Passive Warming Chambers Decrease Abundance of Flying Insects

Philip F. Yang, Caroline Markmann, Kevin Voigt, Robert Braverman

Understanding how organisms and ecosystems will respond to higher temperatures is important for mitigating the effects of climate change on biodiversity and ecosystem functioning. Many ecologists employ open-top chamber warming experiments to assess temperature effects on plants and soils, but often ignore how these chambers might affect multi-trophic interactions in these ecosystems. Plant-animal interactions, or lack thereof, can influence plant responses to warming. Using sticky-traps to measure insect abundance in open-top warming chambers, we sought to understand how chambers influence the abundance of flying insects and vegetated percent cover. Insect abundance was over four-fold higher in control plots than chamber plots. Vegetated percent cover was not different between control plots and chamber plots. Soil temperatures did not differ between warmed and control plots while air temperature was 1 °C higher in chambered plots than controls. Shading effects were noticeable from temperature data. We conclude warming chambers restrict flying insect populations from interacting with primary producers in our plots, but there is no noticeable impact on percent vegetated cover in the time frame for which we collected data. The chamber effect on insect abundance is an important artifact that other studies should consider when measuring ecosystem responses to increased temperature.

Introduction

Global temperatures are projected to rise anywhere from 1.5 ˚C to 4 ˚C by 2100 depending on actions humans take to mitigate climate change (1). Interpreting how ecosystems and organisms will respond to warming will be crucial for developing targeted policies and adaptive efforts to prevent further biodiversity loss, which would negatively impact both humanity and the natural world (2). Many ecologists have explored how warming might influence ecosystem productivity—from fish populations to arctic sedges (3,4). Most studies have examined warming effects at single trophic levels, often focusing on primary producer responses (4–6). To experimentally simulate warming effects in the field, ecologists across the world have commonly used open-top warming chambers (4,6–10). These miniature greenhouses warm experimental plot microclimates but also might restrict certain natural processes from occurring in these plots such as pollination or insect-plant interactions (10–12).

Changing natural species interactions can have possible nocuous effects on primary producer responses to warming. A few studies have found evidence of beneficial mutualistic insect-plant relationships that influence plant responses to warming (10–13). Plant stress decreased when both ant and aphid mutualisms remained intact, and warming decreased herbivore predator abundance and increased aphid abundance (10). Another study found fewer hemipterans outside active warming chambers than within, indicating a possible warming or experimental artifact from the chamber barrier (12). Plant interactions with other plants, herbivores, mutualistic organisms, and herbivore predators will partially determine plant responses to global warming and cannot be ignored (10–14). Underestimating multi-trophic species, or all the different species in the same food chain, ecosystem responses to warming will impede our ability to adapt measures for ecosystem conservation and prevent biodiversity loss in the face of climate change. Furthermore, recognizing experimental artifacts and the drawback of certain methods is important for crafting ecologically relevant studies that accurately simulate the natural world. Previous studies suggest chamber barriers have a stronger influence on insect abundance than higher temperatures (11,12), and generally insect activity has been found to increase in warming temperatures in urban areas (15,16). Vegetated percent cover can indicate changes in insect-plant relations, as insect grazing has been known to decrease percent cover before in a specific insect-plant interaction (17). Measuring percent cover and using it to represent insect impacts on vegetation is not a perfect response variable, but it is worthwhile to investigate because other studies have used satellites (18) and experimental sampling to examine how vegetated growth can change due to insect activity and vice versa (17,19).

The objective of this study was to quantify

elevated temperature and chamber barrier influences on flying insects in an open-top chamber warming experiment through the fall season and compare this data to percent vegetated cover changes. To our knowledge, this is the first time the warming chamber effect has been measured on aerial insect abundance, making this a relevant study for determining the influence of warming chambers on multi-trophic interactions. Yellow sticky traps measured insect abundances in both chambered and control plots because they have displayed success in measuring insect abundance in previous studies (20). To determine warming chamber effects, air temperature and soil temperature were measured. We asked: do open-top warming chambers influence flying insect abundance, and do open-top warming chambers induce noticeable warming over 3 months in autumn? Is soil temperature affected by open-top warming chambers? Will flying insect abundance correlate with vegetated cover? We hypothesized that (a) aerial insect abundance would be higher in control plots because there is no physical barrier compared to chambered plots, and insects would run into the traps more frequently, (b) vegetated percent cover would be lower in control plots with higher insect abundance, (c) soil temperature would be higher in warming chamber plots due to the warming chambers trapping heat in overnight, and (d) air temperatures would be higher in warming chamber plots. If we find fewer insects in warming plots, then the warming effect or the physical chamber barrier might be influencing their abundance. If soil and air temperature are higher in chambers, then the chambers are likely producing a warming effect.

Materials and Methods

Study Site and Experimental Design

This experiment took place in Villanova, PA (40°04'37.2" N, 75°21'47.6" W) on Villanova University's West Campus, which is in Radnor Township of Delaware County, Pennsylvania. The site was originally farmland before the Morris family developed it in the early 20th Century, and Villanova University purchased it from them in 1978 (21). The soil order of the site is alfisol, which is known to be the most productive soil because of the alkalinity of the soil and high clay particle content. Glechoma hederacea (ground-ivy), Persicaria maculosa (spotted lady's thumb), Persicaria longiseta (low smartweed), and Stenotaphrum secundatum (St. Augustine's grass) were identified as key plant species at the site. Air temperature typically ranges from -4 °C to 29 °C with an average between 9-22 °C. Annual average precipitation is 121.92 cm (22).

