as far down as we sampled. This may have also impacted the interaction between warming treatment and depth. In addition, much of the heat from the electric heaters used to warm the plots may have been lost to the atmosphere. One can argue that because the atmosphere around the plots was being warmed, the control plots were also being warmed; however, the long-term nature of this experiment proved a natural warming experiment in and of itself, with or without heat loss to the atmosphere and the inadvertent warming of the control plots. While these two factors may have altered microbial activity in both warming and control plots, the relative differences in microbial activity between the two warming treatments should remain the same.

Although we found no significant differences in gravimetric water content (GWC) with treatment, depth, or the interaction of the two, other studies have found significant differences in gravimetric water content between control and warming treatments, which is the result we would have expected to see. These significant differences are dependent on the way GWC was measured. Our result was marginally significant (Fig. 6) but that is not always the case. As for

the lack of a treatment effect, the climate in the East River Valley of the Colorado Rocky Mountain region is extremely dry and arid; therefore, the soils are extremely water limited. When raised to ideal soil moisture conditions, both the control and the warmed treatments reacted similarly by increasing their microbial activity in response to the now available resource. If we had incubated these samples under field conditions, we may have seen significantly more microbial activity in the warming treatment than the control treatment, since soil microbes would have become better adapted than the control soils over the 30-year duration of the experiment to mineralizing carbon in a drier environment.

Based on the preceding results, we are still unsure of how warming affects soil microbial activity. There is both literature stating that microbial activity increases with warming and that microbes adapt to warming (Classen et al. 2009; Castro et al. 2009; DeAngelis et al. 2015). There may be a shift in microbial community composition with warming toward a microbial community that is better adapted to warming temperatures, which is why we may not see a difference in carbon mineralization rates between warming and control treatments (Classen et al. 2009; Castro et al. 2009). Scientists are unsure whether this is due to thermal acclimation or a community-wide shift (Crowther and Bradford 2013). This warming experiment was one of the longest-running warming experiment in the world; therefore, other studies conducted on a shorter time scale that have looked at microbial activity with warming may have seen an initial increase in microbial activity and may not have been running long enough to see soil microbes adapt to the changing ecosystem (DeAngelis et al. 2015). Based on the available literature (Classen et al. 2009; DeAngelis et al. 2015), we hypothesize that there is a threshold in which accelerated microbial activity peaks and then declines as there is a community-wide shift in composition toward warm-adapted soil microbes. The long-term warming study conducted at the Harvard Forest (DeAngelis et al. 2015) concluded that there are cyclical boom and bust-type patterns in microbial activity (UNH Today 2017); however, this study was conducted in a different ecosystem with a slightly different climate. It is hard to tell whether the patterns observed with our warming study are threshold-related or are cyclical.

There have been other studies conducted during the duration of this warming experiment; however, none have looked at how warming impacts microbial activity across depth (Harte et al. 1995). Because of this, we have no baseline off which to focus our results. There are many other climatic factors that are currently being altered by climate change that scientists are unsure of

how they will affect microbial activity (Castro et al. 2009). Therefore, the results of the incubation may be due to a multitude of other factors that have not been tested in this study or in other studies that may interact directly with each other as well as with the warming and depth treatments to impact microbial activity.

