

## Resilience to Increased Heat of Host *Triticum aestivum* with Arbuscular Mycorrhizal Fungi Rich Soil

Amos R. Samuels

### ABSTRACT

The growing climate crisis is projected to disturb food production, and creative implementation of crop-microbe associations can help secure critical food crops. The common bread wheat, *Triticum aestivum*, has a mutualism with several arbuscular mycorrhizal fungi (AMF) that can help the crop be more resilient to climate disturbances. To assess the potential in AMF presence to *T. aestivum*'s resilience to added heat, I looked at the effects of soil microbe presence, AMF inoculation and added heat on the wheat growth above and below ground over 6 weeks. I found that while native microbe composition decreased growth ( $p\text{-value}=1.27 \times 10^{-2}$ ) and increased overall proportionality of wheat ( $p\text{-value}=1.31 \times 10^{-5}$ ), heat increased both biomass production ( $p\text{-value}=2.74 \times 10^{-6}$ ) and plant proportionality but excess caused plant tissue damage. AMF inoculation was significant in contributing to overall growth and underground mass, especially in conjunction with heat ( $p\text{-value}=5.60 \times 10^{-8}$ ), as well as the best proportionality ( $p\text{-value} = 9.148 \times 10^{-11}$ ). In conclusion, the mutualism between *T. aestivum* and AMF holds potential implications for wheat production in warmer climate conditions. Because the study was limited in precise measurements of water content, nutrient availability and microbe distribution, further research is needed to confirm the findings, as well as to replicate the results in outdoor field conditions.

### KEYWORDS

*Triticum aestivum*, climate change, resilience, arbuscular mycorrhizal fungi, mutualism

## INTRODUCTION

All living things on Earth must adapt to the changing climate in the age of the Anthropocene, including humans as NASA researchers say our food systems could begin to experience failures as early as 2030 (Jägermeyr et al. 2021, UN Report 2019). The world's crops depend on the relatively stable weather patterns we've enjoyed over the last two millennia; but as the globe heats new stressors appear, such as localized waves of powerfully cooler or hotter weather than the historical normal, reducing the ability of most plants to grow in their native ranges (UN Report 2019, Warszawski et al. 2013). It's key that we understand the ability of food plants, such as the wheat fields that feed us in North America, to adapt to these temperature changes and be resilient to them (Ryan et al. 2005). As the world population continues growing and an estimated increase of 70–100% more food is required by 2050, the issue of crop failure should be prioritized so as to fix the sustainability of our food systems as well as grow their capacity (Meena et al. 2016). Agricultural capacity will depend on the farm gate management practices we employ, including potential maintenance or introduction of soil biota mutualisms to these fields (Verbruggen and Toby Kiers 2010). It'll take the cooperation of various life forms, along with wise agricultural practices to be resilient and minimize crop failures in the face of this heating.

A primary variable often neglected in agroecology are the symbiotic relationships between species that enhance their resilience, overall survivability and success (Meena et al. 2016). Resilience is the level of resistance and adaptation a system can withstand or adjust to changes without perishing (Warszawski et al. 2013). A mutualism is such a relationship that benefits both or all the participants in that interaction (Meena et al. 2016). A powerful and ancient example of interspecies cooperation is a plant host and its underground mutualism with mycorrhizal fungi (MF), which are a category of various fungal species that are grouped by their behavior (Abiala et al. 2012). While some fungi can grow similarly in a parasitic fashion, MF all grow in and around roots of host plants in a mutualistic symbiosis (Podila and Karma 2006). MF can either be ectomycorrhizae and grow entirely outside and around the host plant root cells, or endomycorrhizae and have a segment of it grow within the root cells (Podila and Karma 2006, Abiala et al. 2012). By looking at wheat's added resilience due to mutualisms with MF we can change agricultural practices to plan for a resilient food production setting.

The most common group of endomycorrhizae are arbuscular mycorrhizae fungi (AMF) that form arbuscule (tree shape like) structures within the rhizome and root cells (Podila and Karma 2006). The fungi's many fine hair-like hyphae structure and extracellular enzymes can obtain resources such as water, phosphorous & minerals in parts of the soil the plant roots can not reach, and transfer them for the plant's use, while in exchange the plant feeds the fungi sugars produced through photosynthesis (Podila and Karma 2006, Abiala et al. 2012). The full complexity of their interaction is not fully understood (Simard et al. 1997, Verbruggen and Toby Kiers 2010, Vannini et al. 2016, Wang et al. 2016, Neurath et al. 2021). It is clear that AMF and *en vivo* plants have beneficial interactions (Bonfante and Genre 2010) and transfer of carbon-based compounds (Simard et al. 1997). AMF inoculation is already used as a bioprotectant and as a biofertilizer, to protect plants from parasites and also increase plant growth and yield (Abiala et al. 2012). Understanding the ability of AMF to support crops' (such as common bread wheat *Triticum aestivum*) growth and resilience during added stressors is crucial to continue feeding the world's population.

While research was done to understand the scope in which AMF contributes to host resilience in drought situations (Kakouridis et al. 2020) and nutrient deficient soils (Wang et al. 2016), this research seeks to understand the potential resilience gained by *Triticum aestivum* as host to common soil AMF to environmental heat increase. Specifically I ask how does the presence of arbuscular mycorrhizal fungi (AMF) affect *Triticum aestivum* resilience in face of temperature increase inherent to climate change? To answer this question I explore these sub questions; 1) do wheat plants that grow in unsterilized soil with a native microbe community grow longer, thicker and with more mass and/or proportion between roots and stalks than plants that grow in sterilized soil with a minimized microbe community? 2) how much of the above effect can be attributed to AMF specifically or just general soil biota? 3) do wheat plants that grow in unheated environments grow longer, thicker and with more mass and/or proportion between roots and stalks than plants that grow in added heat? My prediction was that AMF presence and unheated environments will grow more due to scavenging and protection services of AMF and its presence increases a host plant's resilience to temperature increase (Ryan et al. 2005, Kothe et al. 2018). However, some research shows MF presence has increased host plants' overall health but not survivability (Pickels et al. 2015).

## METHODS

To investigate the questions above I proxy resilience with growth by comparing the difference in growth of below and above ground structures of *Triticum aestivum* (winter wheat) exposed to the above mentioned three soil microbe treatments (sterilized, AMF, unsterilized) and two heat treatments (heated, unheated).

### Experimental Design

I set the experiment itself at the Oxford Tract by the UC Berkeley campus in Berkeley CA (37°52'32.8"N 122°16'01.7"W). The room was a large greenhouse with a glass ceiling and walls (see appendix for room temperature reading chart). All growing pots were on the same table. To avoid possible confounding variables, all treatment groups had a plastic growing tent of clear polyethylene sheeting fastened around a 4'high x 32"long x 2'deep (feet/inches) wooden skeleton, and a Redi-Heat Heavy-Duty Model No. RHD2105, from Phytotronics inc. heat mat underneath them. All groups received the same watering regiment of watering to saturation once every 7 days, using DI water through a hose. I did not add fertilizers or chemical inputs. Room humidity, light and parent soil were the same for all treatment groups. I fabricated the growing pots from custom cut acrylic sheets and quick-sterilized with ethanol. Their dimensions were 14"high x18"long x1"deep (inches). One side was cemented together while the other was just joined with clear tape for easy removal at harvest.

### Soil Origin

I sourced the soil from Little Buck Field (38.992938° N, 123.067714° W), a managed pasture that is grazed by sheep, at the Hopland Research and Extension Center (HREC), Hopland, CA. The soil was collected in March 2014, just before the start of the summer dry season. This field site is well characterized (Sudderth et al. 2012), and the microbial community has been studied extensively by the Firestone Lab at Berkeley. The soil, a fine-loamy, mixed, active, mesic Typic Haploxeralf (Sudderth et al. 2012), was sieved to <2 mm in the field, dried to 1.1 volumetric water content, and stored at 4°C. Field soil had a field bulk density of 1.2 g-cm<sup>-3</sup>, a pH of 5.4 ± 0.03, and 23.3 ± 2.3 mg/g total carbon (Neurath et al. 2021). It was kept in 5 gallon buckets with plastic liner at a 4 Celsius cooler. Note the soil was given to me identified, sieved but otherwise unprocessed, and I didn't test for these metrics myself. Instead I used the

soil and location to conduct the following treatments.

### **Soil Microbe Treatment: Native Versus Sterilized**

To understand soil biota on wheat's success during growth, I compared plants' growth in sterilized and unsterilized soil. I placed  $\frac{1}{3}$  of the total amount of soil aside in the cooler to serve as an unsterilized treatment. I double sterilized the remaining  $\frac{2}{3}$  of the soil in an autoclave during two consecutive runs with a 48 hour hiatus between them, to kill all microbes present including cysts and spores. I expected the soils to become recontaminated through air borne spores and other microbes but the head start of established native biota should offer added services to the wheat plants as they encounter challenges (Bonfante and Genre 2010, Vannini et al. 2016).

### **AMF Inoculation Treatment: Specific Contribution from AMF**

To understand the specific contribution of AMF to the difference in resilience of host *Triticum aestivum* growth, I inoculated  $\frac{1}{2}$  of the sterilized soil ( $\frac{1}{3}$  of the total soil) with spores of *Rhizophagus irregularis*. A common AMF strain, *R. irregularis* is a robust root cell colonizer and likely to outcompete contamination introduced after inoculation. The spores were isolated by Premier Tech, Canada, sold under product ID PTB297-L-ASP-A 100K (182742) with 100,000 spores per 250mL bottle concentration. I poured 20mL onto the sterilized soil after potting and seeding the soil. I expected AMF presence to significantly increase the wheat's ability to grow in added heat as well as moderately in unheated conditions (Kakouridis et al. 2020).

