Prototyping of an OTC-cable Field Warming Setup for Plant Heat Stress Response Experimentation

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Abstract

Climate change will dramatically impact the physiology of plants, in both ecological and agricultural contexts. Conducting experiments to elucidate plant responses to heat stress are essential to prepare for and understand the impact of coming climatic changes. Ideal experiments are those wherein plants are exposed to higher temperatures while maintaining as close to natural conditions as possible. Field warming setups are the best experimental analog to future climate change temperatures. In this project, heating cables were combined with Open Top Containers to create a field warming setup that can be used to explore plant response to heat stress.

Layman's Summary

In this project, a field warming setup was constructed consisting of an Open Top Container (basically a clear box with no top) with added heating from heating cables. The purpose of this setup was to investigate how plants, especially snowdrops and Arabidopsis, react to increased temperatures. The field warming setup is different from an indoor growth chamber because it is outside where the plants can be exposed to normal environmental processes.

Introduction

Global climate change is a disastrous reality that is bearing down on humans and the planet. Changes in global climate have had and will continue to have wide ranging effects on all aspects of ecosystems (Walther, 2010) and agriculture (Shahzad et al., 2021). Understanding plant responses to climate change is essential as they are the basis for all terrestrial ecosystems as well as the basis of all human food systems.

Climate change will have many effects on plants including changes in phenology (timing of plant responses), reproduction, biomass, susceptibility to disease, and much more (Leisner et al., 2023) (Chaudhry & Sidhu, 2022). Experiments must be carried out to explore the relationship between plant stress response and each potential stressor (i.e., heat, salinity, elevated CO_2). Elevated temperature is one of the primary direct effects of climate change (along with elevated CO_2), and thus experiments around temperature increase are particularly important. Understanding how temperature changes will affect plant physiology and genetics is an essential undertaking that could have direct effects on humanity's ability to salvage cropland, save ecosystems, and develop technologies based on plant heat stress responses (Rivero et al., 2022) (Stuble et al., 2021).

In order to explore plant stress responses to heat, experimental setups that simulate the conditions of a hotter future climate are needed. Much work has been done on this in indoor environments, i.e., in growth chambers in labs. The precise control offered by these experiments is very useful, but it fails to adequately place physiological changes in an ecological context (Kimball et al., 2008).

Given the immense complexities of soil microbiota, precipitation, wind, etc., and the interactions of the above, it is imperative to conduct experiments in more natural conditions. This is the purpose of field warming experiments. These experiments artificially raise the temperature of a given area of land (either in the soil, or the air, or both) to mimic natural temperature increases, without interfering significantly with other climatic parameters. Since the 1990s, many such setups have been created and evaluated. Generally referred to as field warming experiments, these systems have a wide range of designs, efficacies, and energy uses (Ettinger et al., 2019). Some have been combined with CO_2 manipulation and/or precipitation manipulation. Table 1 provides an overview of some of the different types of field warming experiments that have been created/conducted over the past 30 years with example papers.

Table 1. Overview of field warming experiment types. Typically, existing field warming setups can be divided into distinct types based on the type of heating device used and the direct target of warming. A few example papers are provided.

Heating Type	Target	Pros	Cons	Notable Papers
Ceramic Infra-red Heaters	Surfaces (plant and soil)	Excellent recovery time, good heat distribution, minimally effected by weather changes. Most commonly implement in extant literature.	Energy intensive. Does not heat soil well. Can vary in efficacy based on type of plants.	(Kimball et al., 2008) (Han et al., 2014)
Open Top Containers (OTC)	Air	Passive, no power requirement. Cheap to build and implement. Minimal environmental disturbance	No active control. Interferes with wind and potentially other natural variables. Highly weather dependent. High variability in effectiveness	(Bokhorst et al., 2008) (Sun et al., 2013)
Forced air heaters	Air	Good recovery time. Easier to monitor than Infra-red heaters.	Energy intensive and requires more expensive infrastructure up front. Must be coupled with some kind of OTC to be effective. Shown to interfere significantly with other environmental variables.	(Hanson et al., 2017)
Heating cables, buried	Soil	Very efficient.	Potential disruptive to plots. Can create significant temperature gradients	(Hanson et al., 2011) (Patil et al., 2013)

Heating cables, surface	Soil surface and air	Reasonable efficient. Minimally disruptive. Cheap.	Slow response time. Creates heat gradients.	(O'Neill et al., 2019)

These five extant field warming types served as the starting point for deciding how to proceed in building a new field warming setup to be placed in the Botanical gardens of Utrecht University. The setup would allow the Plant Stress Resilience lab group to translate basic lab findings into more realistic experimental conditions. Additionally, this setup would be especially good for experiments with snowdrops (*Galanthus nivalis*), a late winter/early spring blooming flower that needs particularly cold temperatures and therefore does not grow well in growth chambers.

The aim of the setup was to generate three to five degrees of warming above ambient as is in keeping with the current middle to worst case climate change forecasts (Lee et al., 2023). This project was a master's thesis with a limited budget of 1500 EUR and this project took place during a time in the Netherlands when energy prices were very high. This precluded the most energy intensive setups, ruling out IR heaters and most forced air setups. Additionally, the goal of this project was to change air temperature, thus eliminating buried heating cable options. This left ground surface cables and OTCs. The basic OTC design and concept is low cost and relatively easy to execute, but alone is ineffective at warming in most situations (except when placed in direct sunlight) (Johnson et al., 2013) (Sun et al., 2013). Final inspiration came from papers by Sun et al. (2013) and Frei et al. (2020) who combined Open Top Containers (OTCs) with suspended heating cables. This combination was shown to be highly effective in both papers. This option of combining heating cables with an OTC was deemed a good place to start for this project.

In the current field warming literature, areas outside of the US and Europe are significantly underrepresented (Stuble et al., 2021), leaving huge gaps in understanding of plant response to warming. Despite this, many of the most widely used systems (IR heaters and forced air heaters), are high in both material and energy cost, thus precluding their use outside of well-funded (usually western), institutions. The OTC-cable setup is relatively low cost in materials and energy expenditure, making it a useful option for areas and institutions that cannot afford to build large and intensive IR or forced air setups. Thus, our design also offers the additional benefit that it aligns with principles of open-source and more equitable science.

Moreover, there are very few systems of this type documented in the literature as most OTCs have been deployed without additional heating into existing ecosystems (Bokhorst et al., 2008) (Johnson et al., 2013). This project was therefore a good opportunity to test how OTCs operate with additional heating and expand the portfolio on field warming setups described in literature.

Results

Field warming setup design considerations

The goal of this project was to create a functioning field warming prototype setup. After a careful literature survey (see introduction) it was decided to use a combination of OTCs and heating cables. Additional requirements were: 1) the prototype should be built within a budget of 1500 EUR and 2) the protype be scalable and extendable, both in physical size and technological capability (i.e., more sensors, remote monitoring, etc.).

The inspiration for the OTC design came from Sun et al. (2013) and Bokhorst et al (2008) but needed modification and adaptation to fit with the requirements of the project. The differences in design are as follows. 1) The Sun et al. (2013) design used UV transparent PMMA. The current design used regular transparent PMMA because UV transparent PMMA is almost double the cost (and PMMA is already very expensive). Because the wall of the OTC is angled, up to 40% of the ground area of the OTC can be in the UV shadow of the OTC sides. Because the PMMA used in this design was not UV transparent, these areas will have different UV exposure than the areas closer to the middle of the OTC. In the experiments conducted by Bokhorst et al. (2008) and Sun et al. (2013), the OTCs were placed over an existing vegetation, meaning that every square cm of OTC area was experimentally relevant. For the current setup, the plants were meant to be planted after the OTCs were installed and thus could be planted closer to the middle of the OTC, i.e., the experimental design can fit the setup limitations. In addition, proper control plots were included (a warmed OTC, a non-warmed OTC and a field plot lacking OTC and warming altogether). Because of this, the low UV shadow area of the OTC PMMA sides was not considered a major issue as it could be corrected for. 2) The dimensions of the OTC panels were modified so that all necessary panels of PMMA could be cut from a single standard 2x3m sheet. 3) PMMA thickness was increased from 4mm to 5mm. This was for two reasons. First, the 1mm of extra thickness decreased IR transmissivity of the PMMA without dramatically decreasing the visible light transmissivity, thus increasing the thermal insulation of the OTC. Second, the increased thickness and rigidity allowed for a simplification of the OTC design, removing the top and bottom metal supports present in the Bokhorst et al. (2008) setup, without significantly compromising structural integrity. More detailed construction information can be found in the materials and methods. The OTC that was eventually built is shown in Figure 9. See also Appendix 3 for supplemental images of the setup.

Indoor Tests

After construction, indoor tests were performed using a single OTC to test the efficacy of the OTC-heating system (cables) combination. To this aim, the OTC was placed in the middle of a small storeroom

(N3.12) in the Hugo R. Kruyt building in the Science Park in Utrecht NL and a layer of +/- 1 to 2 cm of potting soil was placed in the OTC. Different cable (wiring) setups were designed and tested. The room had no windows and was well ventilated with a relatively stable temperature. Additionally, these experiments allowed for the investigation of the other parameters of the system including heat retention, heating speed (how fast the cables heat the OTC), and thermal distribution within the OTC. The results of these indoor experiments would allow for the testing of different cable layouts in a stable setting to see which one would be most ideal for the inherently less stable conditions of the outdoors. This was an iterative set of experiments. First, the OTC was tested without any cables to see if there were any temperature effects (this was unlikely as the literature suggested that the OTC is only effective at passive heating when exposed to thermal radiation). Second, a cable layout used in Sun et al. (2013) was tested. When this was found to be unsatisfactory another layout was tried and so on until a satisfactory layout was found. In total one unheated control run, and 14 heated experimental runs were conducted using 5 different cable layouts. Results of one experiment per layout is shown in this section as replicate runs were similar. An overview of the total experimental runs can be found in table 2.

Table 2. Overview of all indoor experiments. Start time is the time at which the temperature recording equipment and the heating cables were turned on. Cables Off is the time at which the heating cables were turned off. End time is the time at which the data were collected and the run was officially over. Data of the runs indicated with an asterisk (*) are provided as case examples in the reminder of this results section (figures 1-6).

