

The Impact of Warming and Species Removal on Soil Respiration at Low and High Elevations

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Abstract

Warming temperatures are altering ecosystems, including the plants and soil communities that regulate the carbon cycle. Warming can directly alter the carbon cycle by altering plant and soil community respiration; however, altering plant communities can also have impacts on the carbon cycle. The dominant species in ecosystems also play a large role in ecosystem carbon dynamics because of the unique plant traits present in the ecosystem and plant and soil communities being formed by plant interactions. Soil respiration (carbon efflux) is produced by plant roots as well as soil community respiration and thus is a good integrator of how the soil and plant system may respond to warming and species removal. This study is a part of a long term (6+ year) warming and species removal in Mountains (WaRM) experiment to explore how warming (+2°C) and species removal altered soil respiration. Open-top chambers increased the overall air and soil temperatures and species were removed by cutting all dominant individuals at the soil surface. At each experimental site, a total of 32 plots implemented four treatments (control, warming, removal, and warming + removal) where soil respiration, temperature, and moisture was measured at approximately peak biomass. We found a significant site by warming interaction ($df = 1/184$, $F = 27.60$, $P < 0.001$) where warming increased soil respiration by 104% at the low elevation site, but not the high site. We also determined while the removal treatment at the two sites had no significant impact on soil respiration ($df = 1/184$, $F = 0.14$, $P = 0.71$). These results suggest that warming has a large impact on soil respiration compared to plant community interactions at lower elevations.

Introduction

Due to an increased amount of carbon present in the atmosphere, climate change causes alterations to the structure and function of the soil and plant communities. Within temperate mountain ecosystems, the CO₂ present in the soil is a combination of carbon in the atmosphere and carbon produced by respiration from plant roots, bacteria, fungi, and other soil organisms. Soil carbon exchange is controlled directly by the surrounding climate and indirectly by soil communities. An increase in overall soil respiration (SR) will increase the output of CO₂ into the atmosphere contributing to the effects of global warming. It is unknown what directly regulates SR rates therefore this study's objective is to determine if experimental warming and/or dominant species removal has a significant effect on SR rates. This lack of knowledge will also contribute to the uncertainty in predicting the effects climate change may have on these soil and plant systems.

Soil respiration may increase or decrease with warming temperatures. Climate warming stimulates net primary productivity and increases soil temperature causing soil microbes and enzyme activity to rise, carbon input from leaf litter, and metabolites produced by roots which enhances substrate availability for microbial respiration (Nyberg et al., 2020). Temperature increases have also been shown to extend the growing season causing shifts in plant and soil communities because of the increase of organic matter found in the soil (Nyberg et al., 2020). These warmer temperatures also are believed to decrease the chemical and physical protection of soil organic matter resulting in increased microbial respiration and higher SR. However, SR

rates increase when volume water content (VWC) ranges from 35-50% because more moisture enhances microbial activity (Nyberg et al., 2020) but warmer temperatures decrease VWC. Decreased VWC levels will also reduce microbial activity therefore reducing the amount of carbon produced from plant roots, fungi, bacteria, etc. However, research has shown that soil moisture and temperature are correlated with soil respiration. There are multiple theories on how SR is regulated but there is no consensus on whether warming truly increases or decreases SR.

Plant roots, bacteria, fungi and soil organisms all respire and contribute to soil respiration. Different plant species have different root traits with different respiration and exudation rates, thus plant diversity and biomass can significantly impact soil respiration. Interactions between plants often transition from competitive to facilitative at higher elevation because of the harsher conditions, therefore removing the dominant species may result in overall less biomass (Sundqvist et al., 2019). Removing dominant plant species may also decrease the plant respiration rates therefore impacting the overall SR rate. Dominant species removal and experimentally warming are important variables to research together because interspecific interactions influence plant community composition and ecosystem function, but their impact often fluctuates with climate.