### **Heat Treatment: Heated Versus Unheated Environment**

To understand the effect of increased ambient heat present in the *Triticum aestivum* grow environment on its success, I placed heat mats under the pots so the whole planter was on top of the mat and the ambient temperature within the plastic tent would be homogenous. For the added heat treatment (n=12) I set the heat mat for 30.0 Celsius, and self-regulate to turn off at that temperature through a sensor in the soil at the middle planter. For the room temperature treatment (n=12) I left the heat mat unplugged. I placed a mercury thermometer in the middle of the center pot in each heat treatment to read the temperature weekly at 1pm. I expected the results to show that increased heat decreases the wheat's speed and vitality in growth compared to unheated growing conditions.

### ***Triticum aestivum* Plants**

I obtained *Triticum aestivum* seeds from TrueLeafMarket.com and kept them sealed in a dark drawer until planting. I planted them on March 11th by placing them in even intervals and pushed about ½ inch into the soil to begin germination and growing period. I put 10 equally spaced seeds in each pot. I harvested the plants on April 22nd. I would like to note that an initial common approach attempt of sterilizing the seeds in 1% commercial bleach followed by 70% ethanol, and a direct seed AMF inoculation through submersion (Ryan and Angus 2003, Salamon et al. 2020) resulted in extremely low germination rate (n=16 of 240) after two weeks, and so all seeds were extracted and unsterilized seeds planted instead. The planters were plowed over the top 5cm of soil and then fresh seeds placed about 3 cm deep. For germination rate of initial sterilization attempt see Appendix A. Each soil microbe treatment had 4 pot replicas, totaling 24 pots with seed count n=240.

### **Measurement of Plants Growth**

To record the effects from the various treatment groups on wheat growth, I took measurements of aboveground growth once a week in the afternoon. I measured the wheat length using a ruler, to the nearest 0.1cm, from soil to highest point. At harvest I measured the longest root length the same way. The harvesting was done on April 22nd by unearthing the plant, carefully keeping as many small roots and rhizobia as possible via tapping and shaking. I shook the coarse earth off, let the moist dirt dry and shook again. After harvesting and patting, I cut the plant where the soil interphase was, then measured the biomass using an analytic scale to the nearest .01 g for above ground half, and under ground half.

### **Analysis of Data**

Once I had all my measurements in the tabular format (Appendix C), I checked for normality using R Commander version 2.7-2 (Fox J) to analyze these data. I ran ANOVA tests to see if the differences between the growing treatments were significant. The averages of all plants in each planter are given Table 1 below.

## RESULTS

The unsterilized seed germination rate was much higher (n=202 of 240) and so considered a success. Most planter boxes had 8 to 9 plants successfully growing. The longest wheat planter average measured at 76.4 cm, and the heaviest at 5.44 g. Overall significant differences were observed between the treatments as follows.

### Soil Microbe Treatment: Native Versus Sterilized

I found that plants in the sterilized soil treatment grew more height than native biota in both heat treatments (ANOVA,  $p\text{-value}=1.27 \times 10^{-2}$ ) (Figure 3 & 4). Sterilized average height above ground was 48.5 cm and native was 41.7 cm. Their underground length was insignificant ( $p\text{-value}=9.33 \times 10^{-2}$ ) with sterilized average length 29.2 cm and native 27.2 cm. For a complete chart of average plant growth in each pot see table 1. Biomass told a similar story (ANOVA,  $p\text{-value}=1.91 \times 10^{-2}$ ) with sterilized soil plants being heavier (average 1.96g) and native soil plants being lighter (average 1.74g). An interesting observation was that native microbe soils facilitated a much higher interaction and entanglement between the root systems of the plants, than did the sterilized soil.

**Table 1: Summary data of plant growth averages by planter box.** LEGEND: A=Heat, B=Unheated, S=Sterilized, A=AMF, N=Native microbes, digit: 1-4 number of replica ID (four replicas per treatment). UG= Under Ground, AG=Above Ground, TL/TB= Total Length / Total Biomass as a measurement of plant fitness.

Planter	Avg. AG Length	Avg. UG Length	Avg. Total Length	Avg. AG Biomass	Avg. UG Biomass	Avg. Total Biomass	Avg. TL/TB
AS1	44.1	28.1	72.2	3.5	1.9	5.4	15.0
AS2	40.9	27.9	71.7	3.5	1.9	5.4	15.0
AS3	45.6	27.7	71.0	3.1	1.7	4.8	19.0
AS4	43.2	26.2	68.1	2.9	1.7	4.5	24.1
AA1	45.0	26.5	68.2	2.7	1.6	4.4	24.9
AA2	48.5	26.7	68.5	2.6	1.5	4.1	27.6
AA3	48.9	25.8	66.6	2.6	1.6	4.2	26.6
AA4	44.7	25.8	65.9	2.4	1.6	4.0	27.3

AN1	44.7	27.6	68.3	2.4	1.7	4.2	27.3
AN2	46.4	27.0	67.4	2.1	1.5	3.6	28.5
AN3	41.7	27.5	68.7	2.2	1.2	3.5	28.7
AN4	33.6	27.7	70.4	2.4	1.6	4.0	24.9
BS1	56.5	31.6	76.4	2.9	2.1	5.1	18.7
BS2	52.4	31.0	75.1	2.7	2.1	4.8	22.3
BS3	54.0	31.4	75.3	2.8	2.2	5.1	19.3
BS4	51.7	29.9	72.6	2.6	2.1	4.7	31.5
BA1	49.5	29.4	73.8	2.6	2.0	4.7	31.9
BA2	49.5	28.0	72.6	2.7	2.0	4.6	31.7
BA3	46.6	28.2	73.8	3.0	2.2	5.1	30.7
BA4	48.4	29.2	74.7	2.8	2.3	5.1	30.9
BN1	42.8	29.8	74.0	2.9	2.2	5.1	30.7
BN2	43.6	27.1	71.3	2.7	1.9	4.6	31.2
BN3	43.7	25.8	70.5	2.7	1.9	4.6	30.0
BN4	37.8	25.1	69.2	2.7	1.8	4.5	30.1

### AMF Inoculation Treatment: Specific Contribution from AMF

While microbe treatment alone proved insignificant for the difference in biomass ( $p$ -value $>0.08$ ), plants in the AMF treatment in conjunction with the heat treatment significantly grew the most biomass (ANOVA,  $p$ -value= $5.60 \times 10^{-8}$ ) (Figure 5). Their average total mass was 5.91g. Their underground length was significantly longest as well (ANOVA,  $p$ -value= $3.43 \times 10^{-3}$ ), and averaged 30.3cm (Table 1). AMF was most proportional (ANOVA,  $p$ -value =  $9.15 \times 10^{-11}$ ) of the microbe treatments with heat (Figure 7).

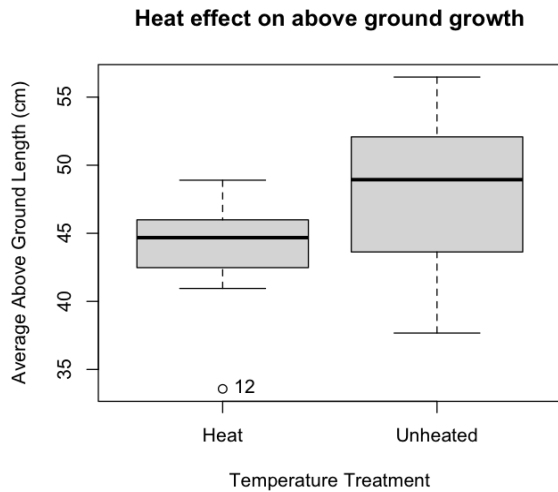
### Heat Treatment: Heated Versus Unheated Environment

Maximum daily temperatures in the heated treatment ranged from 26 to 35 Celsius, while in the unheated treatment from 22 to 31 Celsius. I measured the best biomass production in the heated treatments all around (ANOVA,  $p$ -value= $2.74 \times 10^{-6}$ ), with the heated average biomass at

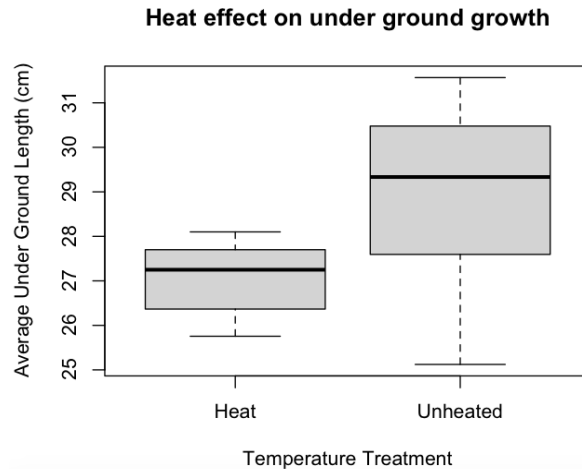


5.16g and unheated average biomass at 3.11g (Table 1). Total length was insignificant ( $p$ -value $>0.8$ ). The tallest plants on average, however, were in the unheated treatment (ANOVA,  $p$ -value= $2.35 \times 10^{-3}$ ) and averaged 48.0cm versus 43.7cm in heated treatment (Table 1). A comparison of growth ability shows the unheated treatment plants grew much more in length than the heated did (Figure 1 and 2). During a heat wave, several plants in sterilized and AMF heat treatment were damaged, but not in native microbe heat or unheated treatments. Furthermore most wheat plants that reached seed stage were in the heated treatment, and within them mostly AMF treatment.

