**Effects of elevated soil temperature, irrigation, and residue application on soil microbial community structure, soil respiration, and plant physiology in the semi-arid region.**

**Introduction**

The increase in atmospheric temperature has emerged as a challenging issue in recent years because of its potential effects on ecosystem diversity and the earth’s climate. According to Collins et al. (2013), the earth’s average temperature will continue to rise over the twenty-first century unless global carbon emission subsides dramatically; they projected that by 2081-2100, the mean global surface temperature would increase by 1.5oC to 2oC. As the atmospheric temperature increases, it will alter the structure and functioning of a terrestrial ecosystem (Rosenzweig et al., 2007), affects plant growth and reproduction, and changes ecosystem carbon budgeting through the change in the amount of CO2 emission and subsequent absorption to the ecosystem. (Zhao et al., 2017).

Soil microbes have a crucial role in determining the CO2 flux in the terrestrial ecosystem (Allison et al., 2010). Through decomposition and heterotrophic respiration, soil microbes control the amount of CO2 emission from soil to the atmosphere. However, climate change, especially warming, alters the rate of C emission to the atmosphere depending on how soil microorganisms respond to climate change (Treseder et al., 2012). Warming enhances soil microorganisms’ metabolism, a significant contributor to organic matter decomposition in soil, and accelerates the microbial decomposition process, resulting in the higher release of microbial CO2 from the soil (Lloyd & Taylor, 1994). However, the degree of response may not be consistent across the studies. Microorganisms are highly sensitive to environmental change, such as warming. So, microbial response to climate change may also cause a shift in soil microbial diversity and level of carbon-climate feedback (Balser et al., 2010). The relationship gets further complicated when more than one climate change drivers come into play. For example, warming, coupled with decreased precipitation, was reported to have more profoundly impacted soil microbes’ diversity, soil respiration, and overall soil microbial functions than warming did alone (Oliveira et al., 2020). Therefore, to better understand how ecosystems respond to climate change, it is necessary first to understand how soil microbial communities behave with changing environmental parameters.

The soil microorganisms, especially because of their huge diversity and functional role, are essential in determining the terrestrial carbon cycle and climate change feedback across various ecosystems (Schindlbacher et al., 2011). Microorganisms carry out several biological processes, including organic matter decomposition and soil nutrient cycling, from which they derive their essential nutrients to synthesize cellular macromolecules. However, the rate of such processes depends on environmental factors such as temperature, soil moisture, pH, and salinity (Wan et al., 2012). Soil microbes are usually adapted to prevalent temperature conditions. Therefore, the change in existing temperature causes a shift in the microbial community structure (Rinnan et al., 2009). However, the community composition of soil microbes does not seem to follow a predictable pattern of change.

Various short-term warming studies have shown the conflicting trend of soil microbial community composition and their relative abundance. According to Yoshitake et al. (2015), increased soil temperature increases soil organic matter decomposition but decreases soil microbial biomass. Zhang et al. (2005), Frey et al. (2008), and Rinnan et al. (2007) also found similar results in their warming studies. Some other warming experiments (Zhou et al., 2012; Margesin et al., 2009), on the other hand, showed increased soil microbial biomass when soil temperature was increased. Allison and Treseder (2008) reported the significant shift in fungal community structure during a warming experiment in the Alaskan boreal forest; the relative abundance of dominant thelephroid fungus was decreased, and the abundance of Ascomycetes and zygomycetes was increased over three years. De Angelis et al. (2015) found that a 5-degree increase in soil temperature marginally reduced fungal biomass. Fujimura et al. (2007), from 5 years of warming experiment in the tundra ecosystem, reported no significant change in composition, diversity, and evenness of soil fungi. There are also evidences that mycorrhizal fungi were dominant over other groups of fungi in warmer soil (Mohan et al., 2014; Deslippe et al., 2011).

Similarly, Rui et al. (2015) reported increased relative abundances of *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* with soil temperature, while *Betaproteobacteria* and *Acidobacteria* were negatively correlated to soil temperature. The shift in bacterial community structure was also detected when the soil was warmed by 2-degrees Celsius; the relative abundance of *Betaproteobacteria* was mainly altered by soil warming (Zhang et al., 2016). The soil microbes became more responsive when warming was coupled with precipitation treatment. Warming mainly affected the community structure, while precipitation affected the alpha diversity, and the interaction between warming and precipitation affected both diversity and structure (Olivera et al., 2020). Sheik et al. (2011) demonstrated that the warming treatment significantly increased microbial population size by 40–150% in average precipitation years but decreased diversity and significantly changed the community’s composition. However, during drought conditions, the microbial population size was decreased by 50-80% compared with the control.