Conducting this experiment at low vs. high environments was important because increased elevation correlates to lower temperatures and higher precipitation, the biodiversity is large, and these ecosystems are very sensitive to fluctuating climate therefore changes in SR can be easily observed. As higher elevations become warmer and the climate fluctuates, plant communities may transition to a more competitive survival technique resulting in an increase in SR. Another benefit of conducting this study at two different elevational gradients is because temperature often changes systematically with elevations therefore research is often performed on elevational gradients when exploring the response of plant and soil communities with climate. These WaRM sites used in this study must be as constant as possible in slope, geology, vegetation growth form, aspect, and hydrology while varying in an elevation of 500 meters (at minimum) to separate temperature differences

We investigated the interaction of species removal and warming at two elevations to help us understand the roles soil and plant communities have on SR, to determine the impact harsh conditions have on plant and soil communities, and whether temperature or plant interactions play a large role in SR rates. During this study, we investigated if experimental warming via open-top chambers and if dominant species removal has a significant effect on SR rate. Our research question was: How does removal of a dominant species and experimental warming directly and interactively impact soil respiration in a montane ecosystem? Our hypotheses were as follows:

1. As open top warming chambers are introduced to the environment causing surrounding air and soil temperature to increase, soil respiration will increase because microbe activity in the soil and plant roots will increase with the warmer temperatures.

2. As the dominant species is removed from the environment, soil respiration will decrease because soil microbes, plant roots, bacteria, fungi, and mycorrhizal symbionts are being removed from the environment.
3. When dominant species removal and experimental warming are both introduced to the environment, soil respiration will slightly increase because soil temperature has a larger impact on soil respiration than species removal.
4. Dominant species removal and experimental warming will have a larger impact at higher elevations compared to low elevations because at high elevations, temperature is more of a limiting factor and species interactions are more facilitative than competitive.

Overall, this experiment provided insight on how climate change could ultimately modify important ecosystem cycles and above/below ground communities in the future.

Methods

Experimental Sites

This experiment was performed at two of the WaRM (Warming and Removal in Mountains) sites set up previously by the Classen and Sanders research group (Figure 1). Each of the sites have 32 2m x 2m plots to test four different treatments: ambient control (AC), ambient removal (AR), warming control (WC), and warming removal (WR). The warming treatment was implemented by placing hexagonal open-top chambers (1.5m in diameter, 2 feet in height) constructed out of fiberglass into the designated plots which raised the mean average temperature inside the open-top chamber by ~2°C (Figure 2). The dominant species was removed by cutting at the soil surface level with scissors and collecting for later biomass measurements. The dominant species at the low elevation site is the forb *Wyethia amplexicaulis* while the dominant species at the high elevation site is the grass species *Juncus drummondii*. For the warming + species removal treatment all of the dominant species was removed from the 2m x 2m plot and then the OTC was installed. The two WaRM sites used during this experiment were a low elevation site located near Almont, CO (Figure 2) and a high elevation site located on Cinnamon Mountain at the top of Paradise Divide near Gothic, CO (Figure 3). At the low elevation site, the open-top chambers were installed, and the dominant species was removed on June 7th, 2020 while at the high elevation site, these procedures were performed on July 1st, 2020 due to snowpack.

Data Collection

To collect the soil respiration data, PVC collars (~4.25 inches in diameter, ~2 inches in height) were installed in each plot into the soil approximately 80cm from the plot edge. The PVC collars were hammered into the ground until ~1 cm of the collar was visible and the vegetation inside and around the collar (1 inch) was cut at the soil surface. The PVC collars were placed into the soil for at a minimum of 5 days before any SR data was taken. Near peak biomass, the SR at each plot was measured using a CO₂/H₂O infrared gas analyzer (IRGA, LI-COR, model LI-6400) with an attachment of a LI-COR 6400-09 soil chamber, which attached to PVC collars in the soil

using the following parameters: target=400, delta= 5(high elevation), 10(low elevation), cycles= 3. During this time, soil temperature was also measured using a soil thermocouple probe (LI-COR 6000- 09TC) which was attached to the LI-6400 (Nyberg et al., 2020) and three temperature data points were collected. Soil moisture (Volumetric Water Content Range, 0- 50%) was also measured randomly throughout each of the plot three times using a hydrosense II soil moisture probe. These measurements occurred on July 6th, 2020 at the low elevation site and on July 16th, 2020 at the high elevation site.