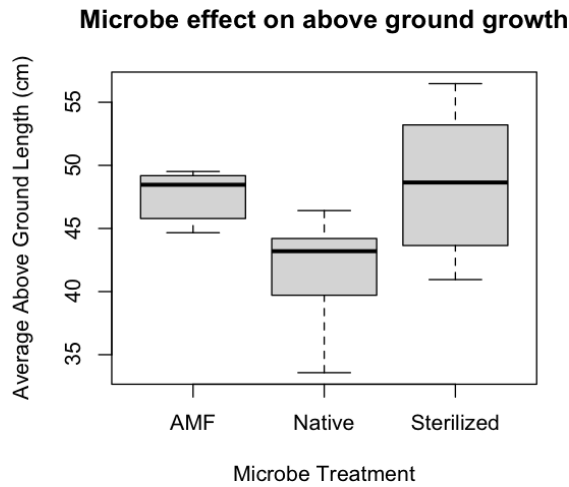
**Figure 1. Box Plots of heated vs unheated comparing above ground growth at harvest of *Triticum aestivum* in added heat vs. unheated ambient room temperature.**



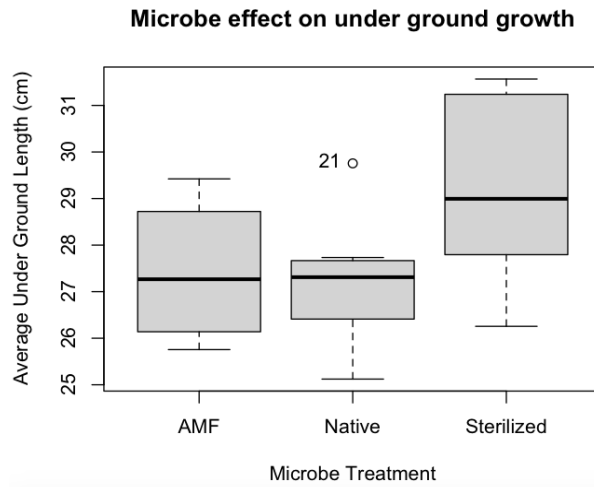
**Figure 2. Box Plots of heated vs unheated comparing underground growth at harvest of *Triticum aestivum* in added heat vs. unheated ambient room temperature.**



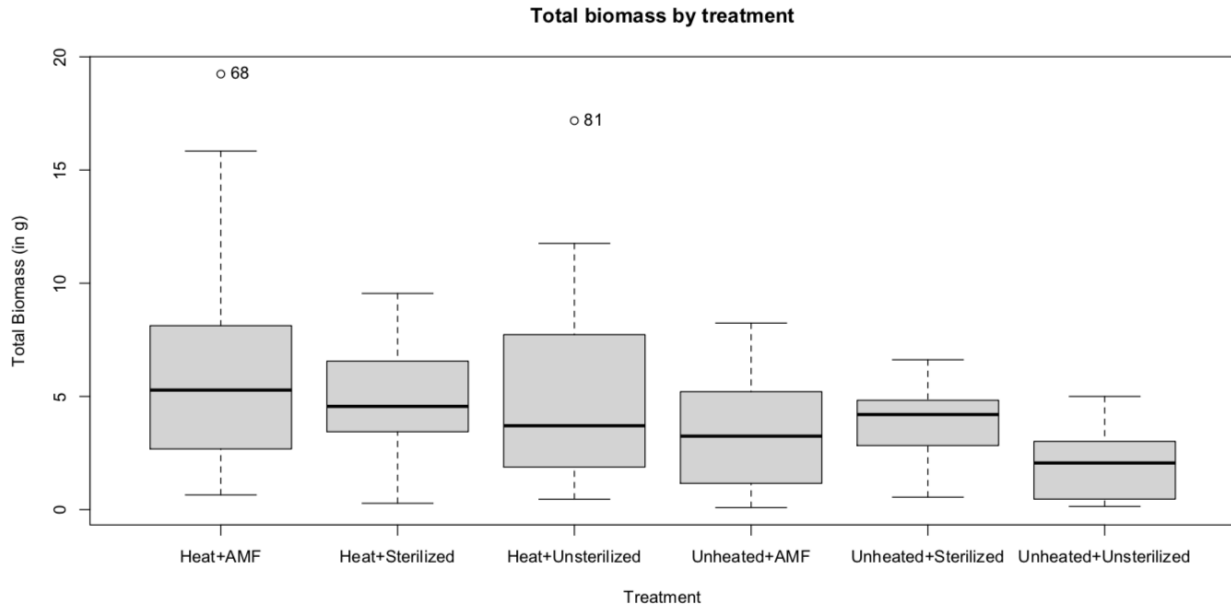
**Figure 3. Box Plots of microbe treatments comparing above ground growth at harvest of *Triticum aestivum* in minimized (pre-sterilized) soil microbe presence AMF encouraged microbe soil presence and original native microbe presence.**



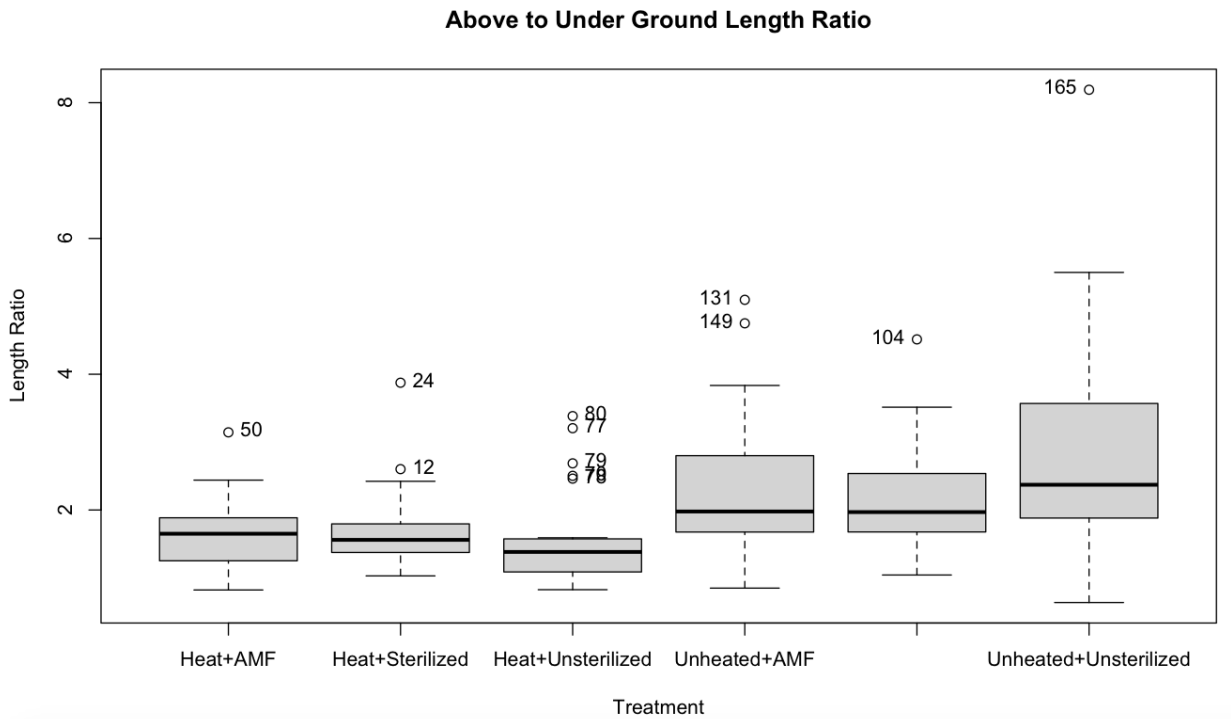
**Figure 4. Box Plots of microbe treatments comparing underground growth at harvest of *Triticum aestivum* in minimized (pre-sterilized) soil microbe presence AMF encouraged microbe soil presence and original native microbe presence.**