Conclusion

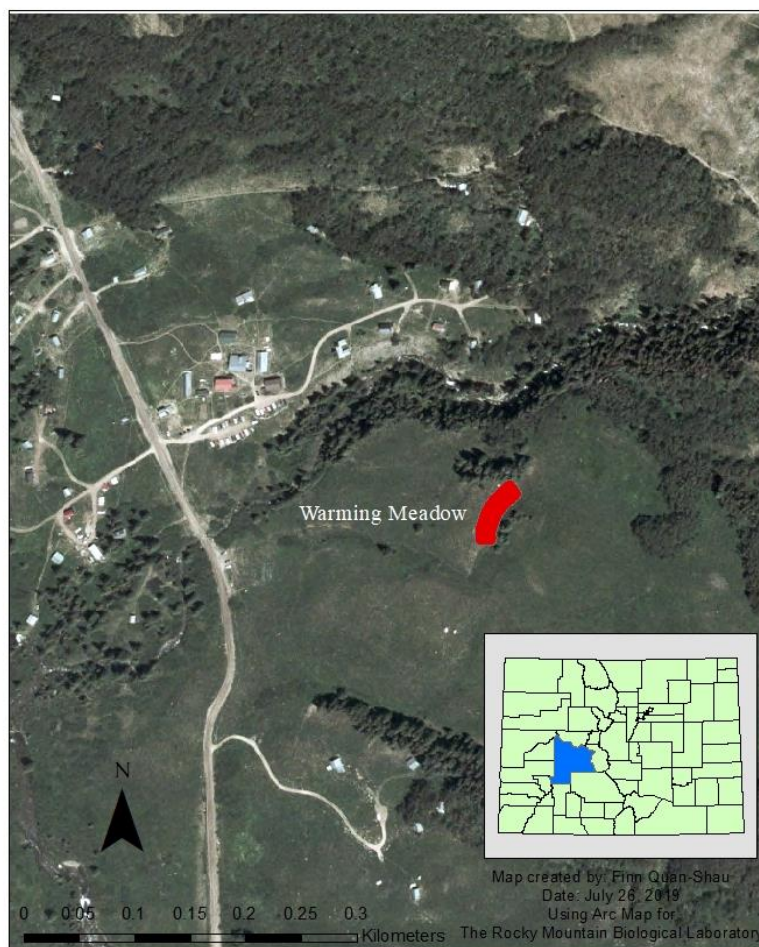
Climate change and increases in temperature can affect the carbon storage capacity of soil, which decreases the ability of soil microbes to control carbon fluxes and mitigate carbon dioxide and methane emissions into the atmosphere (Kardol and De Long 2018). Since we are sampling at the 30-year mark of this experiment, the results we have gathered are just a snapshot of the processes that have been occurring over the past few decades. There may be other patterns that occurred earlier on that we missed in the analyses that may reveal different patterns and drivers of those processes than what we are currently witnessing. Although these carbon fluxes represent a fraction of global carbon emissions on a local scale, these fluxes scale up to create huge fluxes globally that can dramatically affect the global carbon cycle because emissions from microbial respiration far outweigh anthropogenic emissions (Gougoulas et al. 2014). Therefore, it is imperative to further investigate what a warming climate means for soil microbes, and by extension, global emissions.

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Warming Meadow



Map 1: John Harte's Warming Meadow study site in Gothic, Gunnison County, Colorado, USA.