Run #	Cable layout	Date	Start	Cables Off	End
0 *	No cables	21-4-2023	14:50	NA	24-4-2023 14:50
1	Layout 1	8-5-23	13:00	14:30	16:00
2	Layout 1	9-5-23	11:34	14:08	14:08
3 *	Layout 1	11-5-23	10:25	16:35	18:30
4	Layout 1	12-5-23	11:23	13:00	13:40
5	Layout 2	16-5-23	13:30	15:40	17:40
6	Layout 2	17-5-23	10:00	14:00	16:30
7	Layout 2	19-5-23	10:05	11:15	13:05
8	Layout 2	22-5-23	9:58	13:00	17:00
9 *	Layout 2	23-5-23	11:15	15:30	18:30
10 *	Layout 3a	1-6-23	13:10	16:05	18:15
11	Layout 3b	5-6-23	12:15	16:00	18:20
12 *	Layout 3b	6-6-23	12:10	15:30	17:00
13	Layout 4	8-6-23	11:00	14:05	16:00
14 *	Layout 4	9-6-23	10:55	14:00	16:30

For each experimental run temperature data were collected for the air and the ground. Air temperatures were collected with thermocouples and ground temperatures with a thermal camera. five thermocouples were used for all experiments. The thermocouples were type T and had an accuracy of ±0.5˚C. For the first nine experiments three thermocouples were placed inside the OTC (#1-3) and two (#4 & 5) were placed outside the OTC. After experiment 9 one (#5) of the outside thermocouples was moved inside the OTC because it was determined that the room temperature was extremely stable and collecting an additional data point within the OTC would be of more value to the project. The thermal camera captured only the ground temperature within the OTC.

For the heated experiments, the camera and thermocouples were activated 15-30 minutes before the cables were turned on to capture the ramp up period. Once the cables had run for 1-3 hours they were switched off and the system was monitored for at least an hour to capture heat dissipation.

No cables unheated Test

The first indoor experiment (Table 2, Run 0) that was conducted was a 3-day run of the OTC with no heating from the cables. The purpose of this experiment was to make sure there were no unexpected effects of the OTC alone as well as to assess the stability of the room temperature. Both air (inside the OTC) and ground temperatures during this experiment were very stable with a total temperature variation of 2˚C (Figure 1). Of note, there was some minor variation in temperature between night and day. The difference between the temperatures seen in Figure 1A of thermocouples 1,4 & 5 and 2 & 3 can be explained by the accuracy of the thermocouples themselves. The accuracy is ±0.5˚C, which is exactly the difference between the two temperature groups. The ground temperature data showed an identical temperature range for the room and similar stability.

Cable layout 1

Cable layout 1 consisted of a hexagonal PVC frame wrapped with heating cables along the bottom inside perimeter of the OTC (Fig 11A). This layout was a replicate of the one described in Sun et al. (2013). The purpose of this experiment was to duplicate the results of this paper and to determine next steps for improved cable layouts. It was hoped that this layout would give between three and five degrees of warming indoors, which would give some room for the outdoor environment to dampen that heating efficiency and still achieve at least two degrees of heating. This layout (Table 2, Run 3) showed some warming in the ground and air, but consistently less than the three degrees average that was considered the minimum desired level. There were also gradient effects in both the air and ground. In the air, the thermocouple that was furthest from the heating cables (thermocouple 1) consistently read a degree cooler than thermocouples 2 & 3, which were closer to the heating cables.

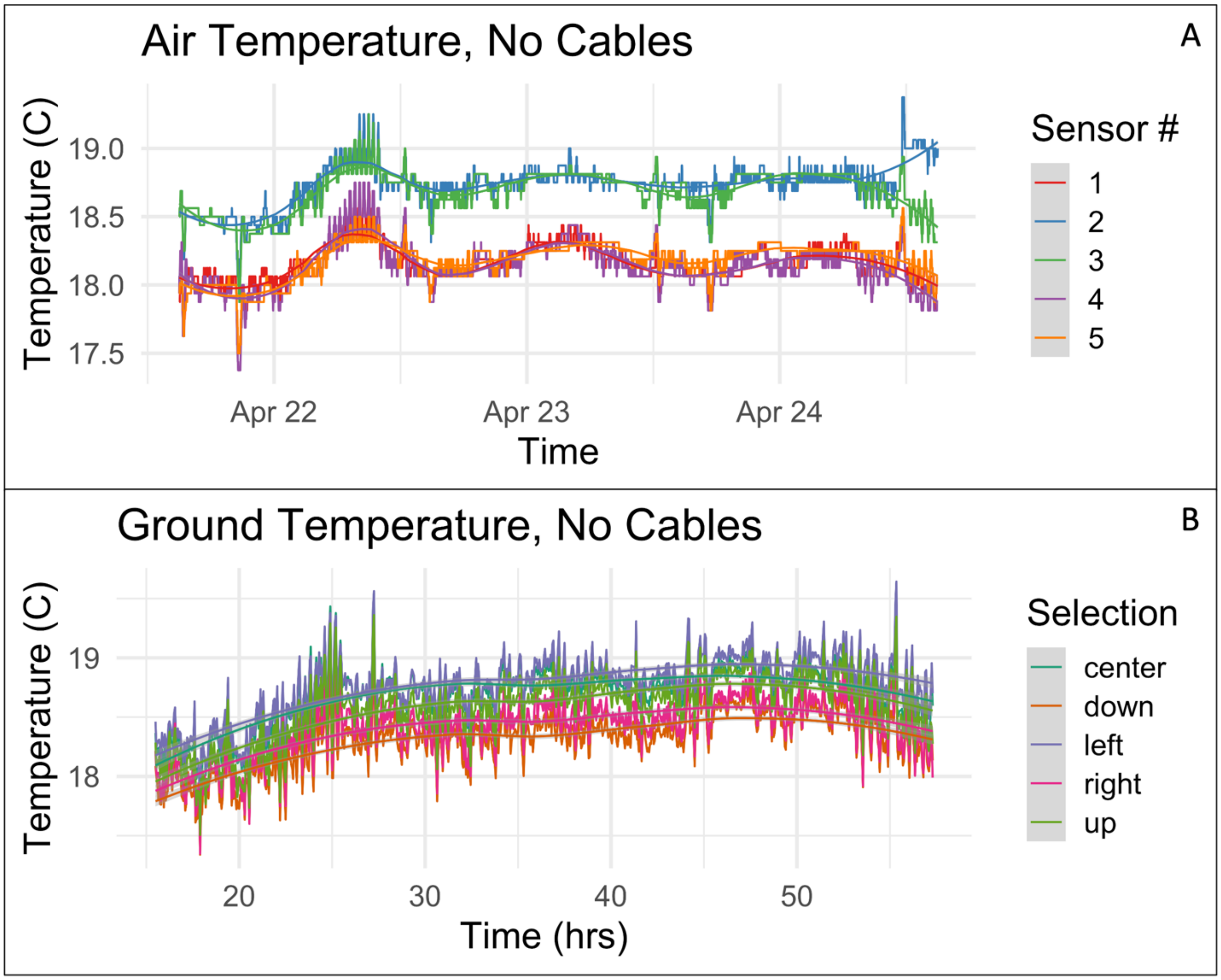

Figure 1. Air and ground temperatures during a three-day experiment (Table 2, Run 0) with no heating cables. Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. Gray shading indicates the 95% confidence level. A) Air temperature and B) ground temperature graphs. A) shows data of 5 thermocouples, numbered 1-5 and each provided with a color in the graphs. Thermocouples 1-3 were inside the OTC; 4 & 5 were outside the OTC. B) spot measurements were taken from the overall thermal picture capturing the whole OTC, in the center (blue-green) of the OTC, and four spots perpendicular to each other in between the center and the edge of the OTC, indicated as down (orange), left (purple), right (pink) and up (green).

This suggests an air temperature gradient of higher heat at the edges of the OTC and lower heat in the middle. On the ground, this same gradient was seen with the center section reading the coolest and the edge selections reading warmer temperature. There was however an unexpected trend in the ground temperature data in which the ground on the left and top of the OTC was warmer than the ground on the bottom and right of the OTC. One possible explanation of this is the presence of an incubator on the other side of the left wall of the experimental room. The exhaust from this device heated the cinderblock wall and a bit of floor around it, possibly accounting for a slight increase in temperature as seen here. This trend was consistent across all cable layout 1 tests but disappeared in later tests with more distributed cable layouts, suggesting that it was only an effect seen with low amounts of OTC warming.

Both the heating speed of the system and the low heat retention were immediately obvious in layout 1 and held true for all subsequent setups. The cables very quickly reach near peak heating once turned on (10-30 minutes) and the heat very quickly dissipates from the OTC once the cables are shut off (10-30 minutes). This means that the system is highly dynamic which would allow for fine control of temperature in future instances with feedback regulation (see discussion).