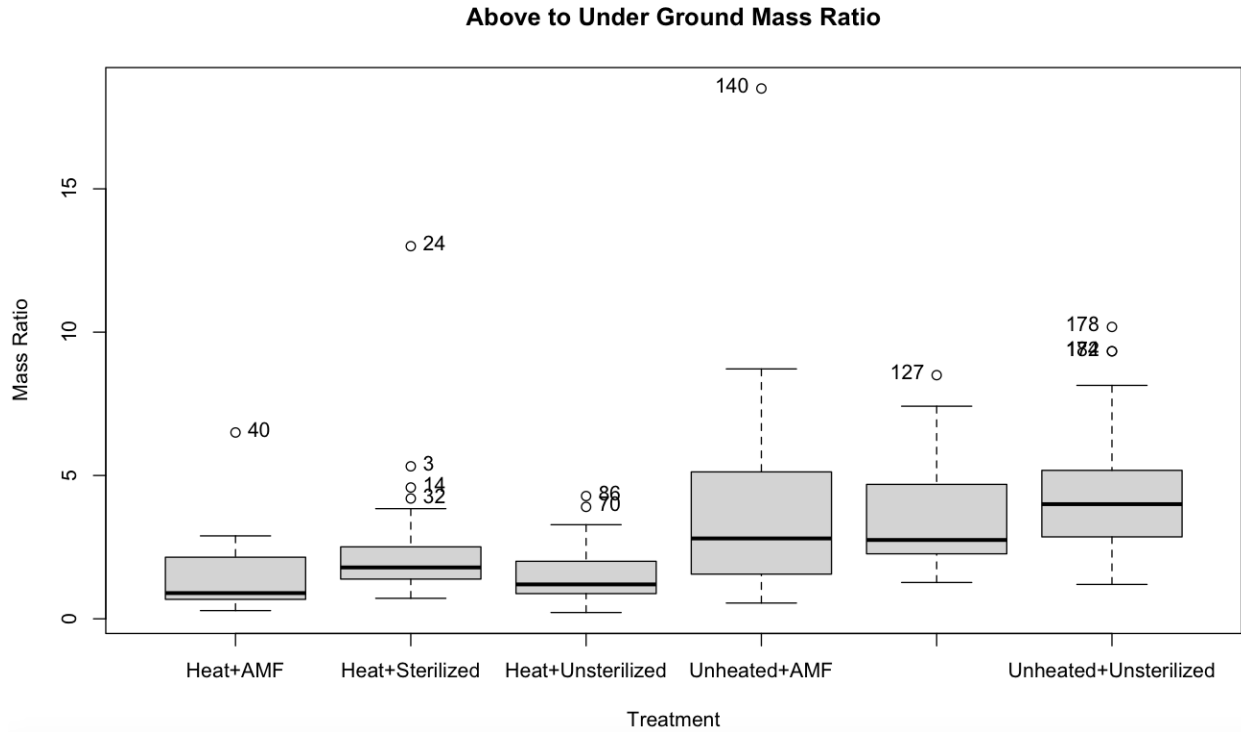
**Figure 5. Total biomass across all plants in each treatment.** AMF in conjunction with heat was the most productive treatment, while native microbes were significantly ( $p\text{-value}=5.60 \times 10^{-8}$ ) least productive.



**Figure 6. Above to Underground Plant Length Ratio.** The closer the ratio is to 1, the more proportionate the plant is above and below ground. The figure shows that heated treatment significantly increased proportion between above ground plant growth and below ground root growth compared to the unheated treatment. It also shows that unsterilized was most proportional of the microbe treatments with heat, but least proportional without heat, suggesting the heat increases native microbe contribution to plant proportion in heat ( $p\text{-value}=1.31 \times 10^{-5}$ )



**Figure 7. Above to Underground Plant Mass Ratio.** The closer the ratio is to 1, the more proportionate the plant is above and below ground. The figure shows that heated treatment significantly increased proportion between above ground plant production and below ground root production compared to the unheated treatment. It also shows that AMF was most proportional of the microbe treatments with heat, and unsterilized least proportional without heat, suggesting the heat increases AMF contribution to plant proportion in heat ( $p\text{-value} = 9.15 \times 10^{-11}$ ).



## DISCUSSION

These results demonstrate that AMF presence causes wheat's resilience to heat to increase, but not as projected in the introduction. The results did not follow my expectations, especially in that the heat treatment was favorable for wheat growth rather than a stressor. The microbe treatment was insignificantly varied between each treatment superficially, but in a deeper look or with conjunction with heat, each of them provided an advantage or disadvantage in some merit as described below. Through the plants proportionality of above and below ground structures, overall biomass production and plant maturation in the 6 weeks given to grow, it is apparent AMF is a boost to *T. aestivum* growth in the heated environment, but not in an unheated environment. Furthermore, in this section I will show there were multiple caveats to my methodology such as microbe contamination, lack of other variables standardization or

expression of full AMF capacity as it is in field conditions.

### **Soil Microbe Treatment: Native Versus Sterilized**

At first glance, my results seem to show that sterilization is the best treatment for crop production, but that is inaccurate. While sterilization increased above ground height and reached similar biomass to AMF, it does not necessarily indicate better resilience. Inherent to the microbe-plant mutualisms is the transference of photosynthesized resources from plant to rhizosphere, which can go to explain the added resources for plant growth within the experimental period with minimal microbe presence (Johnson and Graham 2013, Hossain et al. 2021). Additionally, there were potentially less parasitic microbes to slow the plant growth, which present false representation of field conditions where the soil would be recolonized quickly and without beneficial microbes to compete with parasitic ones the plants would suffer and fail more (Juroszek and von Tiedemann 2013).

The native microbe community seemed to reduce plant height, probably due to the above discussed resource costs to plants, but increase resilience compared to the reduced microbe community by offering additional community resources such as added water scavenging and retention, facilitation of support between different individuals through inter-root networks, and extracellular enzymatic immobilization of nutrients (Podila and Karma 2006, Vannini et al. 2016, Kakouridis et al. 2020). The increased root entanglement and interaction supports this hypothesis, and could also explain the insignificant root length difference that does not account for horizontal and wrapped growth. To reinforce this, I compared the above to the underground plant length ratio (Figure 6). I saw that unsterilized was most proportional of the microbe treatments with heat, but least proportional without heat, suggesting the heat increases native microbe contribution to plant proportion in heat ( $p\text{-value}=1.31 \times 10^{-5}$ ) which could translate to overall fitness in face of stressed conditions i.g water scarcity and temperature swings (Podila and Karma 2006, Kothe and Turnau 2018).

### **AMF Inoculation Treatment: Specific Contribution from AMF**

The AMF treatment showed a hybridic effect of the other two microbe treatments without heat but overall proved significantly useful for plant success in conjunction with heat. On one hand, it did not consistently outperform the other treatments in length without heat; the AMF rich soil did not increase the above ground length more than sterilized in the unheated environment,

and had a similar shorter under ground lengths as the native microbe treatment did. On the other hand, its total length in the unheated environment was much closer to the sterilized treatment even though the plants sacrificed resources to the mutualism with the fungi, which can indicate that the AMF services to the plant helped it more than the native microbe community did (Bonfante and Genre 2010, Wang et al. 2016). Furthermore in the heated environment the AMF treatment plants reached significantly ( $p\text{-value}=8.25 \times 10^{-5}$ ) longer total lengths than did other microbe treatments. They also had much higher biomass average than any other treatment ( $p\text{-value}=5.60 \times 10^{-8}$ ), which places them as the best performing plants in the study (Figure 5).

Heated AMF rich treatment reaching best production performance, along with significant root interaction (less than native microbes but a lot more than sterilized), suggests that AMF richness become a valuable resource for *Triticum aestivum* in added heat environments. AMF was most proportional of the microbe treatments with heat, suggesting the heat increases AMF contribution to plant proportion in heat ( $p\text{-value} = 9.15 \times 10^{-11}$ ) (Figure 7). The fact the AMF didn't contribute significantly to plant growth in the unheated environment suggests the AMF can become more important in hotter environments (Ryan and Angus 2003). It's further supported by the mycorrhizal mutualism-parasitism continuum described in Johnson and Graham's paper; when growing conditions are ideal for host plant growth, the fungi can demand more resources from the host than it helps scavenging for and so function as a parasite, and when the conditions are difficult for the host, the fungi plays a more crucial role and contribute as much or more than it consumes (Johnson and Graham 2013). As heat increased, conditions became more difficult for the plants, they relied further on interactions with AMF, and were able to outcompete the plants that didn't have these interactions. Therefore it supports my hypothesis that AMF can increase resilience and success for *T. aestivum* in increased heat.

### **Heat Treatment: Heated Versus Unheated Environment**

The heat treatment was significant in increasing wheat biomass growth, most of all in the presence of AMF, but also facilitated plant tissue damage in excess heat. When temperatures passed 33 Celsius, the leaves of most plants in the heated AMF and heated sterilized treatments were damaged and wilted, while the leaves of other treatments remained vigorous. This goes to show the potential in microbe presence to extreme heat swings, but not its effect on daily crop growth operations (Ryan and Angus 2003).

While heat was insignificant in total plant length, heat treatment significantly ( $p$ -

value= $8.91 \times 10^{-4}$ ) increased underground length, and unheated treatment significantly (p-value= $2.35 \times 10^{-3}$ ) increased above ground length. This is possibly a strategy to seek resources the plant must undertake to find water available once the superficial water is evaporated in hotter environments, as well as minimize leaf evaporation surface potential (Kakouridis et al. 2020, Neurath et al. 2021). That is supported by the heat treatment having consistently both better above ground to under ground length ratios (p-value= $1.31 \times 10^{-5}$ ) and mass ratios (p-value= $9.148 \times 10^{-11}$ ) across all microbe treatments, showing heat encouraged more root exploration to support stalk growth. Even though the heat might've challenged the wheat's ability to grow above ground length, it still facilitated heavier both above ground biomass (weakly, p-value= $3.38 \times 10^{-2}$ ) and below ground biomass (strongly, p-value= $1.49 \times 10^{-9}$ ). This phenomenon, along with how a vast majority more plants reached seeding stage at 6 weeks in heated pots, indicates heat has potential to increase crop productivity when it doesn't dry out (Jägermeyr et al. 2021).

## Synthesis

Proportionality, rate of maturation to seed and overall mass production of *Triticum aestivum* was highest in the presence of AMF rich soils in a heated environment. In unheated environments, AMF was insignificant in aiding plant growth. Because of this clear difference in contribution between temperature environments, I conclude that AMF holds potential to increase host wheat resilience to heat but can act as a limitation of wheat growth in unstressed environments. This further proves the mycorrhizal mutualism-parasitism continuum (Johnson and Graham 2013), gives possible explanations for AMF lack of contributions found in previous studies (Ryan and Graham 2018), and clarifies the success of AMF contribution in other previous studies (Hijri 2016).