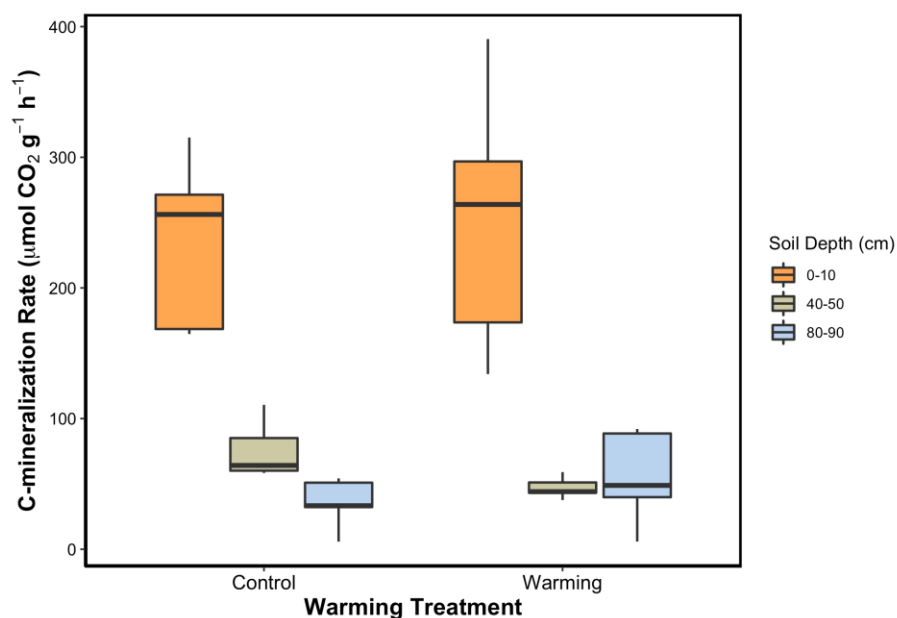


Figure 1: There are no significant differences in day 1 carbon mineralization rates between warming treatments ($p=0.90$) or the interaction between warming treatment and depth ($p=0.53$); however, there is a significant difference in carbon mineralization rates across soil depths ($p<0.01$) ($r^2=0.74$). Blank 1 was removed during analysis because it had a CO_2 concentration higher than some of the samples.

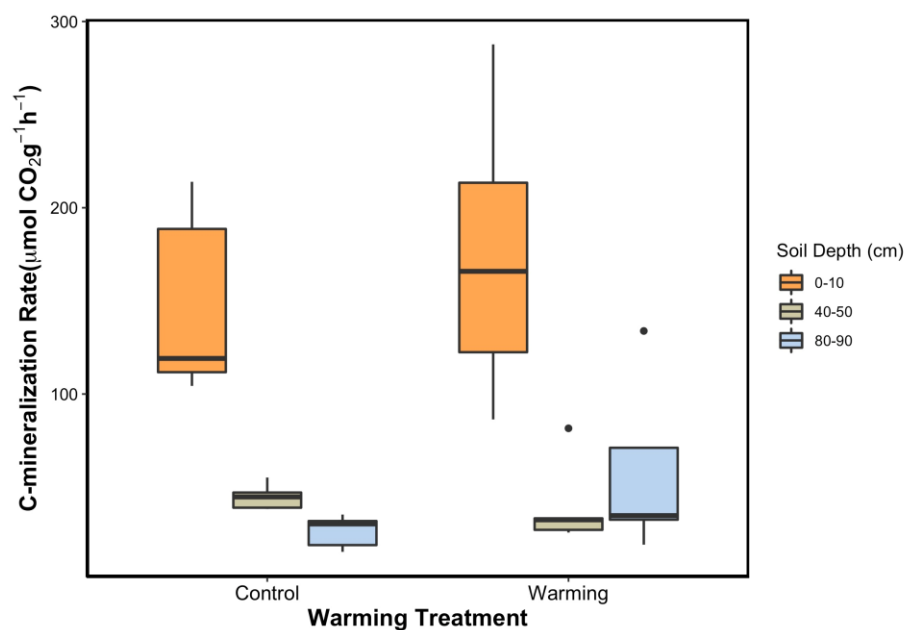


Figure 2: There are no significant differences in day 2 carbon mineralization rates between warming treatments ($p=0.27$) or the interaction between warming treatment and depth ($p=0.60$); however, there is a significant difference in carbon mineralization rates across soil depth ($p<0.01$) ($r^2=0.62$).