Data Analysis

Excel and JMP software were used to analyze the impacts of warming and species removal at low and high elevation sites on soil respiration rates, soil moisture, and soil temperature. 3- way ANOVA statistical tests were used to determine the significance between the SR rates based on the interactions of warming x removal x elevation. LSMeans Difference Tukey HSD analysis was also performed on each of the response's variables (SR, soil moisture, and soil temp) to determine what treatments were causing significantly different results at both the low and high elevation sites. Linear regression models were also used to determine if soil respiration was influenced by soil moisture and soil temperature. Various graphs were then constructed in excel to visualize and compare soil respiration, moisture, temperature at the two experimental sites.

Results

When comparing all warming treatments to non-warming treatments at both experimental sites, warming caused a significant difference in SR rates ($df=1/184$, $F= 32.9030$, $P< 0.001$). Warming treatments impacted SR differently at both of the sites, causing an overall increase of SR by 104% at the low elevation site ($df=1/184$, $F= 27.60$, $P< 0.001$). However, when comparing all removal treatments to non-removal treatments at both of the elevation sites, removal did not cause a significant difference in SR rates ($df=1/184$, $F=0.02$, $P=0.89$). Similarly, we found no significant effect of the removal treatment at each site ($df=1/184$, $F=0.14$, $P=0.71$) and no significant effect when warming + removal treatments occurred ($df=1/184$, $F=0.66$, $P=0.42$). The interaction of the warming + removal treatments at each site had no significant difference on SR rates ($df=1/184$, $F= 0.11$, $P=0.74$). The mean soil respiration rate and standard error for each treatment at each sites was determined along with a LSD Means Tukey analysis to show what treatments were significantly different from one another: the WC and WR treatments at the low elevation site are different from the AC and AR treatments at the low elevation site (Figure 4).

We determined when comparing the warming to non-warming treatments, there was an overall significant increase in soil temperature ($df=1/184$, $F=21.95$, $P<0.001$). Soil temperature was overall significantly different between the low and high elevation experimental sites, with an overall increase in soil temperature by 6.2% at the low elevation site ($df= 1/184$, $F=1021.12$, $P<0.001$). However, there was no significant difference in soil temperature when the following treatments and interactions occurred: removal treatments

(df= 1/184, F= 0.29, P=0.59), warming treatments at each site (df= 1/184, F= 0.61, P=0.44), removal treatments at each site (df= 1/184, F= 0.01, P=0.94), warming + removal treatments (df= 1/184, F= 0.02, P=0.90), and the interactions of the warming + removal treatments at each site (df= 1/184, F= 0.23, P=0.63). The mean soil temperature and standard error for each treatment at each site was determined along with an LSD Means Tukey analysis to show what treatments were significantly different from one another: all treatments at the low elevation site are different from all treatments at the high elevation site (Figure 5).

The soil moisture data collected showed a significant difference between all of the warming treatments (df= 1/184, F= 16.96, P <0.001), but showed no significant difference for the removal treatments (df= 1/184, F= 0.004, P=0.95), or any of the following treatments and interactions: between each site (df= 1/184, F= 0.16, P=0.69), warming + removal treatments (df= 1/184, F= 1.38, P=0.24), warming treatments at each site (df= 1/184, F= 2.25, P=0.14), removal treatments at each site (df= 1/184, F= 0.35, P=0.55), and the interaction of the warming + treatments at each site (df= 1/184, F= 0.19, P=0.66). The mean soil moisture and standard error for each treatment at each site was determined along with a LSD Means Tukey analysis to show what treatments were significantly different from one another: the AC and AR treatments at the high elevation site are different from the WC and WR treatments at the high elevation site (Figure 6).

A linear regression model was also used to determine if soil moisture and soil temperature can be used to predict SR. The soil moisture and SR linear regression has a goodness of fit (R^2) of 0.0037 while the soil temperature and SR linear regressions had a goodness of fit (R^2) of 0.012 showing no significant correlation between these variables and SR.

Discussion

Warming impacted soil respiration, but the impact varied at high and low elevations. Warming at the low elevation site increased soil respiration rates by 104% relative to control. When the same warming was implemented at a high elevations site, no significant difference in SR rates was observed. These results are supported by Rustad (2001) and Nyberg's (2020) results which states that SR rates often increase with warming temperatures. SR is believed to increase with warmer temperature because soil microbes and enzyme activity may be stimulated with increased soil temperatures. Experimental warming is also believed to extend the growing season therefore increasing biomass and overall SR.