Overall, AMF presence still holds much potential in added resilience for wheat fields that are experiencing stressors such as heat. The conditions will become rougher for global wheat production in coming years (Juroszek and von Tiedemann 2013), and hotter temperatures will introduce new pests and disease to wheat growing regions, deplete water resources faster and dramatize patterns of drought and flooding (Warszawski et al. 2013). However, one of the many solutions to these pending crop failures can be the management of soil microbes in deliberate ways to protect and empower our crop plants, specifically AMF such as *Rhizophagus irregularis*.

This study shows that AMF inoculation, supervision and cultivation can be one of these solutions. The results of the study are significant in pushing for more inspection of this as a solution, but before any recommendations are made for farmers, further research on a larger scale is needed.

### **Limitations/Future Analysis**

This study and its scope were limited due to various restrictions in time and resources, and so its conclusion is not ready to be taken on to implementation in the crop fields but rather to a larger research. The core flaw with the research design is the proximity of microbe treatments. In this study I could only give a 'head start' for the specified soil microbes in each treatment. The sterilized treatment was fully sterilized at the point it exited the autoclave on the second time but afterwards was exposed to many sources of contamination, specifically the airborne spores, viruses, bacteria, etc. from the unsterilized treatment sitting mere inches away (Druzhinina et al. 2011, Abiala et al. 2012). Moreover, the very effect of baking the soil in the autoclave could have been a confounding factor, especially in changing the water content in the soil (Podila and Karma 2006, Druzhinina et al. 2011).

Another source of contamination was introduced through the unsterilized seeds after the failure of the sterilized ones to germinate. While it was necessary to allow sufficient data to be collected to discuss the experiment at all, reseeded gave a shorter growth period so plants had less time to interact with the soil microbes and so became less meaningful to the research question. They also introduced unknown microbes through the non sterilized surface of seed, which is a potential confounding factor that is altering my results completely without my knowledge (Podila and Karma 2006).

A similarly critical limitation of the study is that in order to limit confounding factors it was conducted in a greenhouse, in pots, in sieved soil with no animals, macro-aggregation, other plant interactions, unexpected weather conditions, etc. that exist in true field conditions (Gdanetz and Trail 2017). More importantly, the effect of AMF (and perhaps soil fungi in large) cannot be truly measured *in vitro* due to the limited capacity to form a mycelial network and to interact with other members of the ecosystem in the way it can on the field (Hijri 2016, Ryan and Graham 2018).

Another element to expand on in future research is the interaction of the studied variables



in this study with other factors I did not study; water availability, nutrient content (primarily phosphorus) and disease presence. While I watered all pots with similar quantities from the same source at the same time, I did not measure how much exactly entered so some discrepancy is possible, especially considering the importance of water content to AMF beneficial behavior (Kakouridis et al. 2020). Phosphorus content as well as vital micronutrients should be measured before growth and monitored in soil and plant tissue at harvest (Ryan and Angus 2003, Ryan et al. 2005).

Furthermore a gradient of water availability as well as nutrient gradient should be made to see the interaction between drought, soil nutrient depletion, heat and soil microbes to host plant success (Salamon et al. 2020). Further research would take these factors into account, use cultivated soils to have a real life application, compare indoor and field study groups, and expose part of each treatment group to disease or parasites and allow the AMF to form wider spread mycelial networks to fully unleash its potential (Simard et al. 1997, Gdanetz and Trail 2017, Ryan and Graham 2018, Hossain et al. 2021).

### **Broader Implications**

While the initial use of the study was to give context onto which policymakers and farmers can discuss the application of AMF as a tool to make crops more resilient, after the above mentioned limitations were considered the primary implication of this research is the fine tuning of the research tools and methodology that can be used to explore the possibility of this resilience (Ryan and Graham 2018). The comparison of *Triticum aestivum* plant growth is an excellent model for the efficacy of AMF services in heat stress conditions, and a similar study can be done with more effective microbial and water content control (Hijri 2016, Hossain et al. 2021). If the results of that study merit further research, an *in vivo* research over a couple of years where microbes are harder to control is also implicated. Eventually this direction of inquiry can imply the benefit in either the limitation, or proliferation and introduction of AMF to crop fields around the world (Hijri 2016, Ryan and Graham 2018, Hossain et al. 2021).

## ACKNOWLEDGMENTS

Dr. Tina Mendez for endless support, logistic gate opening and structuring; Jessie Anna Moravek for editing advice, content shaping and encouragement; Dr. Javier Ceja Navarro for advising, lab access, resources and problem solving; Dr. Ignacio Chapela for guidance, challenging my project to be better and his mentorship; The Firestone Lab, Dr. Jennifer Pett-Ridge, Katrina Estera-Molina, Rachel Hestrin, The Oxford Tract, Ella Sieradzki, Leslie Fleming for inspiring me to believe in myself as a scientist, and to my partner Eirik Juel for being ever-supportive especially when I don't see the light at the end of the tunnel.

## REFERENCES

- Abiala, M. A., O. J. Olawuyi, J. O. Oyelude, A. O. Akanmu, A. S. Killani, O. Osonubi, O. O. Popoola, and A. C. Odebode. 2012. Harnessing the Potentials of Vesicular Arbuscular Mycorrhizal (VAM) Fungi to Plant Growth – A Review. *International Journal of Pure and Applied Sciences and Technology* 14:61–79.
- Bonfante, P., and A. Genre. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications* 1:48.
- Druzhinina, I. S., V. Seidl-Seiboth, A. Herrera-Estrella, B. A. Horwitz, C. M. Kenerley, E. Monte, P. K. Mukherjee, S. Zeilinger, I. V. Grigoriev, and C. P. Kubicek. 2011. Trichoderma: the genomics of opportunistic success. *Nature Reviews Microbiology* 9:749–759.
- Fox J, Bouchet-Valat M (2021). *Rcmdr: R Commander*. R package version 2.7-2, <https://socialsciences.mcmaster.ca/jfox/Misc/Rcmdr/>.
- Gdanetz, K., and F. Trail. 2017. The Wheat Microbiome Under Four Management Strategies, and Potential for Endophytes in Disease Protection. *Phytobiomes Journal* 1:158–168.
- Global Table. 2019. Digging into carbon sequestration with Guy Webb. Melbourne, Australia.
- Guo, X., Z. Wang, J. Zhang, P. Wang, Y. Li, and B. Ji. 2021. Host-Specific Effects of Arbuscular Mycorrhizal Fungi on Two Caragana Species in Desert Grassland. *Journal of Fungi* 7.
- Hermosa, R., A. Viterbo, I. Chet, and E. Monte. 2012. Plant-beneficial effects of Trichoderma and of its genes. *Microbiology* 158:17–25.

- Hijri, M. 2016. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza* 26:209–214.
- Hossain, A., M. Skalicky, M. Brestic, S. Maitra, M. Ashraful Alam, M. A. Syed, J. Hossain, S. Sarkar, S. Saha, P. Bhadra, T. Shankar, R. Bhatt, A. Kumar Chaki, A. EL Sabagh, and T. Islam. 2021. Consequences and Mitigation Strategies of Abiotic Stresses in Wheat (*Triticum aestivum* L.) under the Changing Climate. *Agronomy* 11:241.
- Jägermeyr, J., C. Müller, A. C. Ruane, J. Elliott, J. Balkovic, O. Castillo, B. Faye, I. Foster, C. Folberth, J. A. Franke, K. Fuchs, J. R. Guarin, J. Heinke, G. Hoogenboom, T. Iizumi, A. K. Jain, D. Kelly, N. Khabarov, S. Lange, T.-S. Lin, W. Liu, O. Mialyk, S. Minoli, E. J. Moyer, M. Okada, M. Phillips, C. Porter, S. S. Rabin, C. Scheer, J. M. Schneider, J. F. Schyns, R. Skalsky, A. Smerald, T. Stella, H. Stephens, H. Webber, F. Zabel, and C. Rosenzweig. 2021. Climate impacts on global agriculture emerge earlier in new generation of climate and crop models. *Nature Food* 2:873–885.
- Johnson, N. C., and J. H. Graham. 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. *Plant and Soil* 363:411–419.
- Juroszek, P., and A. von Tiedemann. 2013. Climate change and potential future risks through wheat diseases: a review. *European Journal of Plant Pathology* 136:21–33.
- Kakouridis, A., J. A. Hagen, M. P. Kan, S. Mambelli, L. J. Feldman, D. J. Herman, J. Pett-Ridge, and M. K. Firestone. 2020. Routes to Roots: Direct Evidence of Water Transport by Arbuscular Mycorrhizal Fungi to Host Plants. preprint, *Microbiology*.
- Kothe, E., and K. Turnau. 2018. Editorial: Mycorrhizosphere Communication: Mycorrhizal Fungi and Endophytic Fungus-Plant Interactions. *Frontiers in Microbiology* 9:3015.
- Lace, B., A. Genre, S. Woo, A. Faccio, M. Lorito, and P. Bonfante. 2015. Gate crashing arbuscular mycorrhizas: in vivo imaging shows the extensive colonization of both symbionts by *T richoderma atroviride*. *Environmental Microbiology Reports* 7:64–77.
- Meena, S. K., A. Rakshit, and V. S. Meena. 2016. Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. *Biocatalysis and Agricultural Biotechnology* 6:68–75.
- Neurath, R. A., J. Pett-Ridge, I. Chu-Jacoby, D. Herman, T. Whitman, P. Nico, A. S. Lipton, J. Kyle, M. M. Tfaily, A. Thompson, and M. K. Firestone. 2021. Root carbon interaction with soil minerals is dynamic, leaving a legacy of microbially-derived residues. [bioRxiv:2021.03.23.436628](https://doi.org/10.1101/2021.03.23.436628).