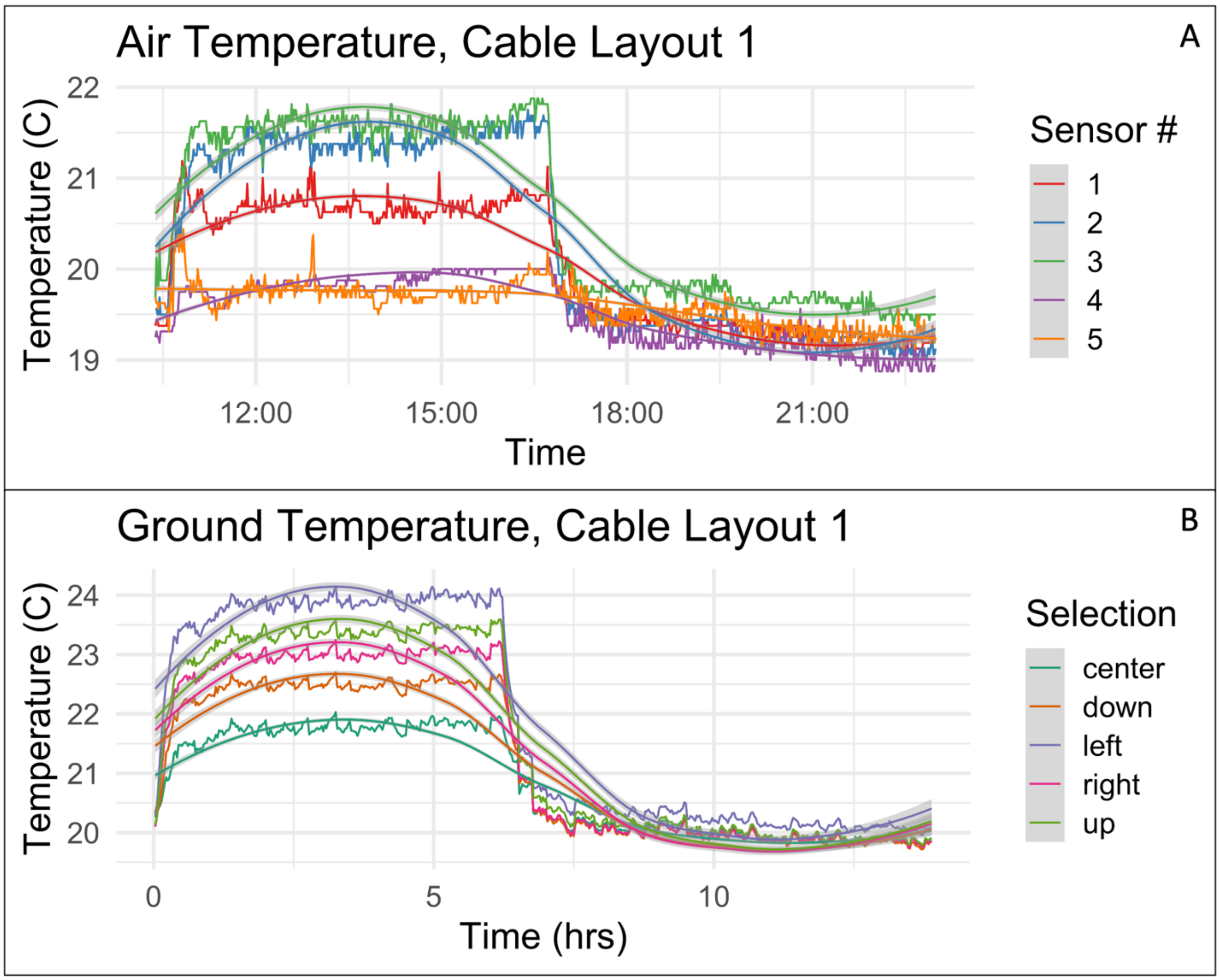

Figure 2. Graphs of the air and ground temperatures during an experiment with cable layout 1 (Table 2, Run 3). Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. A) Air temperature graph with 5 thermocouples B) Ground temperature graph. For details see legend of figure 1. The moments of switching on and off the cables can be seen by the following rapid increase and decline in temperatures, respectively. Note that thermocouples 4 and 5 were placed outside the OTC and represent the (baseline) test room temperature.

Cable Layout 2

Cable layout 2, was the first layout to break from the cable layouts presented in the literature. This layout was a close to random laying of the cables over a suspended PVC and wire frame to create a spiderweb like cable structure (Fig 11B). This layout was never intended to be a final layout as the randomness of the cables makes it nearly impossible to access the planting surface of the OTC. The idea was to see if the thermal gradient issues of layout 1 could be circumvented and to see if there was a greater overall heating effect from a more distributed cable layout. The distributed layout (Table 2, Run 9) was marginally more effective at heating the air (1.52 degree increase in mean) and marginally less effective at heating the ground (0.64 degree decrease in mean), compared to layout 1 (Figure 1). It did however prove extremely effective at reducing the thermal air gradient but did not affect the ground thermal gradient. The ground gradient may also have been enforced by the randomness of the spiderweb layout. The selection areas were in the same general areas for every experiment and in this experiment, the left and up selection areas had, by chance, greater densities of cables within them than the others.

Cable Layout 3a

Cable layout 3a was a more orderly distributed layout. In this layout the cables were laid directly on the ground in a spoke a wheel pattern (Fig 11C). This was the first attempt at a distributed layout that could be practically useful, because the spoke and wheel layout provided areas of exposed ground in which to plant. For air temperature, this layout (Table 2, Run 10) performed worse than layout 2 (Figure 3) both in terms of absolute temperature and in terms of thermal gradient (decrease of 1.52˚C from layout 2). A possible explanation for the dip in temperature was the distance from the thermocouples to the cables. The thermocouples were raised about 10 cm from the ground. In layout 2 the cables were much closer to the thermocouples than in this layout (cables lying on the ground). A likely explanation for the increase in thermal gradient is that unlike layout 2, there were larger gaps in between the cables. The thermocouples were distributed around the OTC at various distances from the cables and that is reflected in the differences in thermocouple temperate readings.

The ground temperature was dramatically affected by the cables, but not in a distributed manner. The selection at the center of the OTC, where the most cables crossed, heated up dramatically reaching temperatures of 29.56˚C. The left and right sections, which had cables running through them, also showed significant warming of up to 24.13˚C. The upper and lower selections, which lacked cables running directly through the selection area, showed only slight warming. This localized effect of the layout is not surprising, considering that the cables were lying directly on the soil. The soil in these experiments was also dry thus decreasing the ability for the soil to transfer heat. This combination meant that the

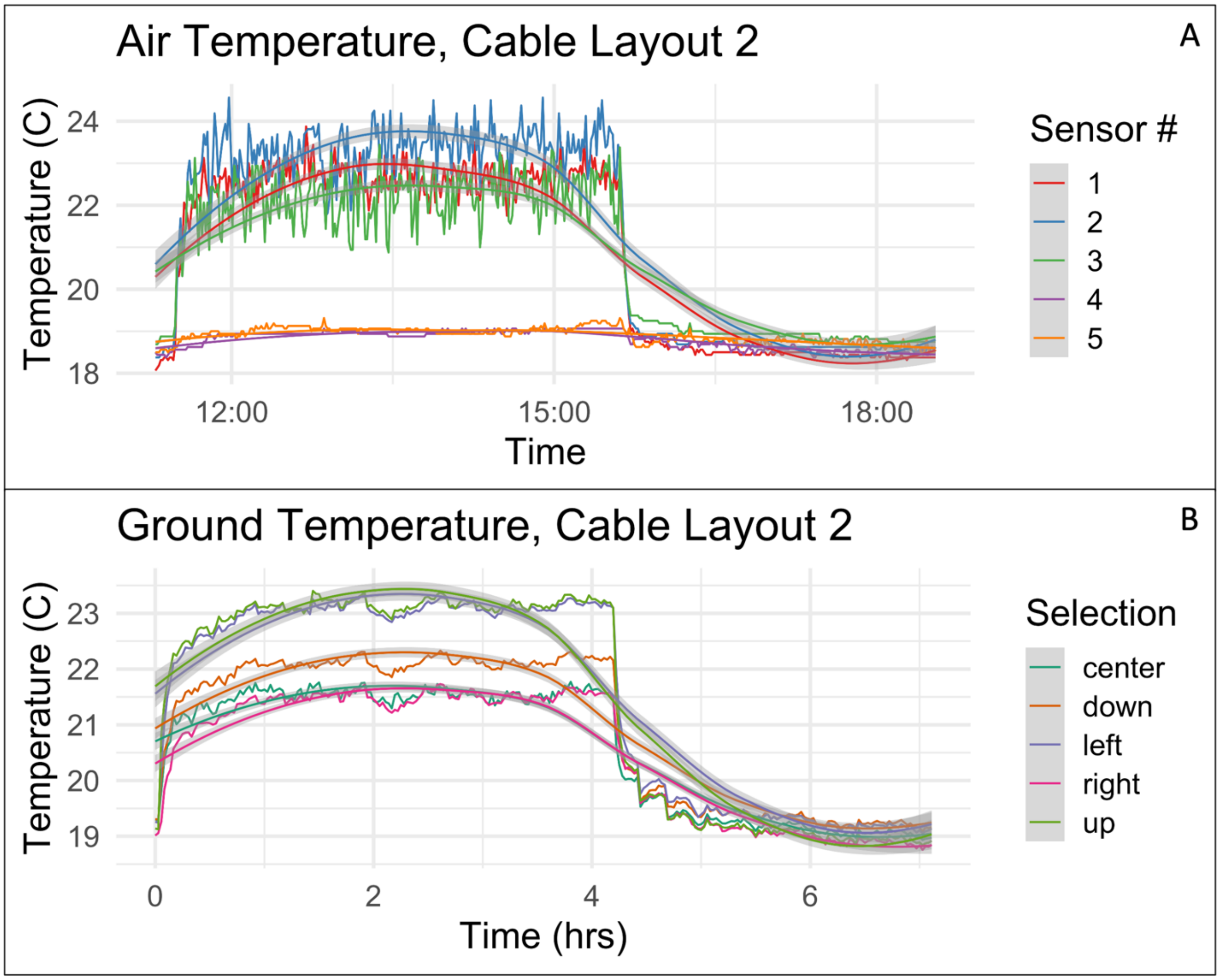

Figure 3. Graphs of the air and ground temperatures during an experiment with cable layout 2 (Table 2, Run 9). Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. A) Air temperature graph with 5 thermocouples B) Ground temperature graph. For details see legend of figure 1. The moments of switching on and off the cables can be seen by the following rapid increase and decline in temperatures, respectively. Note that thermocouples 4 and 5 were placed outside the OTC and represent the (baseline) test room temperature.

cables heated up the areas immediately around them and the air above, but temperature did not spread out evenly across the OTC volume.

Cable Layout 3b

Cable layout 3b was the exact same as cable layout 3a (Figure 4) except being suspended 10 cm above the ground (Fig 11D). This layout performed almost exactly the same as layout 3a (Table 2, Run 12) for both air temperature and ground temperature. However, the key difference for air temperature was that the gradient as observed in layout 3a (Figure 4) was not seen in layout 3b. This suggests that the raising of the cabling layout improved the air circulation within the OTC substantially. For ground temperature, the pattern of increased temperatures where the cables ran through the sections continued, but with slightly lowered temperatures as the cables were not in direct contact with the ground.