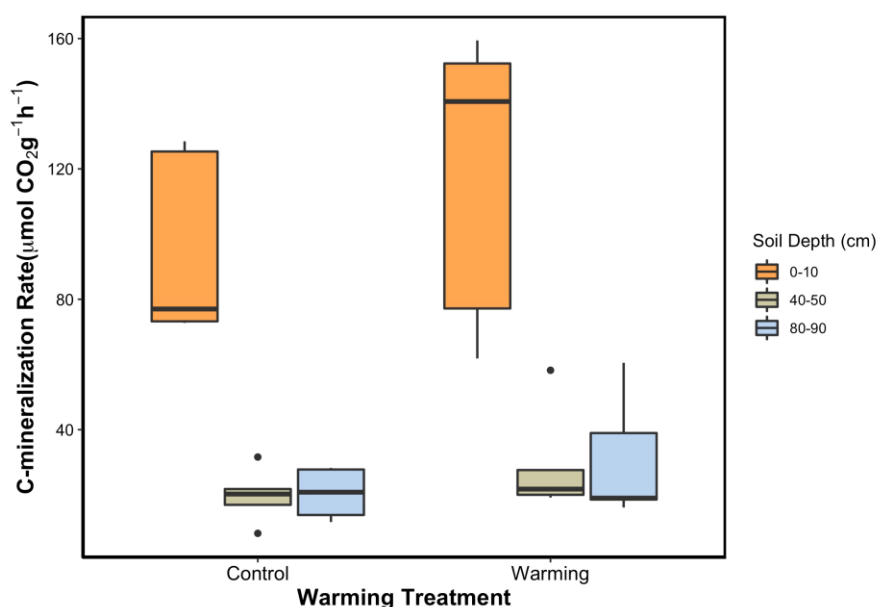


Figure 3: There are no significant differences in day 4 carbon mineralization rates between warming treatments ($p=0.13$) or the interaction between warming treatment and depth ($p=0.80$); however, there is a significant difference in carbon mineralization rates across soil depth ($p<0.01$) ($r^2=0.71$). Blank 2 was removed from the analysis because the LiCOR did not finish recording its CO_2 concentration.

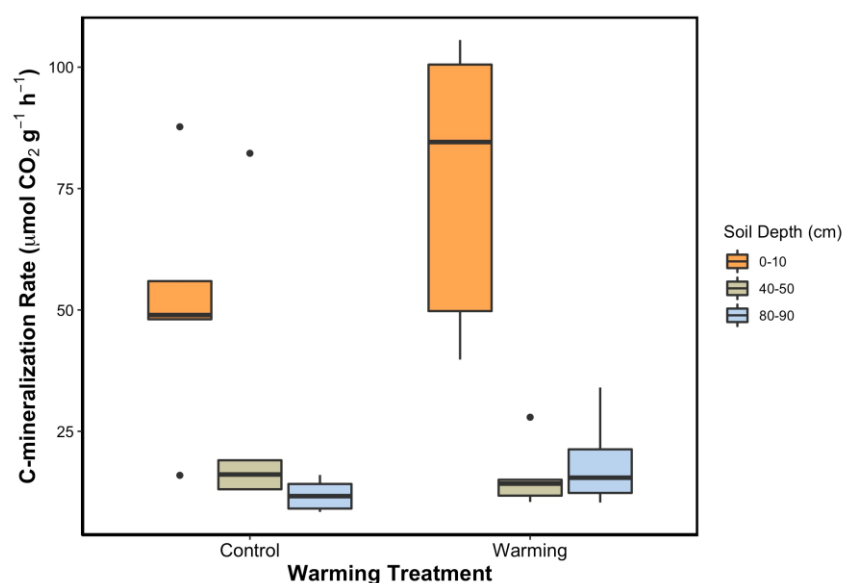


Figure 4: There are no significant differences in day 8 carbon mineralization rates between warming treatments ($p=0.42$) or the interaction between warming treatment and depth ($p=0.15$); however, there is a significant difference in carbon mineralization rates across soil depth ($p<0.01$) ($r^2=0.52$).