- Perez, F., and B. E. Granger. 2007. IPython: A System for Interactive Scientific Computing. *Computing in Science & Engineering* 9:21–29.
- Pickles, B. J., B. D. Twieg, G. A. O'Neill, W. W. Mohn, and S. W. Simard. 2015. Local adaptation in migrated interior Douglas-fir seedlings is mediated by ectomycorrhizas and other soil factors. *New Phytologist* 207:858–871.
- Pizano, C., S. A. Mangan, J. H. Graham, and K. Kitajima. 2017. Host-specific effects of soil microbial filtrates prevail over those of arbuscular mycorrhizae in a fragmented landscape. *Ecological Applications* 27:1946–1957.
- Podila, G. K., and A. Karma. 2006. *Basic research and applications of mycorrhizae*. Anshan, Tunbridge Wells, Kent, UK.
- Ryan, M. H., and J. F. Angus. 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. *Plant and Soil* 250:225–239.
- Ryan, M. H., and J. H. Graham. 2018. Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. *New Phytologist* 220:1092–1107.
- Ryan, M. H., A. F. van Herwaarden, J. F. Angus, and J. A. Kirkegaard. 2005. Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi. *Plant & Soil* 270:275–286.
- Salamon, S., K. Mikołajczak, L. Błaszczyk, K. Ratajczak, and H. Sulewska. 2020. Changes in root-associated fungal communities in *Triticum aestivum* ssp. *spelta* L. and *Triticum aestivum* ssp. *vulgare* L. under drought stress and in various soil processing. *PLOS ONE* 15:e0240037.
- Simard, S. W., M. D. Jones, D. M. Durall, D. A. Perry, D. D. Myrold, and R. Molina. 1997. Reciprocal transfer of carbon isotopes between ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*. *New Phytologist* 137:529–542.
- Sudderth, E. A., S. B. St. Clair, S. A. Placella, S. M. Swarbreck, C. Castanha, D. J. Herman, M. L. Fischer, M. Kleber, E. B. Sudderth, M. S. Torn, M. K. Firestone, G. L. Andersen, and D. D. Ackerly. 2012. Annual grassland resource pools and fluxes: sensitivity to precipitation and dry periods on two contrasting soils. *Ecosphere* 3:art70.
- Toreti, A., O. Cronie, and M. Zampieri. 2019. Concurrent climate extremes in the key wheat producing regions of the world. *Scientific Reports* 9:5493.

- UN Report. 2019. UN Report: Nature's Dangerous Decline "Unprecedented"; Species Extinction Rates "Accelerating." United Nations.
- Vannini, C., A. Carpentieri, A. Salvioli, M. Novero, M. Marsoni, L. Testa, M. C. Pinto, A. Amoresano, F. Ortolani, M. Bracale, and P. Bonfante. 2016. An interdomain network: the endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. *New Phytologist* 211:265–275.
- Verbruggen, E., and E. Toby Kiers. 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems: AMF in agriculture. *Evolutionary Applications* 3:547–560.
- Wang, Z.-G., Y.-L. Bi, B. Jiang, Y. Zhakypbek, S.-P. Peng, W.-W. Liu, and H. Liu. 2016. Arbuscular mycorrhizal fungi enhance soil carbon sequestration in the coalfields, northwest China. *Scientific Reports* 6:34336.
- Warszawski, L., A. Friend, S. Ostberg, K. Frieler, W. Lucht, S. Schaphoff, D. Beerling, P. Cadule, P. Ciais, D. B. Clark, R. Kahana, A. Ito, R. Keribin, A. Kleidon, M. Lomas, K. Nishina, R. Pavlick, T. T. Rademacher, M. Buechner, F. Piontek, J. Schewe, O. Serdeczny, and H. J. Schellnhuber. 2013. A multi-model analysis of risk of ecosystem shifts under climate change. *Environmental Research Letters* 8:044018.

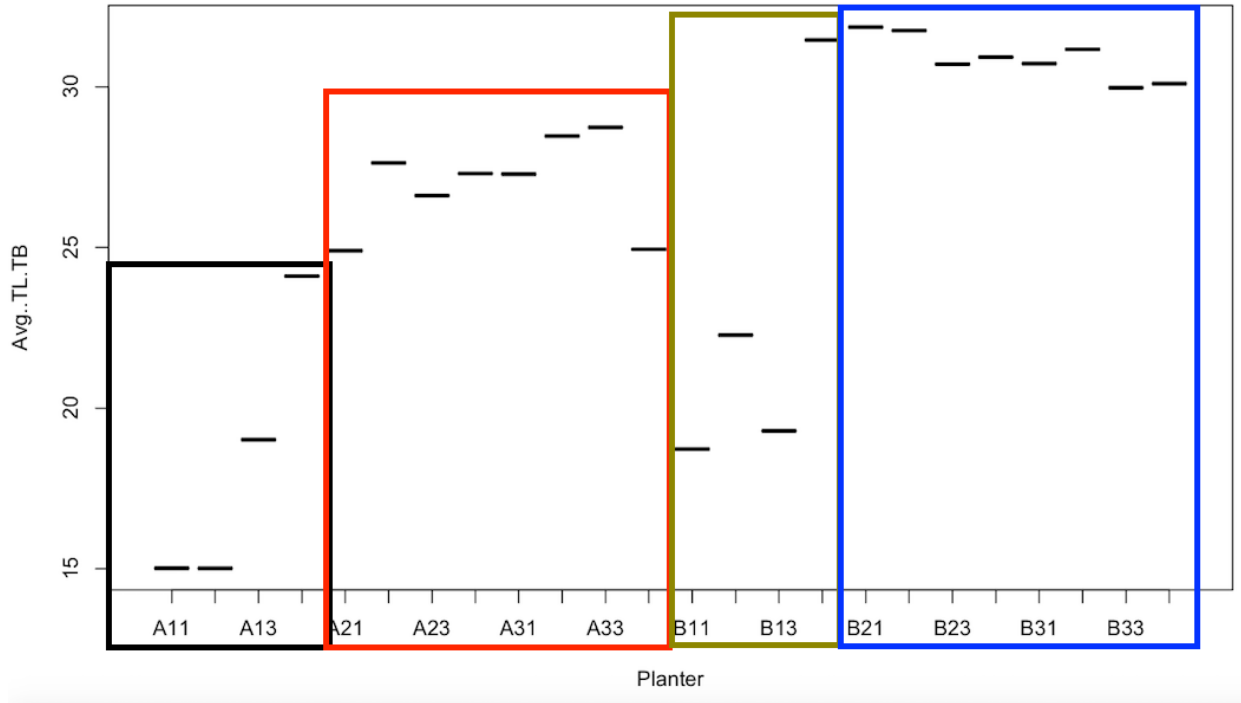
**APPENDIX A**

**Appendix A: Sterilized seeds that germinated.** In number of seeds (over two weeks) of first seeding.

Heated/ Unheated	Microbe Treatment	Seeds Germ- inated	Notes
Heated	Minimal	3	Some localized fungal growth on surface
Heated	AMF	3	Mycelial networking apparent on surface
Heated	Native	1	Some algal growth on the surface
Unheated	Minimal	3	Icy looking fungal growth covered the surface. 4 large weeds sprouted
Unheated	AMF	4	Box next to minimal also icy fungal showing. 2 large weeds sprouted
Unheated	Native	2	Some algal and fungal growth on the surface.

### APPENDIX B

**Appendix B: Average of length to mass ratio by planter.** Black box is sterilized heat treatment, yellow is sterilized unheated, red is both microbe heat treatments and blue is both microbe unheated treatments. Sterilization reduced ratio, their height to width ratio was low and therefore more proportional (ANOVA, p-value=0.001906). This is an interesting finding but not contributing to this paper's story.



## APPENDIX C

**Appendix C: Final Plant Measurements.** Entire data set, without averages or added labels.

Plant number	Treatment	Aboveground Length	Underground Length	Total Length	Aboveground Mass	Underground Mass	Total Biomass	TL/TB
A111	Heat+Sterilized	41	28.7	69.7	3.6	4.79	8.39	8.307508939
A112	Heat+Sterilized	45.1	28.9	74	4.14	2.24	6.38	11.59874608
A113	Heat+Sterilized	39.3	22.5	61.8	2.66	0.5	3.16	19.55696203
A114	Heat+Sterilized	42.3	30.1	72.4	3.72	1.42	5.14	14.08560311
A115	Heat+Sterilized	45.9	27.2	73.1	2.26	1.54	3.8	19.23684211
A116	Heat+Sterilized	49.2	27.4	76.6	2.34	0.72	3.06	25.03267974
A117	Heat+Sterilized	47.1	30.7	77.8	4.38	1.14	5.52	14.0942029
A118	Heat+Sterilized	40.9	25.6	66.5	3.12	1.44	4.56	14.58333333
A119	Heat+Sterilized	46	31.8	77.8	5.32	3.66	8.98	8.663697105
A121	Heat+Sterilized	38.1	27.1	65.2	3.38	4.5	7.88	8.274111675
A122	Heat+Sterilized	41.5	26.6	68.1	1.04	0.39	1.43	47.62237762
A123	Heat+Sterilized	25.5	9.8	35.3	0.36	0.18	0.54	65.37037037
A124	Heat+Sterilized	41.2	32.2	73.4	2.18	1.28	3.46	21.21387283
A125	Heat+Sterilized	48.7	27.1	75.8	1.42	0.31	1.73	43.8150289
A126	Heat+Sterilized	38.5	20.9	59.4	2.26	1.48	3.74	15.88235294
A127	Heat+Sterilized	40.9	31.1	72	2.31	1.24	3.55	20.28169014
A128	Heat+Sterilized	45.6	41.8	87.4	3.52	2.54	6.06	14.42244224
A129	Heat+Sterilized	43.3	26.6	69.9	2.34	1.28	3.62	19.30939227
A1210	Heat+Sterilized	46.2	31.2	77.4	4.64	2.59	7.23	10.70539419
A131	Heat+Sterilized	53.9	28.9	82.8	2.56	3.58	6.14	13.48534202
A132	Heat+Sterilized	45.6	44.3	89.9	4.69	4.86	9.55	9.413612565
A133	Heat+Sterilized	33.4	27.7	61.1	0.57	0.58	1.15	53.13043478
A134	Heat+Sterilized	47.8	30.2	78	2.81	1.78	4.59	16.99346405
A135	Heat+Sterilized	27.9	7.2	35.1	0.26	0.02	0.28	125.3571429