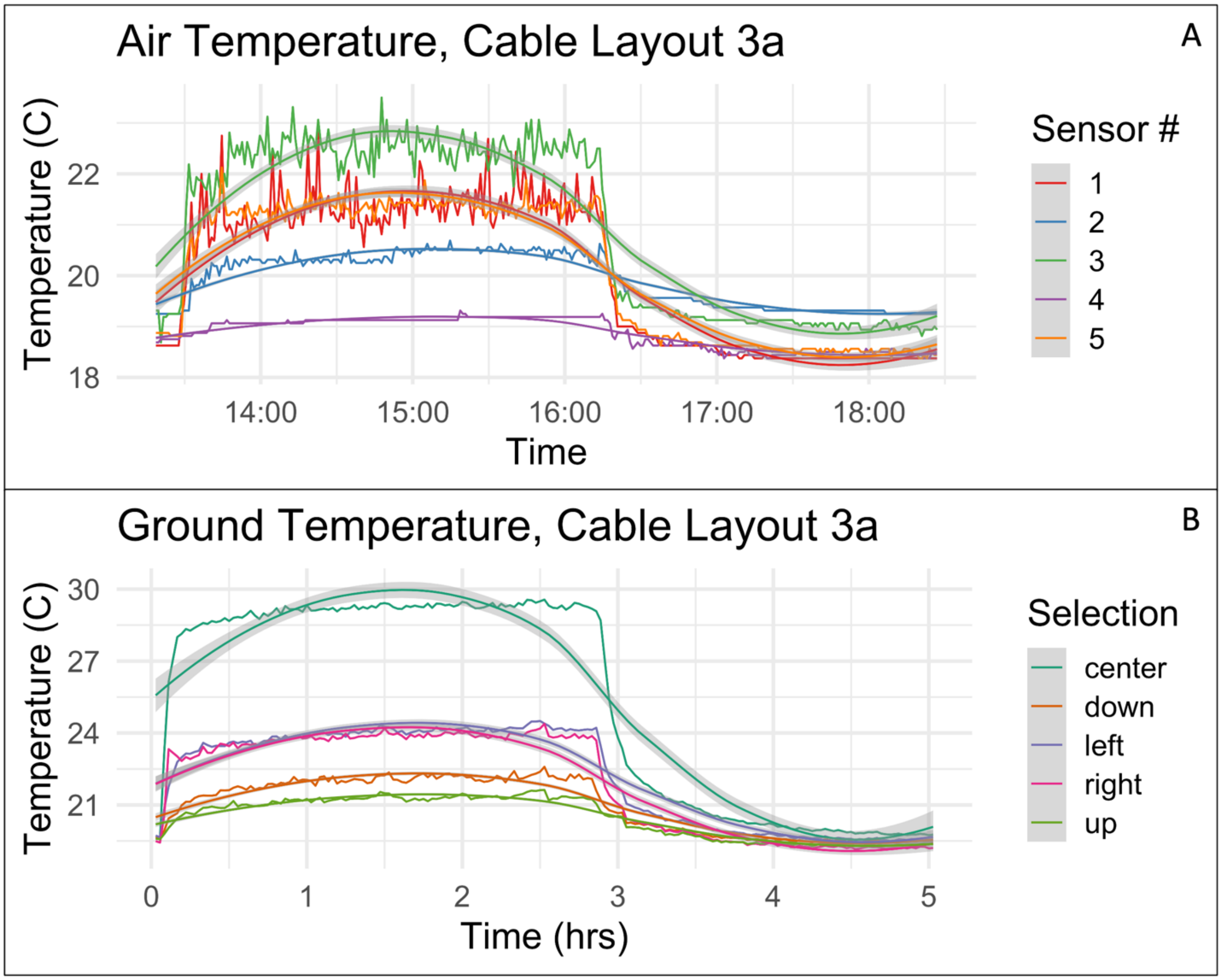

Figure 4. Graphs of the air and ground temperatures during an experiment with cable layout 3a (Table 2, Run 10). Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. A) Air temperature graph with 5 thermocouples B) Ground temperature graph. For details see legend of figure 1. The moments of switching on and off the cables can be seen by the following rapid increase and decline in temperatures, respectively. Note that thermocouple 4 was placed outside the OTC and represent the (baseline) test room temperature.

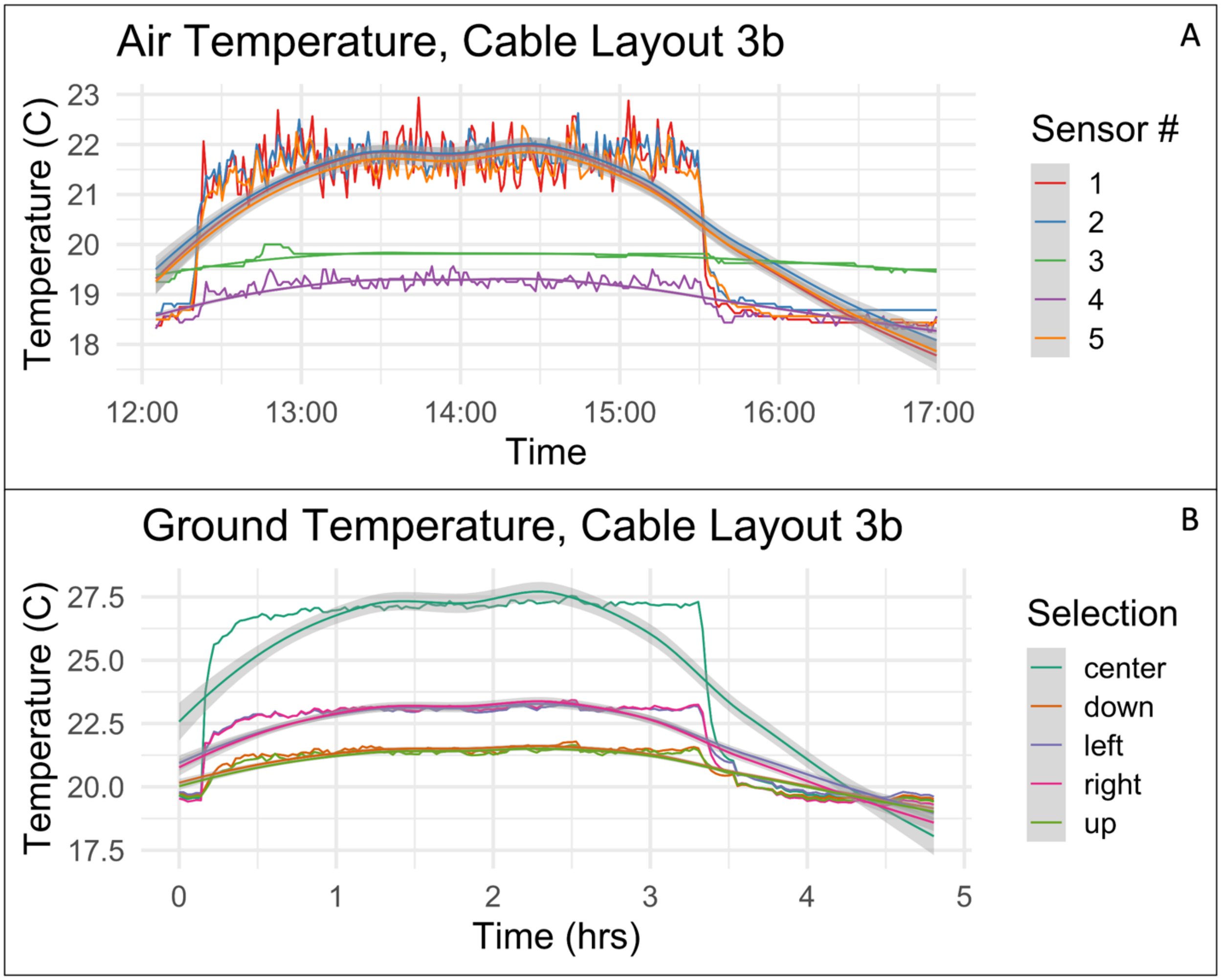

Figure 5. Graphs of the air and ground temperatures during an experiment with cable layout 3b. (Table 2, Run 12) Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. A) Air temperature graph with 5 thermocouples B) Ground temperature graph. For details see legend of figure 1. The moments of switching on and off the cables can be seen by the following rapid increase and decline in temperatures, respectively. Note that thermocouple 4 was placed outside the OTC and represent the (baseline) test room temperature. Note also that thermocouple 3 was malfunctioning during this run.

Cable Layout 4

Cable layout 4 was essentially a combination of the layouts 3a and 3b and was the final layout tested (Table 2, Run 14) (Fig 11D). It proved superior to the other layouts in increasing air temperature and ground temperature in the OTC. This layout was chosen because layouts 3a and 3b were effective at raising the air temperature of the OTC but did not provide enough of a temperature increase. This increase was achieved in cable layout 4 by doubling the number of cables as compared to layouts 1-3b. The air temperatures recorded by the thermocouples were also more uniform than those in 3a. The absence of a clear thermal air gradient and the increased temperatures led to the selection of this layout as the setup to take outside. The ground temperatures of this layout were similar in pattern to those in layouts 3a and 3b but higher.

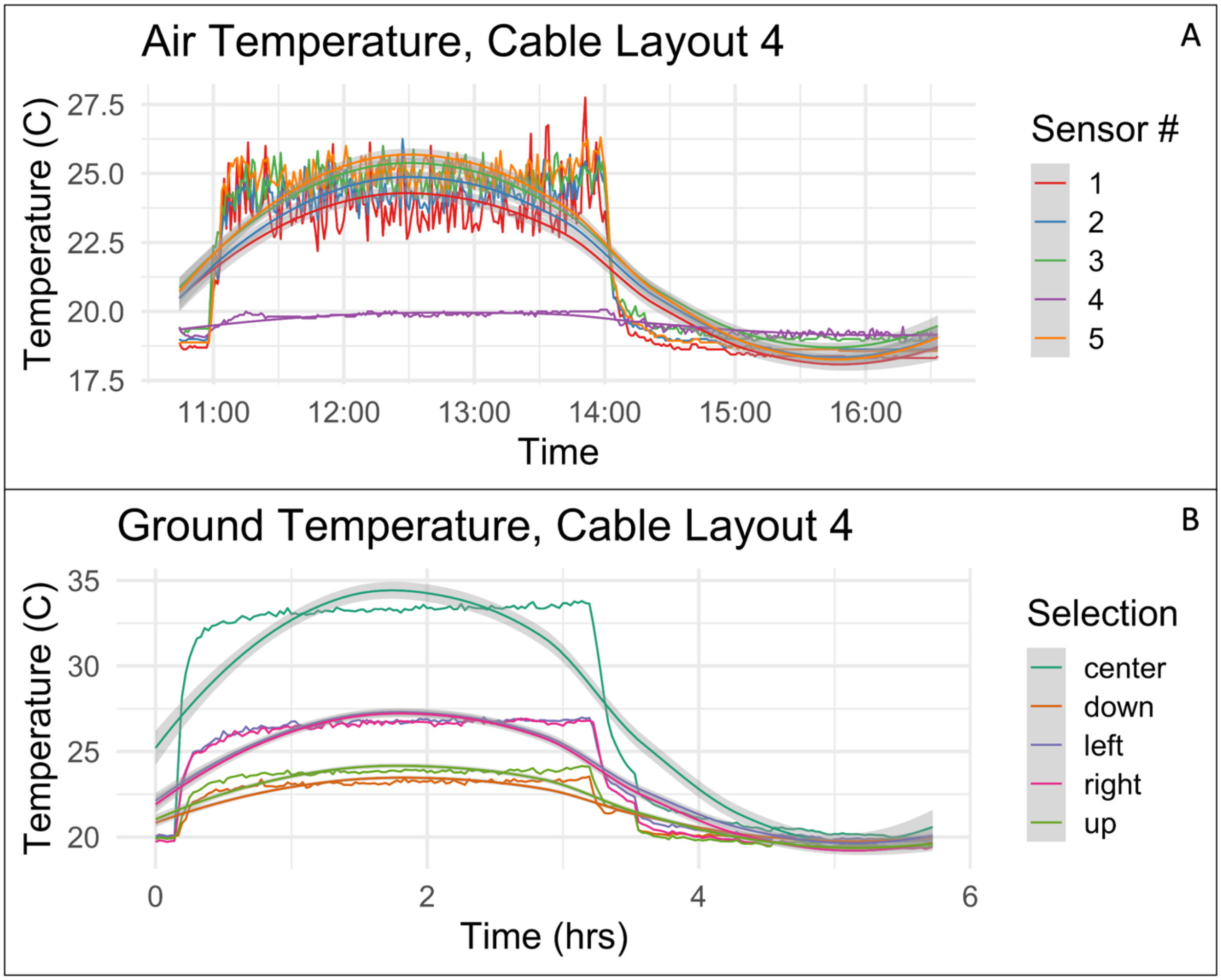

Figure 6. Graphs of the air and ground temperatures during an experiment with cable layout 4. (Table 2, Run 14) Graphs display stochastic temperature data and are fitted with lines of best fit using the Loess method. A) Air temperature graph with 5 thermocouples B) Ground temperature graph. For details see legend of figure 1. The moments of switching on and off the cables can be seen by the following rapid increase and decline in temperatures, respectively. Note that thermocouple 4 was placed outside the OTC and represent the (baseline) test room temperature.