Results indicated that warming had a significant increase in SR only at the low elevation site contrary to Sundqvist (2020) and Rustad (2001). Sundqvist and Rustad's reported that SR will have a larger response at high elevation because of higher temperature sensitivity for soil microbes and species interactions are often more facultative than competitive at high elevations. Increases SR rates at the low elevation site may be due to an early peak biomass season therefore increasing SR earlier in the season, an increase in average precipitation therefore increasing soil moisture and SR, or because the species interactions may be are more

competitive, species must individually response to the increase temperatures quickly in order to survive.

The removal treatments across both low and high elevation sites showed no significant impact on SR which is supported by Nyberg et al. (2020) and Wardle et al. (2005) results. Dominant species removal may have shown no significant impact on SR because other species in this environment have not taken over the area where the dominant species was removed yet. Bokhorst also reported that soil respiration may be unresponsive to changes in plant communities in tundra ecosystems therefore these low and high experimental sites may have similar properties to tundra ecosystems (Bokhorst et al., 2015). Plant communities' interactions are very complex therefore other factors including gross primary productivity, normalized difference vegetation index, total carbon, and total nitrogen may have a larger impact on SR as well as species that grow faster may have a larger contribution to SR than the dominant species that was removed (Wardle et al., 2005). Species removal also had no significant impact on soil temperature and soil moisture which may explained why species removal had no significant impact on SR. Our results were also supported by Nyberg et al. (2020) results which found species removal to have no significant impact on soil temperature.

Soil temperature and moisture are believed to directly correlate to SR rates (Nyberg et al., 2020, Rustad et al., 2001, Sundqvist et al., 2020). However, our results show that there is little to no correlation between these variables. Our results may differ from common literature because the SR data was collected in the morning, potentially causing the soil temperature to be colder than during peak temperature of the day. At the low elevation site, soil moisture was taken a week after the SR data was collected therefore the soil moisture data may have fluctuated over the course of that week. At the high elevation site, soil moisture may vary daily because the data was collected at the beginning of monsoon season causing an increase in precipitation in the area. Overall, when collecting the soil moisture data, the VWC % was variable causing data points within a single plot to be extremely different therefore, causing the mean soil moisture to potentially be inaccurate. Overall, there was no significant evidence that soil moisture and soil temperature had an impact on SR therefore there may be other factors having a larger impact on the response of SR at our experimental sites which can be later studied.

Overall, our results found that experimental warming has a significant impact on the response of SR at lower elevations while removal of the dominant species has no significant effect on SR rates at both low and high elevations. Warmer temperature therefore may be altering plant and soil communities, causing an increase CO₂ in the atmosphere, and contributing to the effects of global warming at lower elevations. In alpine tundra ecosystems, similar to the high elevation site, experimental warming had no significant effects on SR rates making it beneficial for of mountain communities to be more similar to alpine tundra communities. We also found that soil moisture and temperature have no correlation to the response of SR. These results are relevant when investigating what variables, specifically warmer temperatures, impact soil and plant communities. Further experiments may be conducted to explore other biotic and abiotic factors that could be influencing SR rates

including investigating plant cover and diversity effects and collecting other soil variables (TN, TC, root composition, etc.).

Tables and Figures

Figure 1. Coordinates of the low elevation site located in the Almont Triangle and the high elevation site on Cinnamon Mountain near Gothic, CO. Wilderness areas, streams, forestry lands, and country roads are also displayed on this map