A136	Heat+Sterilized	55.3	26.9	82.2	2.46	0.98	3.44	23.89534884
A137	Heat+Sterilized	48.1	29.4	77.5	3.7	2.06	5.76	13.45486111
A138	Heat+Sterilized	52.5	28	80.5	5.02	3.12	8.14	9.889434889
A141	Heat+Sterilized	44.7	40.6	85.3	3.18	3.54	6.72	12.69345238
A142	Heat+Sterilized	43	33.5	76.5	3.3	3.26	6.56	11.66158537
A143	Heat+Sterilized	44.9	20.8	65.7	2.86	2.04	4.9	13.40816327
A144	Heat+Sterilized	37.8	15.6	53.4	0.84	0.42	1.26	42.38095238
A145	Heat+Sterilized	42.8	24.1	66.9	2.98	0.71	3.69	18.1300813
A146	Heat+Sterilized	43.4	30.9	74.3	3.16	1.24	4.4	16.88636364
A147	Heat+Sterilized	43.1	27.8	70.9	2.12	0.98	3.1	22.87096774
A148	Heat+Sterilized	44	35.4	79.4	2.88	1.66	4.54	17.48898678
A149	Heat+Sterilized	45.5	33.1	78.6	3.98	3.88	7.86	10
A1410	Heat+Sterilized	42.9	32.2	75.1	4.12	3.11	7.23	10.38727524
A211	Heat+AMF	44.1	25.9	70	0.96	3.35	4.31	16.2412993
A212	Heat+AMF	49.9	28.5	78.4	1.24	2.29	3.53	22.20963173
A213	Heat+AMF	43.4	24.5	67.9	0.78	0.12	0.9	75.44444444
A214	Heat+AMF	40.6	20.4	61	0.81	0.28	1.09	55.96330275
A215	Heat+AMF	48.3	22.7	71	0.93	0.38	1.31	54.19847328
A216	Heat+AMF	51.3	22.2	73.5	3.11	2.86	5.97	12.31155779
A217	Heat+AMF	42.2	26.8	69	3.14	3.5	6.64	10.39156627
A218	Heat+AMF	40.4	30.1	70.5	3.12	5.68	8.8	8.011363636
A221	Heat+AMF	43.5	27.6	71.1	2.6	3.52	6.12	11.61764706
A223	Heat+AMF	45.2	52.3	97.5	3.18	4.42	7.6	12.82894737
A225	Heat+AMF	49	31.7	80.7	5.42	2.91	8.33	9.68787515
A226	Heat+AMF	63.2	25.9	89.1	2.06	0.96	3.02	29.50331126
A227	Heat+AMF	45.9	14.6	60.5	1.26	0.54	1.8	33.61111111
A228	Heat+AMF	46.7	41.3	88	6.38	9.46	15.84	5.555555556
A229	Heat+AMF	46.1	26.2	72.3	2.36	2.92	5.28	13.69318182
A231	Heat+AMF	51.1	28.9	80	3.68	6.98	10.66	7.504690432
A232	Heat+AMF	46	38.2	84.2	2.9	3.79	6.69	12.58594918
A233	Heat+AMF	59.1	24.4	83.5	2.46	1.08	3.54	23.58757062

A234	Heat+AMF	52	29.2	81.2	1.42	1.06	2.48	32.74193548
A235	Heat+AMF	57.2	26.9	84.1	2.1	0.78	2.88	29.20138889
A236	Heat+AMF	31.2	14.1	45.3	0.48	0.17	0.65	69.69230769
A237	Heat+AMF	46.5	32.9	79.4	3.24	4.74	7.98	9.949874687
A238	Heat+AMF	48.1	38.5	86.6	5.12	6.08	11.2	7.732142857
A241	Heat+AMF	42.4	35.1	77.5	0.84	1.59	2.43	31.89300412
A242	Heat+AMF	50.1	46.5	96.6	3.61	4.66	8.27	11.68077388
A243	Heat+AMF	41.7	33.5	75.2	1.62	2.88	4.5	16.71111111
A244	Heat+AMF	48.9	28.3	77.2	2.97	1.38	4.35	17.74712644
A245	Heat+AMF	37.4	45.5	82.9	5.28	4.62	9.9	8.373737374
A246	Heat+AMF	47.2	28.6	75.8	3.32	2.38	5.7	13.29824561
A247	Heat+AMF	40	31.9	71.9	1	1.08	2.08	34.56730769
A248	Heat+AMF	49.6	36.1	85.7	6.54	12.7	19.24	4.454261954
A311	Heat+Unsterilized	33.2	32.7	65.9	0.5	1.38	1.88	35.05319149
A312	Heat+Unsterilized	35.3	14.1	49.4	0.82	0.21	1.03	47.96116505
A313	Heat+Unsterilized	47.1	46.8	93.9	2.78	5.36	8.14	11.53562654
A314	Heat+Unsterilized	53.3	35	88.3	1.38	1.1	2.48	35.60483871
A315	Heat+Unsterilized	42.9	40.2	83.1	1.24	1.14	2.38	34.91596639
A316	Heat+Unsterilized	51.5	32.7	84.2	5.88	5.88	11.76	7.159863946
A317	Heat+Unsterilized	49.6	35.6	85.2	5.46	5.32	10.78	7.903525046
A321	Heat+Unsterilized	41.4	45.1	86.5	1.39	6.34	7.73	11.19016818
A322	Heat+Unsterilized	39.1	12.2	51.3	0.88	0.46	1.34	38.28358209
A323	Heat+Unsterilized	45.8	18.6	64.4	3.01	1.5	4.51	14.27937916
A324	Heat+Unsterilized	48.1	17.9	66	2.86	1.21	4.07	16.21621622
A327	Heat+Unsterilized	52.1	15.4	67.5	1.62	0.59	2.21	30.54298643
A328	Heat+Unsterilized	52	45.1	97.1	7.82	9.36	17.18	5.651920838
A331	Heat+Unsterilized	36.5	33.2	69.7	1.3	0.58	1.88	37.07446809
A332	Heat+Unsterilized	47.4	29.8	77.2	2.56	0.78	3.34	23.11377246
A333	Heat+Unsterilized	42.5	30.7	73.2	2.88	1.24	4.12	17.76699029
A334	Heat+Unsterilized	46.9	30	76.9	4.32	2.46	6.78	11.34218289
A335	Heat+Unsterilized	48	35.1	83.1	6.08	1.42	7.5	11.08

A336	Heat+Unsterilized	46.6	29.3	75.9	4.98	4.64	9.62	7.88981289
A337	Heat+Unsterilized	27.3	25.1	52.4	0.24	0.36	0.6	87.33333333
A338	Heat+Unsterilized	44.2	40.9	85.1	4.52	3.4	7.92	10.74494949
A339	Heat+Unsterilized	36.3	30.5	66.8	1.27	0.86	2.13	31.36150235
A341	Heat+Unsterilized	19.6	14.2	33.8	0.22	0.24	0.46	73.47826087
A342	Heat+Unsterilized	24.7	29.9	54.6	0.36	0.34	0.7	78
A343	Heat+Unsterilized	37.9	24.1	62	1.52	0.92	2.44	25.40983607
A344	Heat+Unsterilized	39.8	25.4	65.2	2.98	2.6	5.58	11.68458781
A345	Heat+Unsterilized	29.6	26.2	55.8	0.51	0.58	1.09	51.19266055
A346	Heat+Unsterilized	42	32.6	74.6	4.38	5	9.38	7.953091684
A347	Heat+Unsterilized	31.8	29.4	61.2	0.72	0.84	1.56	39.23076923
A348	Heat+Unsterilized	43.2	28.3	71.5	2.64	1.82	4.46	16.03139013
B111	Unheated+Sterilized	57.4	26.5	83.9	3.02	1.32	4.34	19.33179724
B112	Unheated+Sterilized	53.2	25.7	78.9	1.84	0.88	2.72	29.00735294
B113	Unheated+Sterilized	58.6	20.4	79	2.6	0.46	3.06	25.81699346
B114	Unheated+Sterilized	65.1	19.9	85	4.46	2.16	6.62	12.83987915
B116	Unheated+Sterilized	53.9	18.3	72.2	3.38	0.86	4.24	17.02830189
B117	Unheated+Sterilized	50.1	11.1	61.2	1.58	0.31	1.89	32.38095238
B118	Unheated+Sterilized	52.7	19.6	72.3	2.14	0.81	2.95	24.50847458
B119	Unheated+Sterilized	60.8	17.3	78.1	3.46	1.54	5	15.62
B121	Unheated+Sterilized	52.5	32.8	85.3	3.24	0.81	4.05	21.0617284
B122	Unheated+Sterilized	44.1	42.3	86.4	1.7	0.74	2.44	35.40983607
B123	Unheated+Sterilized	54.2	29.7	83.9	3.56	0.76	4.32	19.4212963
B124	Unheated+Sterilized	54.4	30.7	85.1	2.42	0.52	2.94	28.94557823
B125	Unheated+Sterilized	55.5	29.1	84.6	3.52	0.68	4.2	20.14285714
B126	Unheated+Sterilized	51.6	30.1	81.7	2.84	0.62	3.46	23.61271676
B127	Unheated+Sterilized	53.6	34.4	88	1.78	0.24	2.02	43.56435644
B128	Unheated+Sterilized	53.6	32.7	86.3	1.88	0.46	2.34	36.88034188
B132	Unheated+Sterilized	56.4	23.2	79.6	3.86	2.1	5.96	13.3557047
B133	Unheated+Sterilized	52.5	25.1	77.6	3.42	1.74	5.16	15.03875969
B134	Unheated+Sterilized	53.8	39.7	93.5	2.58	1.48	4.06	23.02955665