Direct Temperature Comparison

To fully understand the data collected in these experiments, direct temperature comparisons were made. These were essentially just comparisons of mean temperatures from all experiments. Figure 7 shows the compiled temperature data of the thermocouples and Figure 8 of the ground temperature, for each of the experiments shown in the results above (Figures 1-6). The data used here are a subset of the full experimental runs using only the heated phase of the experimental run. Additionally, the data points from each of the thermocouples that were inside the OTC (thus omitting data of the one(s) outside) were combined into one set of mean temperature values for each experiment. This allows for a direct comparison of both the means and distribution of the air (figure 7) and ground (Figure 8) temperatures respectively for each experiment. The values represented graphically in the boxplots can also be seen in Table 3 and Table 4, which lists the summary data for these compiled data sets.

All of the 4-cable layouts (layout 1-3) had average air temperature increases of 2.83 to 4.35 degrees above ambient (i.e., the no cables layout), whereas the 8-cable layout (cable layout 4) was superior, having a mean temperature increase of 6.15˚C above ambient (Figure 7, Table 3). A similar trend was seen in the ground temperature data. The only slight deviation from this trend is layout 3a which, due to having cables directly on the ground, was warmer than the other 4-cable layouts. Overall, the compiled data thus makes clear that more than any specific cable layout, the primary driver of overall OTC temperature is raw energy input.

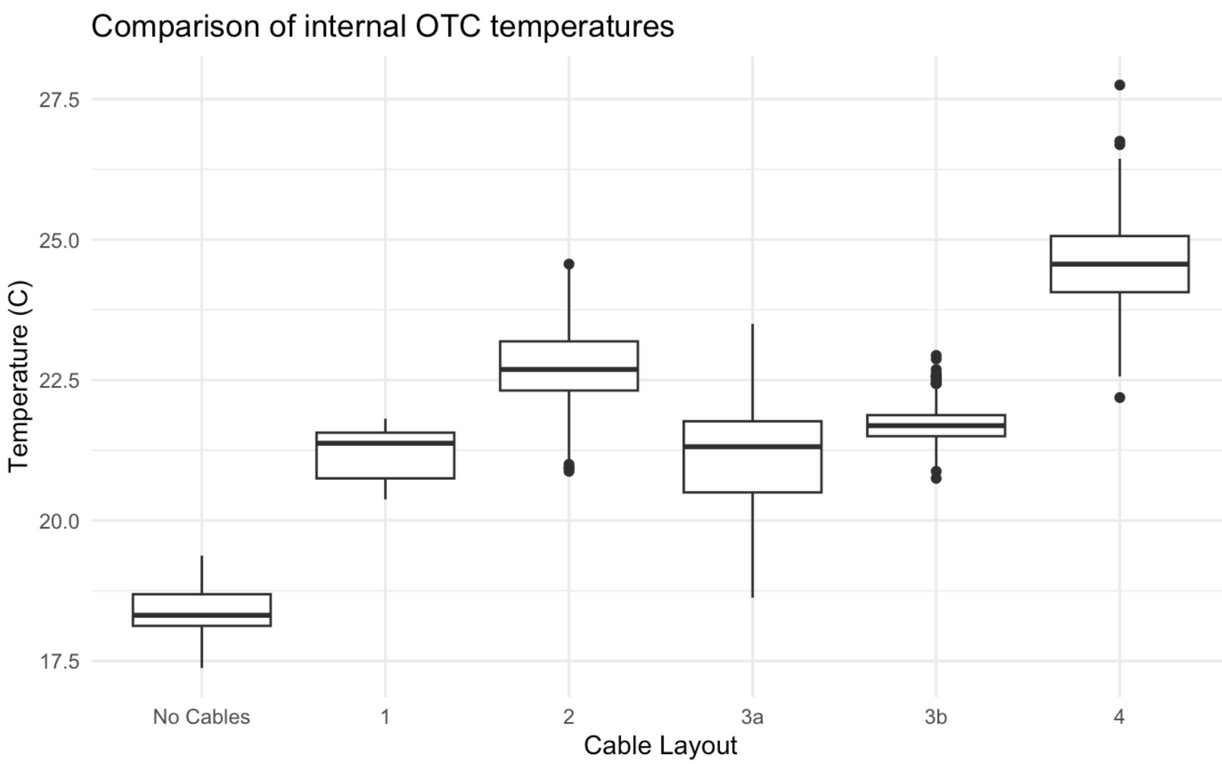

Figure 7. Overview of air temperature means for the 5 cable layouts and no cable experiments. Data are a subset of the total experimental data using only the data points from the heated portion of each experiment. Data points from all thermocouples placed inside the OTC in each experiment are combined to form one set of temperature data for each experiment. Boxes indicate boundaries of the second and third quartiles (Q) of the data distribution. Black horizontal bars indicate median and whiskers Q1 and Q4 values within 1.5 times the interquartile range.

Table 3. Air temperature summary data from the 5 cable layouts and no cable experiments. Data are in degrees Celsius. As in figure 7, data are a subset of the total experimental data using only the data points from the heated portion of each experiment. Data points from all thermocouples placed inside the OTC in each experiment are combined to form one set of temperature data for each experiment.

Cable Layout	Min	1st Q	Median	Mean	3rd Q	Max
No Cables	17.38	18.12	18.31	18.38	18.69	19.38
Layout 1	20.38	20.75	21.38	21.21	21.56	21.81
Layout 2	20.88	22.31	22.69	22.73	23.19	24.56
Layout 3a	18.62	20.50	21.31	21.19	21.77	23.50
Layout 3b	20.75	21.50	21.69	21.71	21.88	22.94
Layout 4	22.19	24.06	24.56	24.53	25.06	27.75

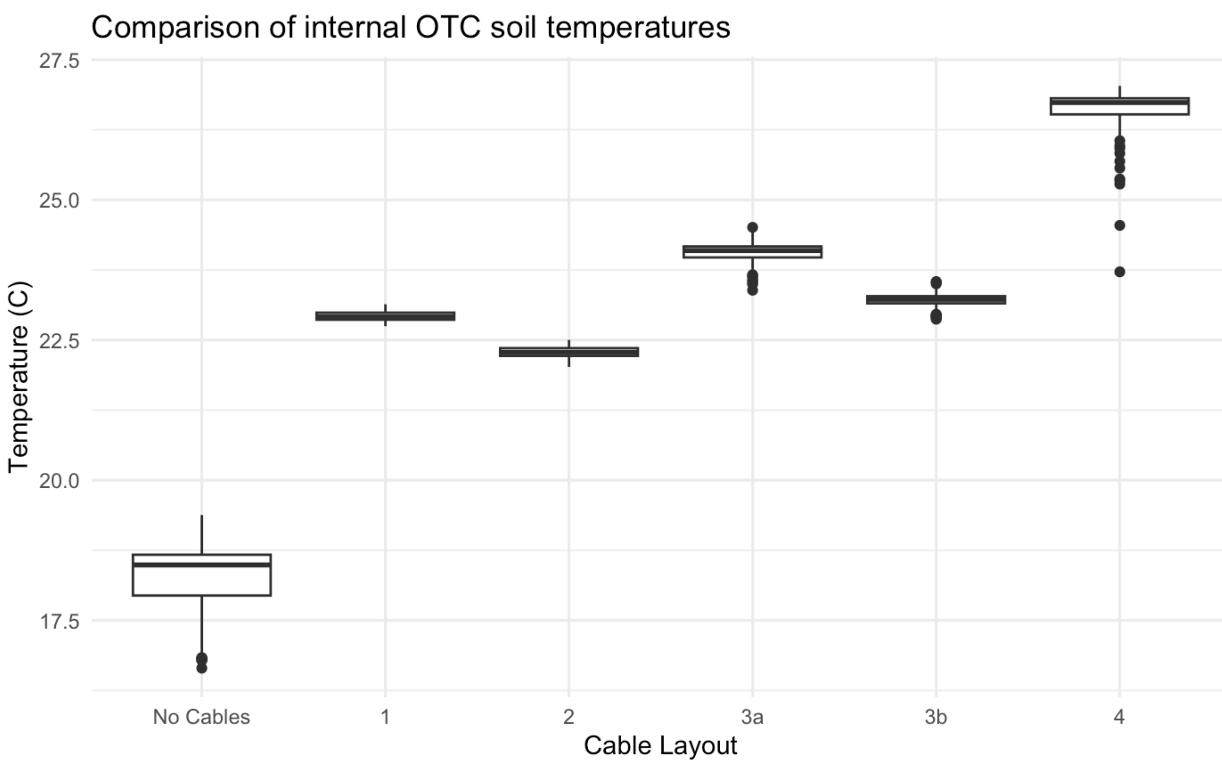

Figure 8. Boxplot overview of ground temperature means for the 5 cable layouts and no cable experiments. Data are a subset of the total experimental data using only the data points from the heated portion of each experiment. Data points from all selections in each experiment are combined to form one set of temperature data for each experiment. Boxes indicate boundaries of the second and third quartiles (Q) of the data distribution. Black horizontal bars indicate median and whiskers Q1 and Q4 values within 1.5 times the interquartile range.

Table 4. Ground temperature summary data from the 5 cable layouts and no cable experiments. Data are in Celsius. As in figure 7, data are a subset of the total experimental data using only the data points from the heated portion of each experiment. Data points from all

selections in each experiment are combined to form one set of temperature data for each experiment.