Bailey Sharon_RMBL2020_Almont/CinnamonSites

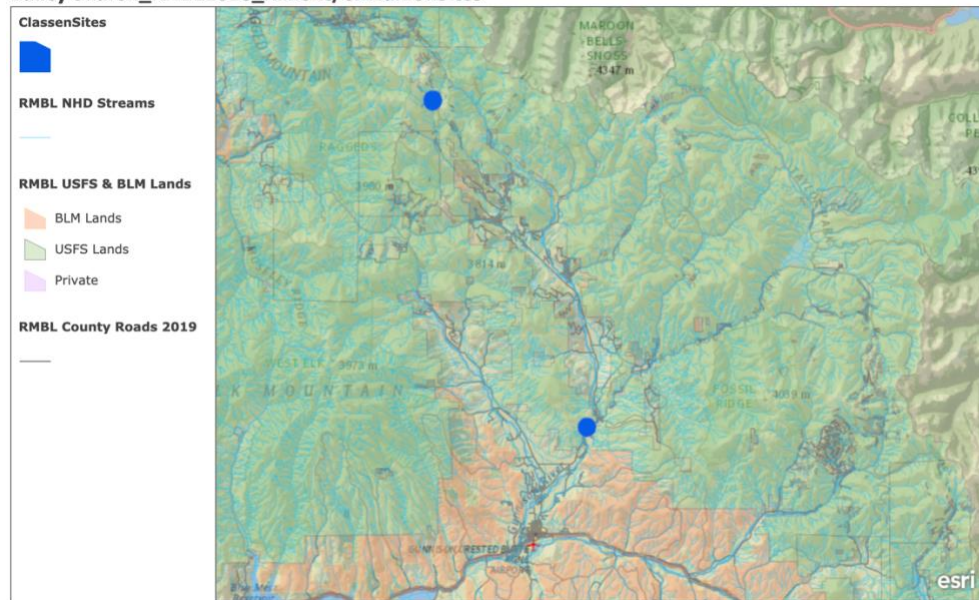


Figure 2. Low elevation site located near Almont, CO where open-top chambers are installed which warms air and soil temperature by $\sim 2^{\circ}\text{C}$.



Figure 3. High elevation experiment site located at the top of Paradise Divide and Cinnamon Mountain near Gothic, CO.



Figure 4. Average soil respiration at each experimental site for four treatments: ambient control (AC), ambient removal (AR), warming control (WC), and warming removal (WR) and an LSD Means Tukey analysis showing various significantly different levels.

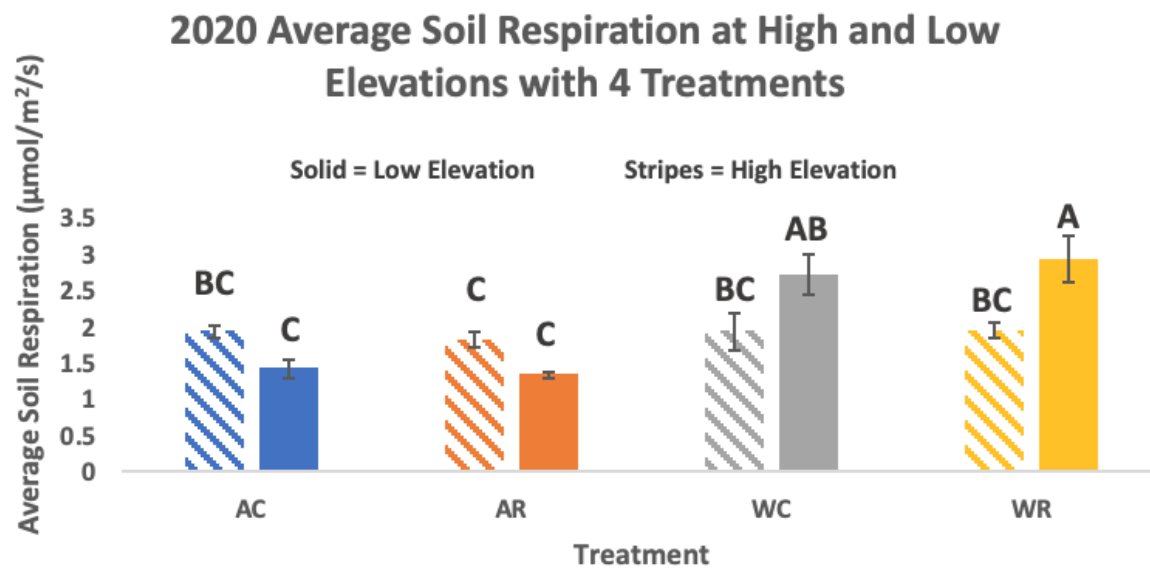


Figure 5. Average soil temperature at each experimental site for four treatments: ambient control (AC), ambient removal (AR), warming control (WC), and warming removal (WR) and an LSD Means Tukey analysis showing various significantly different levels.