In general, warming increases the metabolic activity of soil microorganisms (Schindlbacher et al., 2011). An increase in soil temperature usually stimulates soil respiration, either by accelerating CO2 release through autotrophic respiration or affecting the heterotrophic decomposition of organic matter in the soil (Lamberty & Thomson, 2010). However, the soil respiration response to warming is not consistent across all the temperature ranges; therefore, the interaction between soil respiration and soil temperature remains controversial in ecological research (Exbrayat et al., 2013). Temperature dependency of root respiration and microbial decomposition is also influenced by other environmental factors such as soil moisture, soil nutrients availability, and carbon biomass in soil (Davisson and Janssens, 2006). Soil moisture might not always directly affect soil respiration, but it indirectly affects soil respiration by controlling soil temperature. When the soil moisture level is low, it increases the soil temperature and vice versa (Ding et al., 2007). Therefore, more studies are required to explain the interactive effects of soil temperature and moisture on soil carbon flux.

Warming affects the soil processes and the aboveground plant responses like plant growth, assimilation, transpiration, and plant yield. The net assimilation rate is dependent on the efficiency and activation of the Rubisco enzyme complex. As atmospheric temperature increases, the activation energy required for catalysis of photosynthetic reaction, including Rubisco carboxylation activity, also increases (Moore et al., 2021). Rubisco also has an equal tendency to its alternative substrate oxygen. At elevated temperatures, the solubility of oxygen increases relative to CO2, resulting in a decrease in net photosynthesis because of increased photorespiration (Ainsworth & Ort, 2010). The transpiration (water loss from the leaves) is controlled by stomatal behavior, which correlates with vapor pressure deficit (VPD). The interaction between VPD, transpiration, stomatal conductance, and stomatal behavior is crucial in determining net assimilation rate and overall plant performance. The increase in air temperature lowers the atmospheric humidity (i.e., increase VPD). Greater VPD around the plant canopy results in an increased transpiration rate that induces rapid stomatal closure to prevent excessive water loss from plant tissue (Merilo et al., 2018). The combination of moisture and heat stress further intensify the effects as compared to heat stress alone. With the rising temperature and moisture deficiency, stomatal conductance decreases, limiting CO2 diffusion into the leaves and reducing evaporative cooling, leading to metabolic disruption and lower photosynthesis rate (Perdomo et al., 2017).

The great plain region is a primary hub for cotton production in the US. About 30 to 35 % of the cotton yield of the US and 5 to 8 % of the cotton yield production in the world is produced from the great plain s region. However, in recent years cotton production in Texas high Plain has been decreasing – In 2019 cotton produced upland cotton produced in Texas decreased by 8% from 2018 (USDA-NASS, 2020). According to the (PCG, 2020) report, wet and cool weather during planting season in May and hot and dry condition during the critical growth period in August are the major contributing factors for the decrease in yield. Therefore, it is vital to know how soil temperature change and moisture stress affect the plant physiology and community structure of microbes inhabiting the soil rhizosphere region to effectively speculate the consequences of global warming on soil health and plant production. We designed this study to (i) examine how soil microbial community composition and function respond to temperature change in the semi-arid region; (ii) investigate the role of other environmental variables, e.g., soil moisture, available soil carbon biomass, and residue management practices, in determining these responses; and iii) study the effects of heat and moisture stress in cotton physiology, growth, and cotton yield. We will conduct a field experiment manipulating soil temperature, precipitation, and residue management to achieve the earlier objectives. We plan to answer the following questions:

1. How does soil warming changes soil microbial community structure?
2. Does irrigation and soil residue change the soil microbial response to warming?
3. How does soil temperature impact soil organic carbon and CO2 flux from the soil?
4. Does irrigation and soil residue alter soil respiration?
5. How do warming affect plant photosynthesis, transpiration, and stomatal conductance?
6. Is there any difference in net assimilation rate in dryland vs. irrigated agroecosystem?

**Hypothesis**

* If the soil temperature increases, the relative abundances of soil fungi, bacteria, and actinobacteria change because the temperature required for optimum growth and development of each microbial group is different.
* Irrigation and soil residue increases the microbial abundance because optimum soil moisture favors microbial growth and movement within the soil profile, and soil residue provides the substrate for microbial metabolism.
* If the soil temperature increase, organic carbon content decreases and soil respiration increase because temperature accelerates the microbial decomposition of organic matter in the soil. Moisture and residue have positive effects on soil respiration.
* Warming increases net assimilation under irrigated conditions but decreases in dryland because heat and moisture stress in plants impairs metabolic processes leading lower photosynthesis rate.
* Warming increases transpiration but decreases stomatal conductance because increased temperature increases VPD and causes the closing of stomata.

**Methods**

**Site characteristics**

We are conducting an experiment at the Quaker Research Farm, Lubbock, Texas, to study soil microbial and plant physiological responses to soil warming in a semi-arid region. The Quaker Research Farm is located at 33° 41’ 36.4596” N, -101° 54’ 18.612 “W, the elevation is 992 m above sea level, and the average annual rainfall is 472 mm. During the growing season of 2021 (June – September), the field received an average rainfall of 13.25 inches.

**Experimental Design**

Our experiment has three treatments, each treatment having two levels. The treatments are warming (ambient temperature and elevated temperature), irrigation (irrigated vs. dry land), and residue (residue vs. without residue). So, our experiment has the following treatment combinations.