Cable Layout	Min	1st Q	Median	Mean	3rd Q	Max
No Cables	16.65	17.94	18.49	18.28	18.67	19.38
Layout 1	22.75	22.86	22.92	22.92	22.99	23.14
Layout 2	22.02	22.22	22.28	22.28	22.36	22.50
Layout 3a	23.39	23.97	24.10	24.04	24.17	24.51
Layout 3b	22.87	23.15	23.23	23.21	23.28	23.54
Layout 4	23.72	26.53	26.74	26.57	26.81	27.03

Discussion

Benefits of the new OTC-cable setup

Several things about the setup worked quite well. The OTC construction with thicker PMMA and no additional reinforcements cut down on material and manufacture costs. The final distributed cable layout (Layout 4) removed any thermal air gradients that were present in earlier layouts and proved quite effective at increasing temperature in the OTC. This more distributed layout has advantages over the setup in Sun et al (2013) for more agricultural field warming experiments because of the reduced gradient. This project also had two important findings for future field warming setups. First, that OTCs do not retain heat well when not exposed to external (solar), or internal (heating cables) warming. Second, energy input is the most important factor in determining overall temperature increase in an OTC-cable setup. These two findings taken together with Johnson et al. (2013) who found that OTCs did not provide significant long-term heating, indicate that the primary purpose of the OTC is to provide shelter for the heating cable setup rather than being a significant driver of temperature increase themselves.

Limitations of the current OTC setup

Many of the limitations of the setup are the same as limitations imposed by other field warming setups, namely that every experimental structure, per definition, impacts the plants in the setup and interferes with the outcome of the experiment. The idea of the current setup is that this can be in part corrected for with proper controls, namely; one warmed-OTC, one non-warmed OTC, and a control plot lacking an OTC and warming. Within the scope of this project, the setup was placed outside in the research area of the Botanical gardens of Utrecht University, but not yet tested (switched on) nor were any microclimatic data collected. But the casing of the electronics proved waterproof so far. Therefore, the impact of the experimental structure and the heating cables can only be deduced from extant literature. In other field warming studies, heating caused a decrease in plot moisture and OTCs interfered heavily with wind patterns (Ettinger et al., 2019). Additionally, OTC structures impact rain along the perimeter of the plot, thus further interfering with some natural variables. A particular potential limitation of our current setup is UV shading as non-UV-transparent

Plexiglas was used. Further testing is needed to elucidate the influence of such limitations.

Future experimentation

Because of time limitations on the current project, there were no outdoor experiments conducted with the OTC-cable system. While the setup proved effective indoors, without the same temperature experiments conducted outside, there is no way to know how effective the system remains when exposed to environmental pressures. To elucidate this, heated experimental runs should be carried out with the OTC-cable setup in its outdoor field location. The experiments should mirror the indoor tests with the cables turned on and then off with data collected the entire time. Unlike the indoor tests where the experimental intervals were relatively short, these outdoor tests should have increasingly long experimental intervals, starting with heated intervals (cables on) of a few hours and increasing the heated interval to several days. This would allow for the exploration of how the setup responds to changes in day and night temperature. From these experiments, the following information could be determined: 1) total thermal capacity of the system, 2) thermal response time of the system, 3) thermal gradients present within the OTC, and 4) effect of OTC alone (direct comparison on heated OTC and unheated OTC). During these experiments additional data on soil moisture and air humidity should be collected as these two variables are known to be influenced by heating (Ettinger et al., 2019). The ultimate goal of experimentation with this setup is to test plant responses to heat stress. Not only are plant phenotype responses the target data, but additionally, effective readouts of plant phenotypes and marker genes would be further proof that the system works and can be used to obtain biologically relevant information.

Further improvements

The setup as it exists right now is still a prototype. It is an advanced prototype that is capable of functioning and collecting data over longer periods of time, but it lacks several features that would bring it from prototype to developed research tool. These improvements are in two categories: user interface improvements, and research functionality improvements.

The scope of this project was to create a functioning field warming setup but did not include fully developing the setup. Because of this, it lacks an easy way to extract logged data or monitor the system while it is running, either on location or remotely. Currently the data collected by the system is stored on local micro-SD cards that must be manually removed from the data loggers in order to be read out. This is a less than ideal setup because it is both difficult for the untrained user and can lead to unexpected errors in the system. While removing the card a user could damage the system or the card itself. Additionally, taking the card in and out could cause the data logger system to stop recoding data or otherwise malfunction through no direct user error. To solve this, a separate data offloading system needs to be added. This could be a wired data connection that could be connected directly to a computer, a Bluetooth connector, or the best option, a Wi-Fi hub that would allow users to remotely offload data. There is currently no way to monitor the system while it is running. In order to check the workings of the system, one must connect the microcontrollers to a computer and run them through the Arduino IDE. This has drawbacks. First, it requires a knowledge of Arduino IDE and the code underlying the system functioning. Second, it requires disconnecting the system to run diagnostics. This is disruptive to experiments and there is no guarantee that when the system is connected to the back to main power that the system will continue to function without issue. the solution to these issues would be to install a small lcd screen as part of the system that could provide system readouts on command. Additionally, these readouts could be tied into the Wi-Fi connection so that readouts could be collected from afar. This would also allow for the system to monitor itself and alert the user of any issues.

The primary functionally improvement for the system would be addition of feedback regulation to the heated plot. Currently the cables are either on or off. However, the way the cables are connected to the microcontroller, by MOSTFETs, would allow for the microcontroller to essentially dim the power of the cables. This could be tied together with the thermocouples in the plots to provide a temperature feedback system. The sophistication of this system could be of various levels from a basic feedback loop to a PID system wherein the system would predict the changes in temperature and the response time of the cables, and adjust accordingly. The current configuration of the microcontroller also only allows for the cables to be controlled as one unit. However, if the microcontroller was upgraded to one with more I/O pins, each cable could be controlled individually potentially increasing the complexity of the feedback regulation to one of tailored sectional control. Feedback regulation would dramatically increase the usefulness of this setup because it would allow for nuanced temperature regulation rather than brute force, always on, warming.

Another potential system improvement would be to add an additional source of warming to the OTC. This could be in the form of IR heaters, forced air, or something else. The heating cables are effective, but they are not perfect. In the coldest and warmest periods of the year, it may be necessary to increase the energy input dramatically to maintain a constant temperature increase above the baseline. Typically field warming setups fall short of this (Ettinger et al., 2019), but the combination of more than one heating technique could potentially overcome this trend. Additionally, the cables don’t necessarily provide a perfectly even heat distribution in the OTC. It is reasonably good but could be improved with the addition of forced air. This forced air could be heated, but it could also just be a fan blowing air in a spiral manner within the OTC. At this basic level, the air current within the OTC would serve to distribute the hottest air coming off the heating cables. It would also potentially serve to disrupt any vertical thermal gradients that may form within the OTC. This would have to be extensively tested including gathering higher resolution air temperature data of any developing thermal gradients. In an ideal forced air setup, the ventilation would be connected to a heating element that could turn on and off depending on the energy input needs of the system.

Materials and Methods

OTC Specs

Each OTC is composed of 6 PMMA panels with the following dimensions: 5mm thickness, 49 cm height, 108 cm bottom base, and an 80 cm top base. These panels are connected to form a hexagonal OTC. The angle of the panels relative to the ground is 60 degrees. The panels were connected by strips of stainless steel bolted to the panels. The specifications of the steel connectors are as follows: 5mm thickness, 50cm height, 8cm width. The steel strips have two 5 cm triangular teeth cut into their bases to secure the OTC to the ground and are bent vertically along the midline to an angle of 120 degrees.

Figure 9. Image of OTC outdoors in the test plot.

Heat cable specs

The heating cables used are 18W/m resistance wire cables intended for under floor use (7423418520541, Decochip, Netherlands). The cables used are cut to lengths of 4m with a total of 72W per cable. In the final field setup, there are a total of eight 4 m cables with a total length of 36m and a total wattage of 588W.

Electronics layout

The electronics for this system consist of two parts. The first part is the heating cable power supply and control. The second part is the thermocouple data logger. All components of the setup are controlled by means of an Adafruit Feather HUZZAH ESP8266 microcontroller (Adafruit Industries, New York, NY, USA). The heating cables are powered by 24V DC power supplies (Mean Well Inc). Each power supply powers 4 heating cables. The heating cables are controlled by the microcontroller using IRLZ34NPbF MOSFETs (Infineon Technologies AG, Neubiberg, Germany). These electronic switches can be used to switch the cables on and off and can function as dimmers allowing for future feedback regulation in the system. The thermocouple data logger consists of an SD card logger with a real time clock (Adalogger FeatherWing, Adafruit Industries, New York, NY, USA), thermocouple amplifier boards (Adafruit MCP9600 I2C Thermocouple Amplifier, Adafruit Industries, New York, NY, USA), and type T thermocouples (Labfacility, Dinnington, UK). Circuit diagrams can be found in Appendix 2. For the indoor experiments the electronics were connected using breadboards and snap connectors (WAGO Group, Minden, Germany). For the outdoor setup the components were soldered into circuit boards and wires fitted with more permanent screw terminals. Additionally, the MOSFETs are cooled by two 230v fans (Sinwan, Electric Industries Co, Taipei, Taiwan).

A full list of materials used with relevant information can be found in Appendix 1.