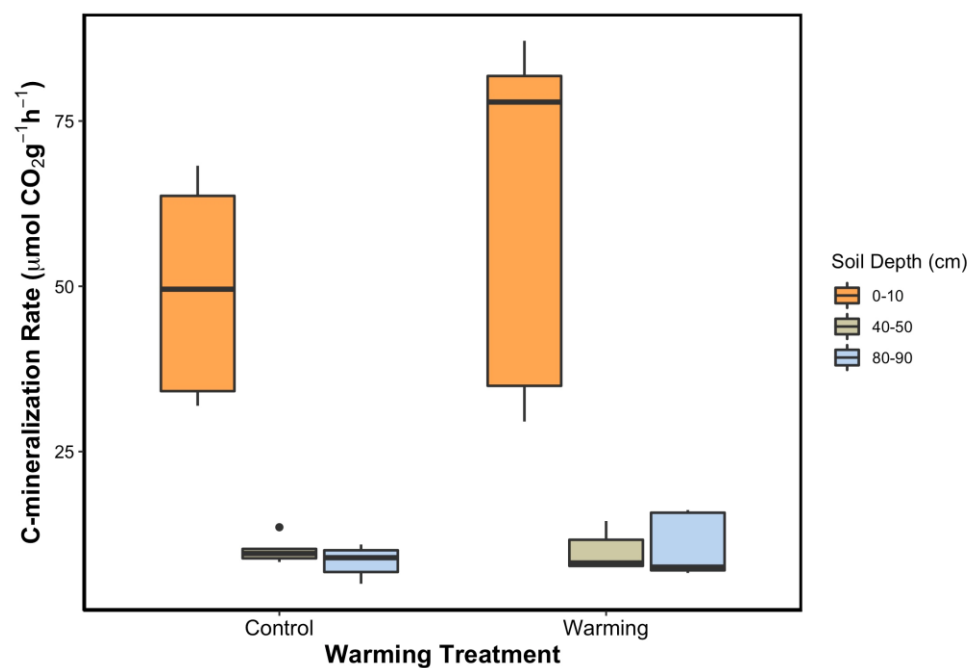


Figure 5: There are no significant differences in day 17 carbon mineralization rates between warming treatments ($p=0.32$) or the interaction between warming treatment and depth ($p=0.53$); however, there is a significant difference in carbon mineralization rates across soil depth ($p<0.01$) ($r^2=0.72$).

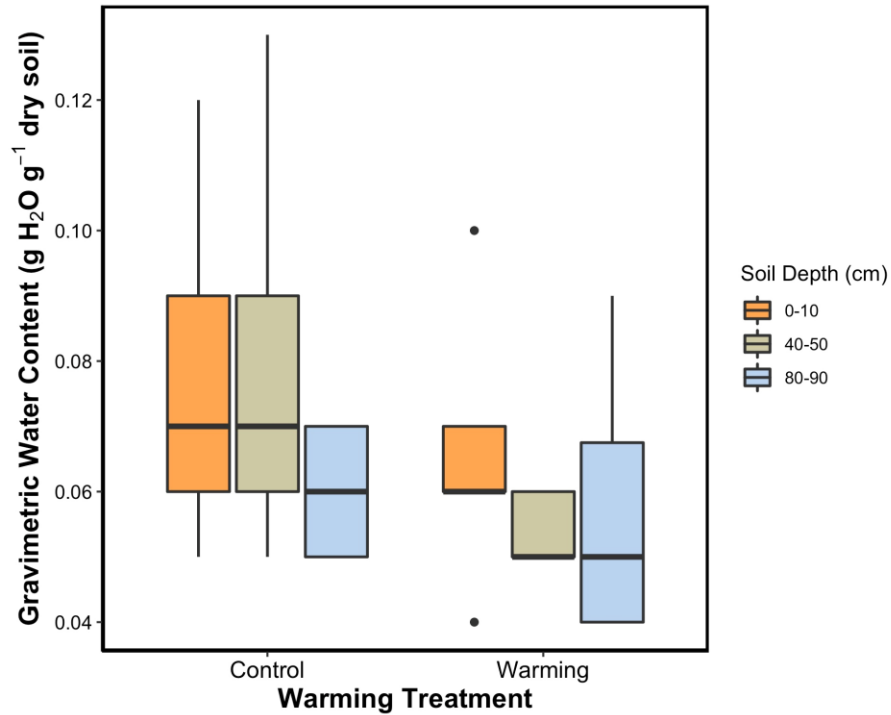


Figure 6: There are no significant differences in gravimetric water content between warming treatments ($p=0.12$), across soil depth ($p=0.40$), or in the interaction between warming treatment and depth ($p=0.52$) ($r^2=0.03$).