B135	Unheated+Sterilized	63.1	27.5	90.6	3.34	1.32	4.66	19.44206009
B136	Unheated+Sterilized	46.9	32.6	79.5	1.28	0.48	1.76	45.17045455
B137	Unheated+Sterilized	53	47.5	100.5	3.32	2.62	5.94	16.91919192
B138	Unheated+Sterilized	52.1	40.9	93	2.86	1.46	4.32	21.52777778
B141	Unheated+Sterilized	56.2	25.7	81.9	3.94	1.48	5.42	15.11070111
B142	Unheated+Sterilized	49.8	26.1	75.9	3.3	1.2	4.5	16.86666667
B143	Unheated+Sterilized	56.6	22.3	78.9	4.32	0.92	5.24	15.05725191
B144	Unheated+Sterilized	53.8	21.2	75	3.39	0.87	4.26	17.6056338
B145	Unheated+Sterilized	42.5	14.7	57.2	0.48	0.07	0.55	104
B146	Unheated+Sterilized	44.9	22.8	67.7	1.36	0.16	1.52	44.53947368
B147	Unheated+Sterilized	48.1	26.3	74.4	3.46	0.73	4.19	17.75656325
B148	Unheated+Sterilized	61.9	31.5	93.4	4.6	1.72	6.32	14.77848101
B211	Unheated+AMF	51.3	30.5	81.8	2.42	0.72	3.14	26.05095541
B212	Unheated+AMF	26.5	5.2	31.7	0.12	0.06	0.18	176.1111111
B213	Unheated+AMF	48.5	27.2	75.7	1.52	0.24	1.76	43.01136364
B214	Unheated+AMF	54.1	30.7	84.8	3.98	1.16	5.14	16.49805447
B215	Unheated+AMF	56.1	39.7	95.8	4.16	1.46	5.62	17.04626335
B216	Unheated+AMF	56.2	24.4	80.6	4.01	0.46	4.47	18.03131991
B217	Unheated+AMF	53.9	30.3	84.2	3.64	0.64	4.28	19.6728972
B221	Unheated+AMF	56.7	47.6	104.3	2.68	0.48	3.16	33.00632911
B222	Unheated+AMF	55.8	32.3	88.1	2.44	0.59	3.03	29.07590759
B223	Unheated+AMF	55.6	14.5	70.1	3.24	0.44	3.68	19.04891304
B224	Unheated+AMF	48.5	15.4	63.9	0.74	0.04	0.78	81.92307692
B225	Unheated+AMF	44.1	15.7	59.8	0.84	0.12	0.96	62.29166667
B226	Unheated+AMF	56.4	29.8	86.2	3.2	0.39	3.59	24.01114206
B227	Unheated+AMF	47.9	56.4	104.3	2.42	0.74	3.16	33.00632911
B228	Unheated+AMF	26.8	9.4	36.2	0.16	0.04	0.2	181
B229	Unheated+AMF	53.4	32	85.4	2.62	0.95	3.57	23.92156863
B231	Unheated+AMF	54.1	33.2	87.3	2.36	2.92	5.28	16.53409091
B232	Unheated+AMF	52.4	25.7	78.1	2.31	1.02	3.33	23.45345345
B233	Unheated+AMF	48.2	22.6	70.8	1.12	0.24	1.36	52.05882353

B234	Unheated+AMF	20.9	4.4	25.3	0.08	0.06	0.14	180.7142857
B235	Unheated+AMF	55.6	37.2	92.8	2.86	1.96	4.82	19.25311203
B236	Unheated+AMF	55.4	28.9	84.3	3.84	2.02	5.86	14.38566553
B237	Unheated+AMF	31.3	9.1	40.4	0.22	0.4	0.62	65.16129032
B238	Unheated+AMF	54.5	42.6	97.1	4.08	4.16	8.24	11.78398058
B241	Unheated+AMF	57.2	23.9	81.1	4.76	2.77	7.53	10.77025232
B242	Unheated+AMF	49.1	14.8	63.9	0.76	0.19	0.95	67.26315789
B243	Unheated+AMF	48.4	23.2	71.6	1.54	1.14	2.68	26.71641791
B244	Unheated+AMF	17.6	8.5	26.1	0.04	0.05	0.09	290
B245	Unheated+AMF	53.2	20.8	74	4.72	2.66	7.38	10.02710027
B246	Unheated+AMF	48.3	17.3	65.6	1.81	0.92	2.73	24.02930403
B247	Unheated+AMF	58.9	33.1	92	3.88	2.34	6.22	14.79099678
B248	Unheated+AMF	54.6	47.5	102.1	3.46	2.82	6.28	16.25796178
B311	Unheated+Unsterilized	48.7	33.1	81.8	1.68	0.54	2.22	36.84684685
B312	Unheated+Unsterilized	27.8	7.8	35.6	0.18	0.06	0.24	148.3333333
B313	Unheated+Unsterilized	52.7	20.6	73.3	3.08	1.12	4.2	17.45238095
B314	Unheated+Unsterilized	34.4	4.2	38.6	0.24	0.06	0.3	128.6666667
B315	Unheated+Unsterilized	55.1	20.9	76	3.12	1.07	4.19	18.13842482
B316	Unheated+Unsterilized	38.4	9	47.4	0.72	0.13	0.85	55.76470588
B321	Unheated+Unsterilized	54.1	27.2	81.3	2.12	0.48	2.6	31.26923077
B323	Unheated+Unsterilized	26.5	7.3	33.8	0.14	0.05	0.19	177.8947368
B324	Unheated+Unsterilized	56.6	15.1	71.7	3.1	0.63	3.73	19.22252011
B325	Unheated+Unsterilized	50.4	14.1	64.5	1.45	0.37	1.82	35.43956044
B326	Unheated+Unsterilized	37.9	8.4	46.3	0.56	0.06	0.62	74.67741935
B327	Unheated+Unsterilized	23.1	4.2	27.3	0.12	0.1	0.22	124.0909091
B328	Unheated+Unsterilized	47.2	19.9	67.1	1.23	0.26	1.49	45.03355705
B329	Unheated+Unsterilized	52.6	24.3	76.9	2.43	0.54	2.97	25.89225589
B331	Unheated+Unsterilized	48.2	25.5	73.7	1.74	0.32	2.06	35.77669903
B332	Unheated+Unsterilized	48.4	22.8	71.2	2.16	0.28	2.44	29.18032787
B333	Unheated+Unsterilized	41.7	26.1	67.8	1.12	0.11	1.23	55.12195122
B334	Unheated+Unsterilized	50.7	47.9	98.6	1.92	0.46	2.38	41.42857143

B335	Unheated+Unsterilized	50.8	26.1	76.9	2.88	0.72	3.6	21.36111111
B336	Unheated+Unsterilized	50.3	28.2	78.5	3.32	0.84	4.16	18.87019231
B337	Unheated+Unsterilized	53	28.3	81.3	3.36	1.26	4.62	17.5974026
B338	Unheated+Unsterilized	19.2	6.4	25.6	0.12	0.02	0.14	182.8571429
B3310	Unheated+Unsterilized	31.1	11.3	42.4	0.28	0.03	0.31	136.7741935
B341	Unheated+Unsterilized	18.3	16.2	34.5	0.1	0.06	0.16	215.625
B342	Unheated+Unsterilized	17.4	27.3	44.7	1.86	0.57	2.43	18.39506173
B344	Unheated+Unsterilized	59.1	48.6	107.7	3.24	1.76	5	21.54
B345	Unheated+Unsterilized	51.4	23.7	75.1	2.08	0.98	3.06	24.54248366
B346	Unheated+Unsterilized	37.2	12.2	49.4	0.54	0.14	0.68	72.64705882
B347	Unheated+Unsterilized	17.9	3.6	21.5	0.08	0.06	0.14	153.5714286
B348	Unheated+Unsterilized	41	18.2	59.2	1.02	0.23	1.25	47.36
B349	Unheated+Unsterilized	59.1	23.8	82.9	2.28	0.28	2.56	32.3828125