1. Ambient temperature + irrigated + residue
2. Ambient temperature + irrigated + without residue
3. Ambient temperature + dryland + residue
4. Ambient temperature + dryland + without residue
5. Elevated temperature + irrigated + residue
6. Elevated temperature + irrigated + without residue
7. Elevated temperature + dryland + residue
8. Elevated temperature + dryland + without residue

The field is divided into two halves, and one half receives irrigation treatment while another half is dryland. Both irrigated and dryland has three plots (eight subplots within each of those plots), and combinations of warming and residue levels are randomized within the plot. There are six replicates of each treatment type (two replicates per plot). Therefore, we have 48 subplots (2 warming levels × 2 irrigation levels ×2 residue levels × six replicates). The subplots are 1m ×1m, and there is a buffer zone of 1m between each subplot.



Figure 1: Diagrammatic representation of the experimental design. Plots are 1m × 1m. Four different colors represent four individual treatments.

At the beginning of the growing season, the soil was finely plowed using the disc plow to make pulverized soil tilth. We collected soil samples and conducted preliminary tests before planting cotton to ensure that the field had uniform soil microclimatic conditions. Then the cotton was planted continuously in rows spaced 1 meter apart. We irrigated both dryland and irrigated plots once right after seeding to maintain uniform soil moisture conditions. Irrigated plots received 8.60 inches of irrigation water during the growing season. Also, the total amount of rainfall recorded since planning was 13.25 inches. Due to the high rainfall total, we had to modify the irrigation amount and frequency. A week after planting, we applied multi-species grasses residue (Bermuda grass, bluegrass, fescue) at the rate of 6.85lb/plot. We performed two manual weedings to keep the plots weed-free – the first one week after planting and the second six weeks after planting.

The open-top chamber (OTC) method is used to achieve soil warming. We constructed 24 open-top chambers of 1m× 1m× 1m using aluminum rods and transparent polycarbonate sheets. Chambers were constructed in the lab and transported to the field. We installed OTC in the field immediately after sowing cotton seeds. We fixed them on the ground using stakes and zip ties to prevent the wind from blowing the chambers away. Our field is instrumented with three different sensors/loggers to take periodic measurements of soil temperature, soil moisture, air temperature, humidity, and light intensity. Each subplot has one 5TM sensor (Meter Group, Inc. Pullman, WA, USA), which measures soil temperature and moisture from 10 cm below the ground surface. Four 5TM sensors are connected to an EM50 (Meter Group, Inc., Pullman, Washington, USA) data logger, which records soil moisture and temperature data every 15 minutes. We also have Ibuttons - a battery-sized logger (Maxim Integrated, California, USA), fixed 75cm above the ground surface using a plastic funnel attached to the PVC pipe. They record air temperature and humidity every 15 minutes from the plant canopy. Half of our plots (n = 3) also has HOBO Pendant Temperature/light data loggers (Onset Computer Corp., Massachusetts, USA), which measure the amount of light intercepted near the leaf canopy. We have also installed a weather station in the field to obtain field-level weather data such as rainfall, wind speed, atmospheric temperature, humidity, and solar radiation.

We have been measuring leaf-level photosynthesis once a month starting from July using Li-6800 portable photosynthesis system (Li-Cor Inc, Nebraska, USA). We randomly selected four individual plants/leaves in a plot during photosynthesis measurement and took a leaf level measurement of net photosynthesis (A) in the field. Apart from photosynthesis, we are also recording stomatal conductance(gs) and transpiration (E) simultaneously. We are taking measurements in the morning from 9 AM - 12 AM to maintain consistency in the data. We will use photosynthesis and stomatal conductance data to calculate intrinsic water use efficiency as:

Where A = net assimilation rate and gs = stomatal conductance.

We have been taking soil respiration measurements using the Li-8100A soil CO2 flux system (Li-Cor Inc, Nebraska, USA). A soil collar of 20 cm diameter was installed in each plot at the start of the experiment. The measurement of soil CO2 flux was taken once a month, and the observation length was 90 seconds. We take measurements in the morning from 9 AM – 12 AM because the temperature difference might affect the CO2 flux during measurement.

We will collect soil samples before harvesting the crop. Samples will be collected from multiple sites within the plot and mixed to make a composite soil sample. We will collect two composite samples from each plot. Therefore, a total of 96 soil samples will be collected from the field. We will take collected soil samples to the lab for processing. We will send processed soil samples to Waters Agricultural Laboratories Inc. for chemical analysis. For microbial analysis, we will measure soil microbial biomass using the chloroform fumigation extraction technique in the lab. Similarly, we will measure the abundance of each microbial group using FAME analysis. Before performing FAME analysis, soil samples will be stored at -80 0C.

We will manually harvest the crop in mid-October. The cotton boll yield will be measured immediately after harvesting. Similarly, we will also record aboveground and biomass yield.

We believe the data obtained from the study will allow us to understand the effects of heat and moisture stress on cotton physiology, soil microbial community structure, soil respiration, and cotton yield in the semi-arid region.

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