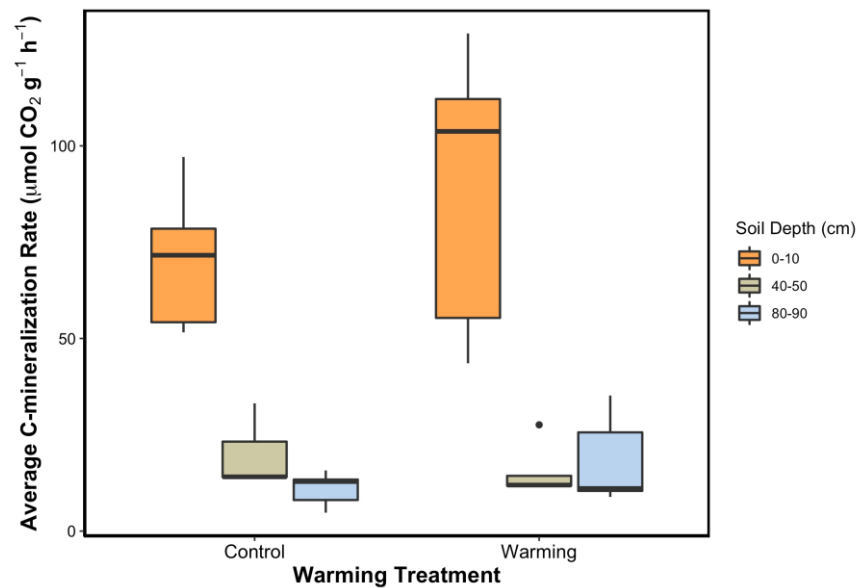


Figure 7: There are no significant differences in the average carbon mineralization rate over the total incubation length between warming treatments ($p=0.30$) or the interaction between warming treatment and depth ($p=0.35$); however, there is a significant difference in the average carbon mineralization rate across soil depth ($p<0.01$) ($r^2=0.72$).