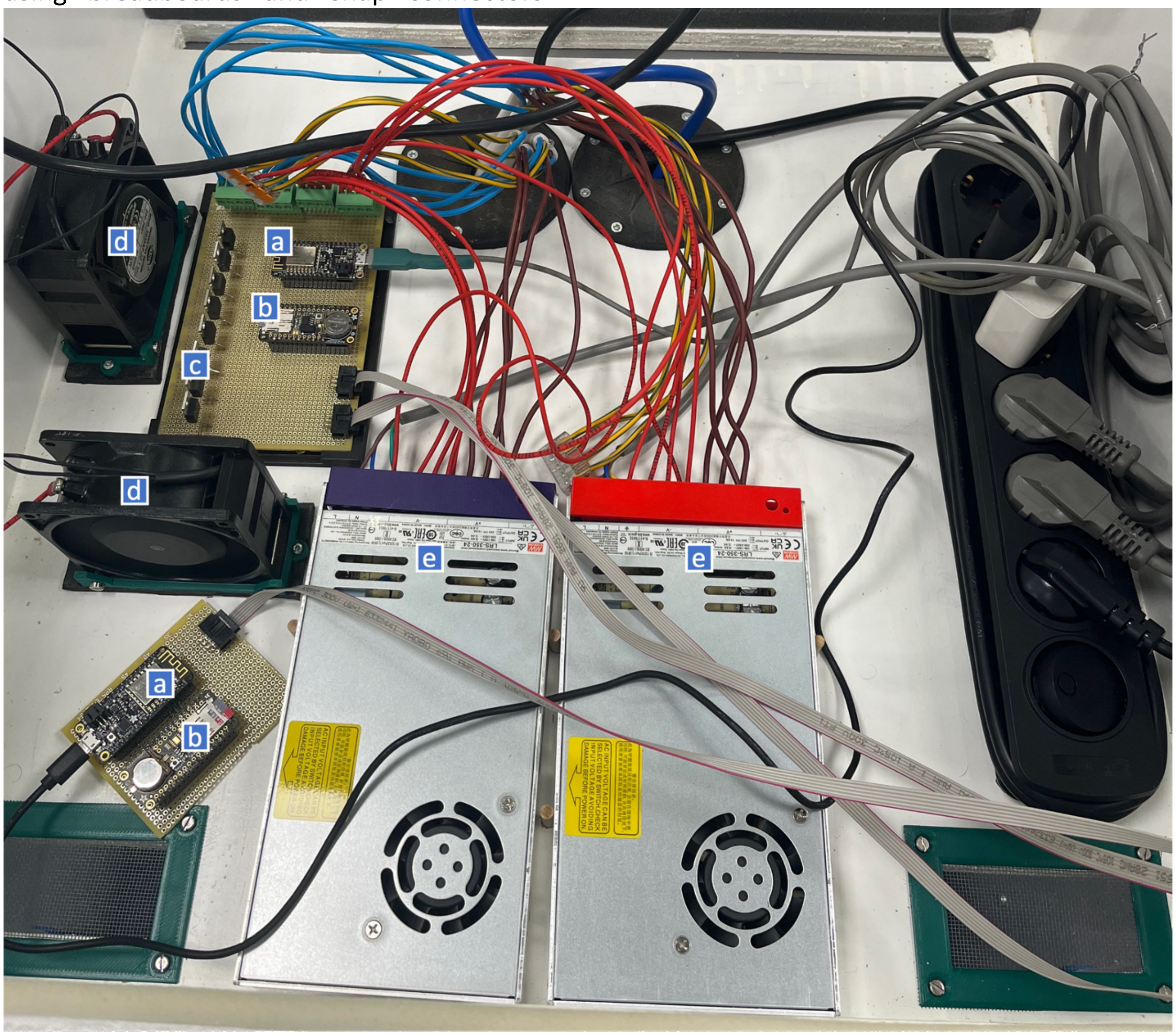

Figure 10. A picture of the electronics layout within the weatherproof housing box. a) ESP8266 microcontroller, b) Adalogger SD card data logger, c) MOSFETs, d) 230v cooling fans, e) 24v power supplies. Not pictured: thermocouples and amplifier boards, 24v computer fan.

Arduino Code

The thermocouple data logger runs using a set of custom-built Arduino code that can be found here.

Indoor experiments

To test the efficacy of the system in the absence of stochastic environmental perturbations, a single OTC with heating cables was setup indoors in a semi-temperature-controlled room lacking windows and direct connection to outside walls. The floor within the OTC was covered with a layer of dirt to simulate a more realistic situation. Over the course of several experiments various cable setups were tried in order to determine a heating cable layout that provided adequate heating.

No heating

To test whether the OTC itself would have an effect on temperature while running the indoor tests, temperature data were collected over the course of three days with no heating present.

Layout 1

The first cable layout was similar to the layout in Sun et al. (2013). 12m of cable was wrapped around a hexagonal PVC support with a perimeter of 5.54m. This PVC support was suspended approximately 10 cm above the ground within the OTC.

Layout 2

The second cable layout was a distributed network of cables suspended on a PVC and wire frame. This cable setup resembled a spider web. Cable distribution was random.

Layout 3a

The third cable layout was a distributed spoke and wheel layout with the cables lying on the ground. One cable went around the perimeter of the OTC and the other cables were laid from vertex to vertex of the hexagon dividing it into 6 smaller triangles.

Layout 3b

This layout was the same as 3a but with the cables suspended from the PVC and wire frame instead of laid on the ground.

Layout 4

This layout was a combination of 3a and 3b and was the final layout tested and was used as the final prototype setup.

Layouts 1-3 each used 4 cables and had a total wattage of 288W. Layout 4 used 8 cables and had a total wattage of 576W.

Indoor experiment Setup

Temperature data for the above 5 setups was collected using thermocouples and a thermal camera (FLIR A600 Series). Additionally, the OTC was tested first without any cables as a comparison. The basic OTC setup with the thermocouples and thermal camera can been seen in Figure 12. The data were processed and visualized using R Studio. A total of 15 trials were conducted, consisting of 1 unheated control trial and 14 heated trials with the various cable layouts.

Analysis of thermal camera data

Each image produced by the camera was exported as a CSV file were each pixel (480x640) was a temperature reading. To analyze these images, an R script was created that took five 75x75 pixel snapshots across each image. These snapshots were laid out with one snapshot in the middle, and one to the left, right, top, and bottom closer to the edge of the OTC as determined by visual inspection of the images. This R script then cycled through all the CSVs for a given experimental run pulling out the min, max, and mean from each of these sections as well as removing any data points above 40 C as temperatures above these were deemed to be only produced by the cables themselves and therefore not to be included in the analysis. This process produced a timeseries of data points for each experimental run that could be analyzed in the standard ways.

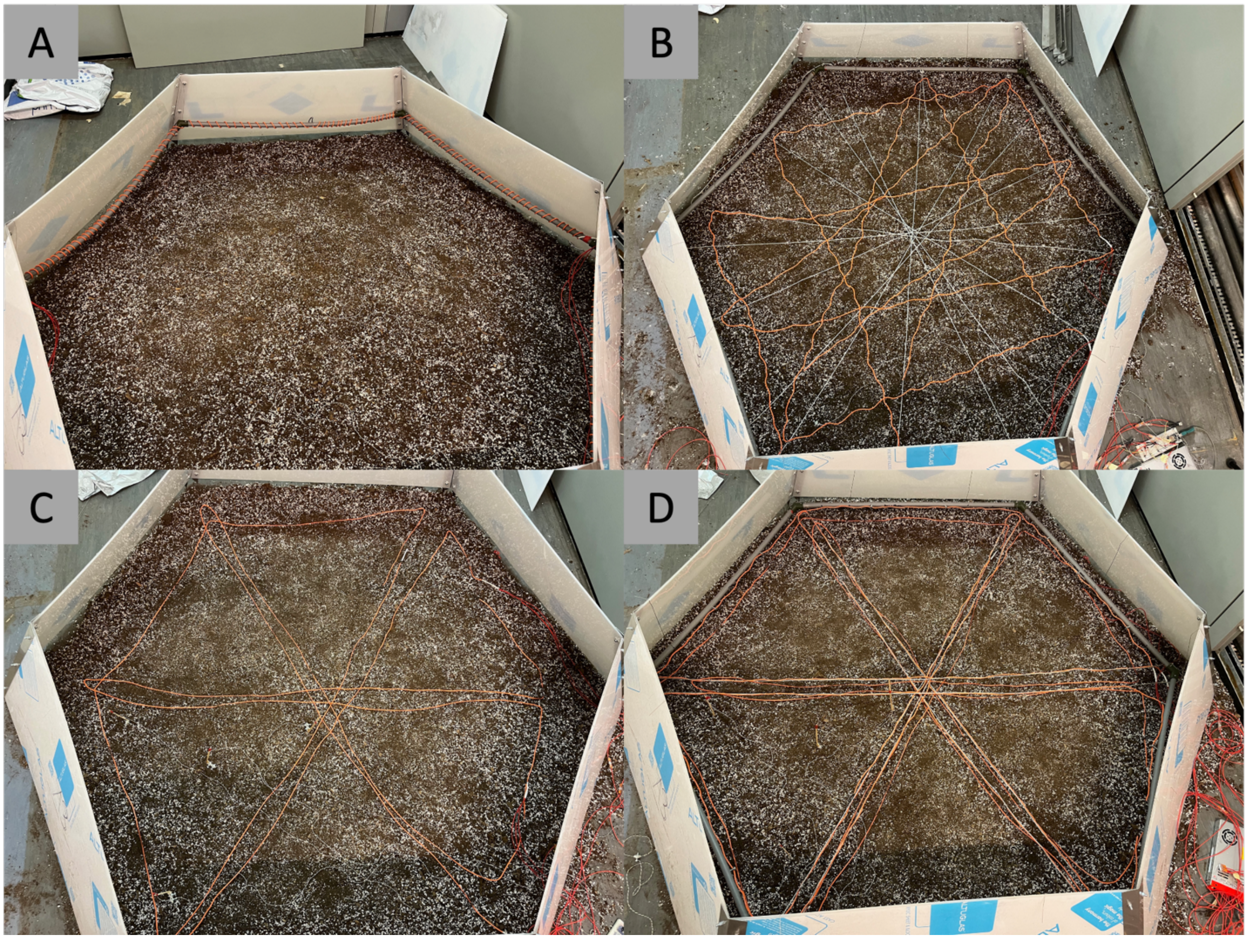

Figure 11. Indoor OTC-cable test layouts. Cables are the orange lines within the OTCs. OTCs are not transparent because the protective plastic was left on until the setup was taken outside to prevent scratching of the material in transit. A) Cable layout 1, b) Cable layout 2, c) Cable layout 3a, d) Cable layout 4. Layout 3b not show in isolation but is present as the top layer of cables in layout 4.

Figure 12. Indoor experiment research setup complete with master's student

Outdoor experiments

In preparation for moving the setup outside the electronics layouts were finalized and moved from prototyping breadboards and snap connectors to soldered circuit boards and more robust connectors. Waterproof cabling was added to the heating cables including custom printed cable connectors (3D printing information can be found here). To house the electronics that could not be directly waterproofed, a white wooden hutch was built. This hutch was made of 18mm plywood. The hutch had base dimensions of 60x60cm and had a sloped roof of 68x68cm, the overhang of which prevented water from seeping into the inside of the hutch. The hutch was raised 10cm off the ground on 45mm dowel legs. Ventilation holes in the bottom of the hutch along with a 24V computer fan located just under the top of the overhang served to provide airflow within the hutch to prevent any electronic components from overheating. Fine screen mesh was fitted over the vents to prevent ingress by insects or other animals. Two ports for wires were cut into the base of the hutch and fitted with flanged rubber discs on both the inside and outside of the base wood to allow cable access but prevent having fully open ports. See figure 10 for inside view of box. See Figure S2 for outside view of the box.