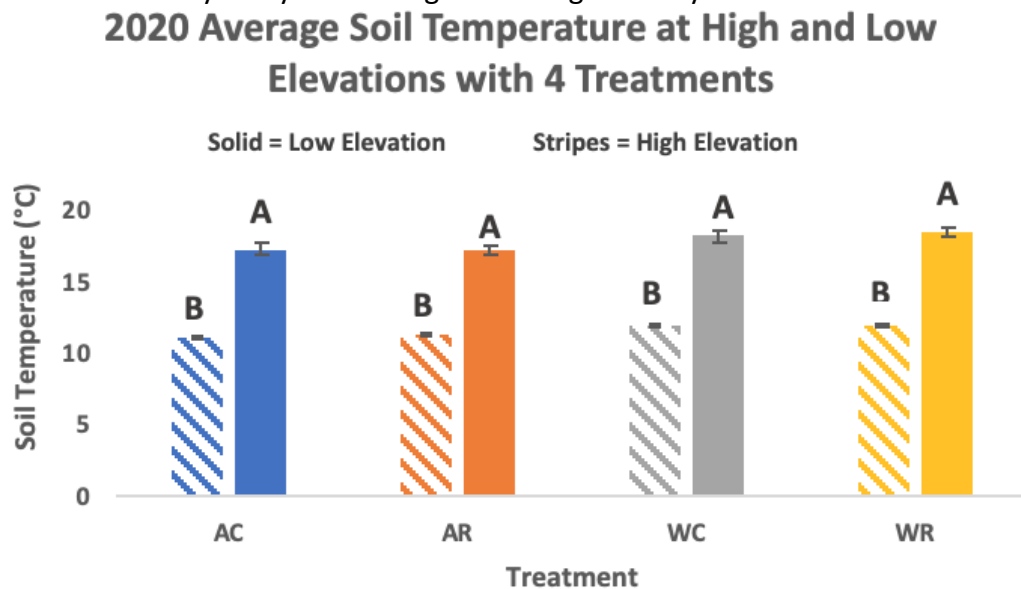
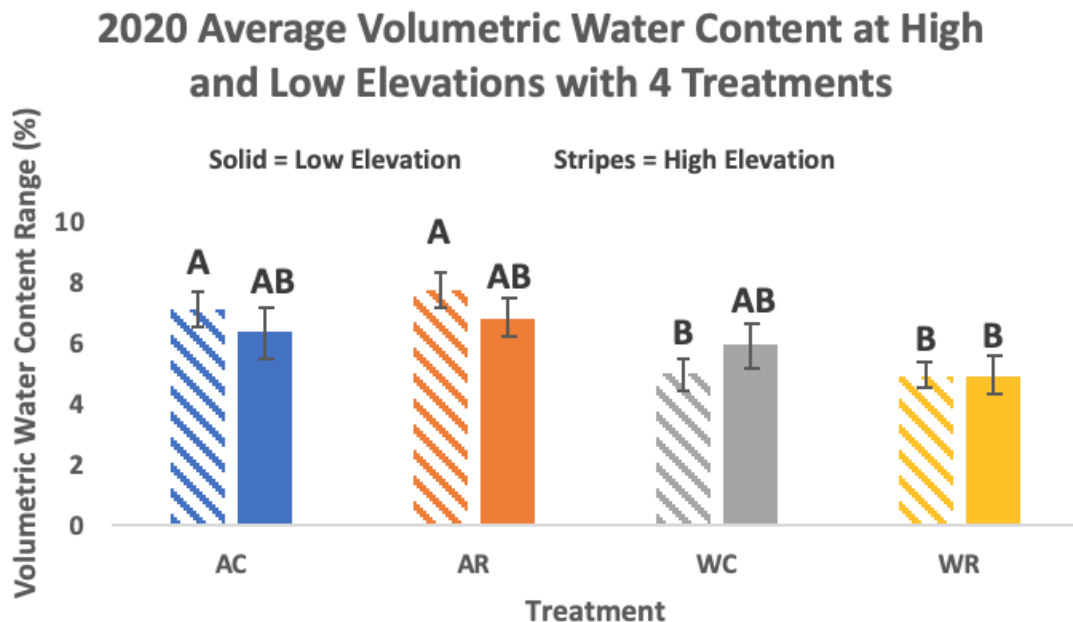


Figure 6. Average volumetric water content at each experimental site for four treatments: ambient control (AC), ambient removal (AR), warming control (WC), and warming removal (WR) and an LSD Means Tukey analysis showing various significantly different levels.



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