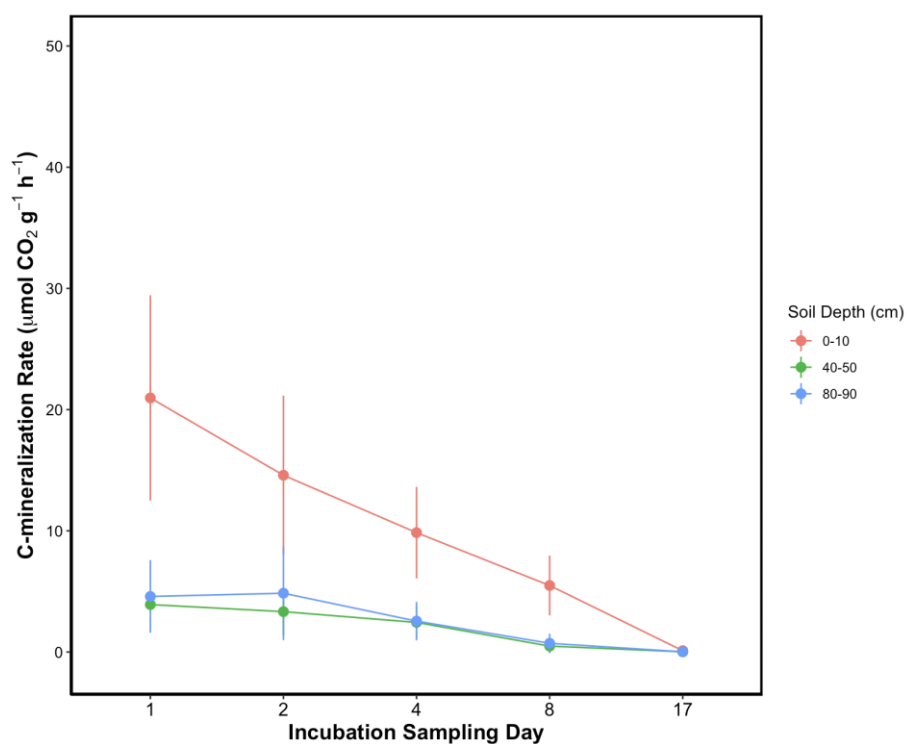


Figure 8: There are no significant differences in the carbon mineralization rate between warming treatments or the interaction between warming treatment and depth in the warmed plots for each of the individual sampling days; however, there are significant differences in the carbon mineralization rate across soil depth. Graph shows a cumulation of individual sampling days. *P* values taken from individual sampling days.

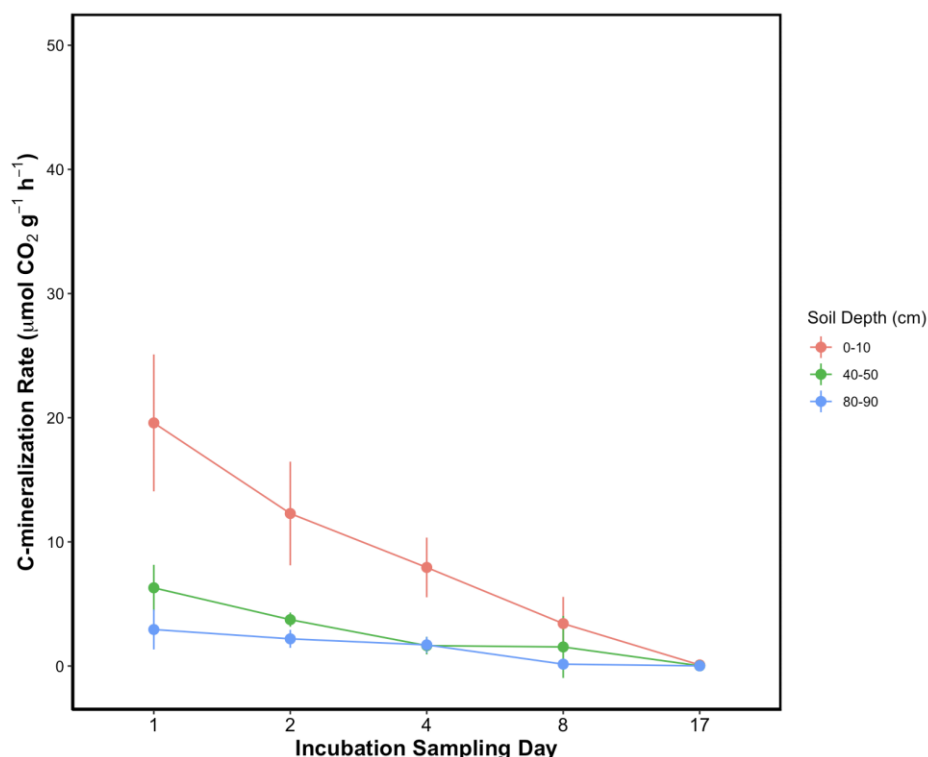


Figure 9: There are no significant differences in the carbon mineralization rate between warming treatments or the interaction between warming treatment and depth in the control plots for each of the individual sampling days; however, there are significant differences in the carbon mineralization rate across soil depth. Graph shows a cumulation of individual sampling days. *P* values taken from individual sampling days.

Brown Forsythe Levene-type Test			
Depth	0-10	40-50	80-90
C-min rate <i>p</i> value	<i>p</i> = 0.14	<i>p</i> = 0.57	<i>p</i> = 0.32

Table 1: There are no significant differences in variation in the median carbon mineralization rates across both treatments by depth.

Brown Forsythe Levene -type Test		
Treatment	Warming	Control
C-min rate <i>p</i> value	<i>p</i> = 0.06	<i>p</i> = 0.46

Table 2: There are no significant differences in variation in the median carbon mineralization rates across depths in warmed or control plots.