The outdoor experiments were setup in the Botanical Gardens at Utrecht University at the coordinates 52°05'23.8"N 5°10'21.5"E. The existing soil (mix of dense and loose clay), was turned over and mixed with some loose highly organic soil to form a plot of 2x6 m. Within this larger plot, the two OTCs and the OTC control plot were assembled with the OTC control on the leftmost side, the heated OTC in the middle and the unheated OTC on the right side. Within each plot several varieties of plants were planted: Arabidopsis (*Arabidopsis thaliana*), basil (*Ocimum basilicum*), winter cabbage (*Brassica oleracea* convar. *acephala* var. *Laciniat.*), broccoli (*Brassica oleracea* var. *italica*), and snowdrops (*Galanthus nivalis*, accession *Nienhof*). See Appendix 3 for supplemental photos of the outdoor setup.

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Appendices

Appendix 1: Materials

Table 1. Materials List. This table contains a list of important electronics components used in the setup with manufacturer name and parts numbers.

Material Description	Material Name	Manufacturer	Location	Parts Number/Product ID	Technical Specs
Heating cables	NA	Decochip	Netherlands	7423418520541	18W/m
Power supply	NA	Mean Well	USA	LRS-350-24	AC/DC converter 24V 350W
Microcontroller	Adafruit Feather HUZZAH with ESP8266	Adafruit	New York, NY, USA	2821	NA
MOSFET	NA	Infineon	Germany	IRLZ34NPbF	NA
SD logger + RTC	Adalogger FeatherWing - RTC + SD	Adafruit	New York, NY, USA	2922	NA
Thermocouple Amplifier	Adafruit MCP9600 I2C Thermocouple Amplifier - K, J, T, N, S, E, B and R Type T	Adafruit	New York, NY, USA	4101	NA
Type T Thermocouple	NA	Labfacility	Dinnington, UK	Z2-T-2M (IEC)	NA
Multicore cable	NA	Multicomp Pro	NA	3183Y-1.50MMWHT	3 core, 1.5mm^2, unscreened
6 core ribbon cable	NA	Pro Power	NA	R2651DTSY06SC85	28 AWG, unscreened
Cooling fans	NA	Sinwan	Taipei, Taiwan	S938AP-22-1	230V AC
Computer fan	NA	Multicomp	NA	MC001581	24V DC
Screw terminal block	NA	Amphenol Anytek	NA	TJ045153000AG	4 wire
Screw terminal header	NA	Amphenol Anytek	NA	OQ045450000AG	4 wire
IDC connector	NA	Amphenol	NA	T812106A100CEU	6 wire
Pin header	NA	Amphenol	NA	T821106A1R100CEU	6 wire

Appendix 2: Circuit Diagram

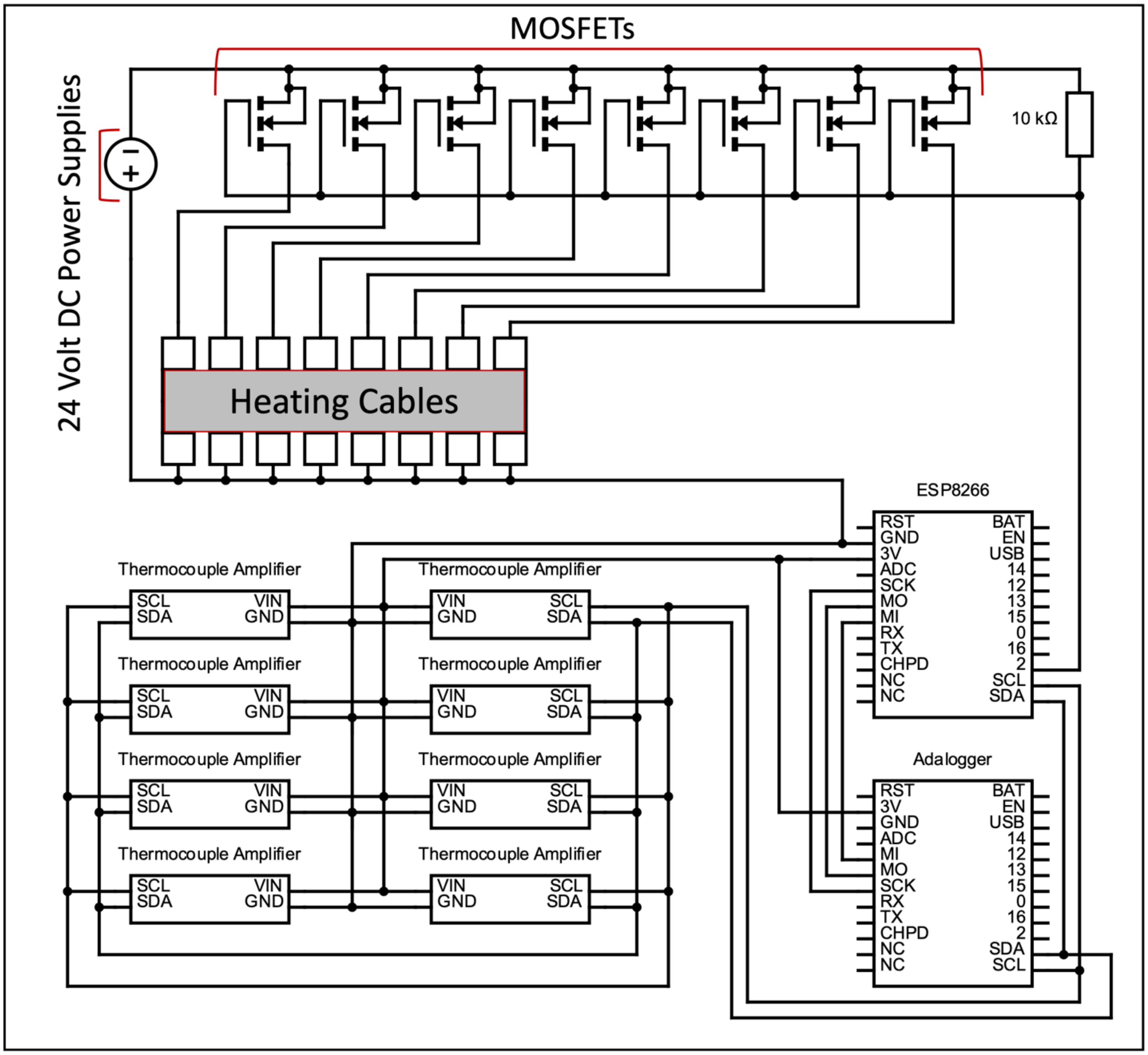

Figure S1. Simplified circuit diagram of the electronics setup. Not pictured: 3V power supplied to the microcontroller and AC power connected to the DC power supply.

Appendix 3. Supplemental Photos

Figure S2. Outside view of the white electronics box on location in the experimental plot. A) Side view of the box. OTCs can be seen behind it. B) Front view of the box. ventilation fan can be seen (blue bracket).

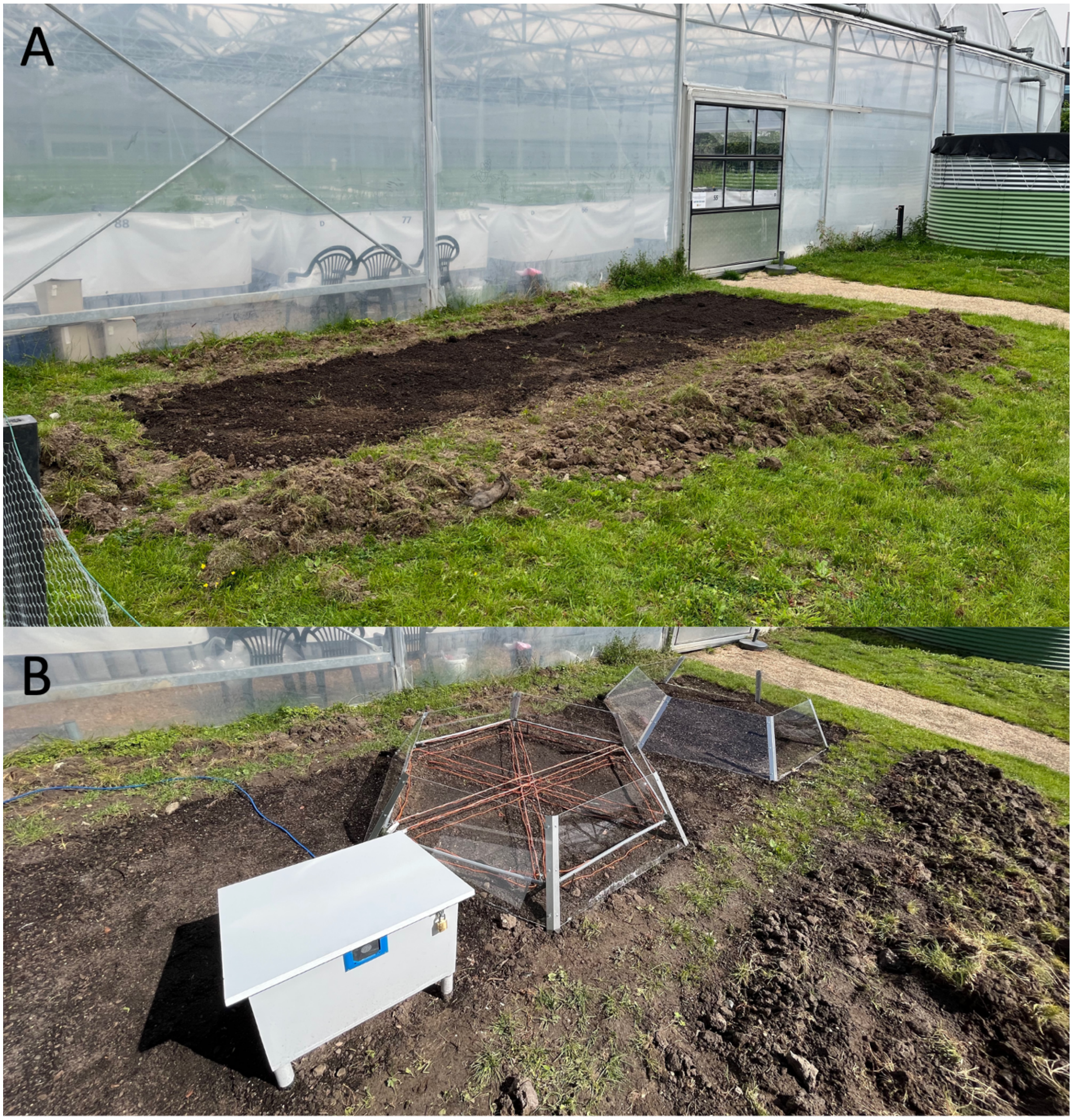

Figure S3. Views of the outdoor experimental plot. A) Empty plot. B) Plot with OTCs, heating cables, and electronics box.

Figure S4. View of middle OTC (heated) with plants.