Experiment design consisted of twelve plots with the dimensions of 1.5 x 0.9 x 0.9 m. Six plots served as controls, while the other six were subject to a warming treatment (Figure 1). An open-top plastic sheet chamber held together with polyvinyl chloride (PVC) piping covered treatment plots to induce passive warming. The mechanism by which warming occurs throughout the chambers is mainly through interrupting radiative and convective heat loss. In unchambered control plots heat can dissipate by radiation to the sky or by advective or convective air currents. The plastic sheets act like a mini greenhouse by trapping solar energy and re-radiating it throughout the chamber and by blocking advective air currents. Each of the twelve plots were further divided in half to impose a nitrogen treatment on one side by addition of fertilizer for a different project. Air temperature was collected hourly using HOBOWARE devices (Onset, Bourne, MA). One HOBO pendant logger was placed in each of the twelve plots inside of a plastic funnel covered with aluminum to prevent any warming effect from direct sunlight. The funnel was then tied to a wooden stake located in the center of the plots, allowing the logger to record air temperatures approximately 15 centimeters above the ground. To measure insect abundance, yellow sticky trap paper was fastened to the same wooden stake as the HOBOs (Figure 2). Sticky trap paper was changed once during the experiment on October 22nd because some of them were so full that there was no more space to collect more insects. Measurements occurred from September to November 2020.

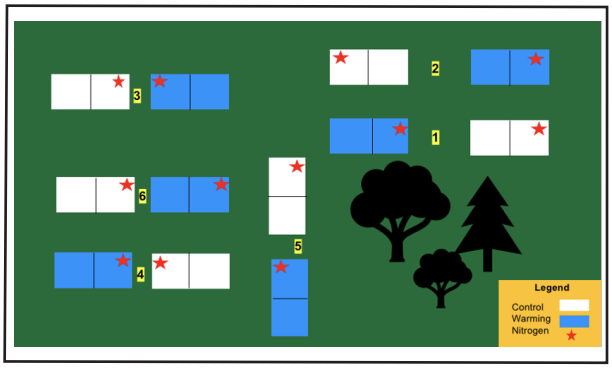

Figure 1. Diagram of plots at the research site in Villanova University, Pennsylvania, USA. Subplots were split in each warmed and control plot to apply a nitrogen treatment that was used for other projects. Trees are placed so possible shading effects can be visualized for all plots. Insect traps were placed in the center of each plot. Picotte Hall at Dundale is due East of this plot with Villanova W-3 Picotte Hall parking lot in between.

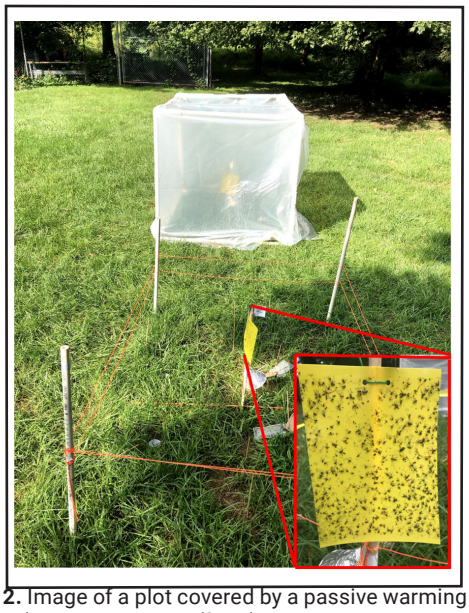

Figure 2. Image of a plot covered by a passive warming chamber (back) and control plot (front) with a zoomed-in image (red outline) of a flying insect yellow sticky trap.

Three measurements were conducted throughout the experiment: insect abundance, air temperature, and soil temperature. Data on percent vegetated cover was collected by another group and used to compare insect abundance trends with vegetated cover. Data collection started on September 11th and continued until November 5th. Insect abundance was collected bi-weekly using ImageJ to count particles from trap images. Temperature data was obtained from the HOBO's halfway through the experiment to confirm they were functioning correctly and once at the end of the experiment. Soil temperature for every subplot was recorded weekly at approximately noon. A soil thermometer was placed 6 cm below the ground and was allowed to be calibrated/self-calibrate before a temperature was recorded.

Calculations and Data Analysis

In order to calculate insect abundance on ImageJ, the following steps were adapted from Parker et al. (23):

1. Converted the original RBG image to an 8-bit image.
2. Changed the color threshold to a range that included all the insects.
3. Selected the image area to be analyzed and clicked analyze particles.
4. Adjusted pixel size to 05-infinity after preliminary trials revealed that was the ideal range to count one insect and checked exclude on edges.
5. Recorded the count and saved the image for future reference or analysis.

Insect counts from the traps stood as proxies for insect abundance. A repeated multiple analysis of variance (MANOVA) test was performed to analyze the effects of the warming treatment on insect abundance. T-tests were performed on air and soil temperature as well as the vegetated percent cover data.

Results

Insect Abundance

A MANOVA test was run on insect abundance data. Traps in control plots had an insect abundance four to five times greater than traps placed inside warming chambers shown in Figure 3 even after the traps were changed on October 22nd ($p<0.05$). Plot location did not have any significant effect on insect abundance ($p=0.62$).

Percent Cover

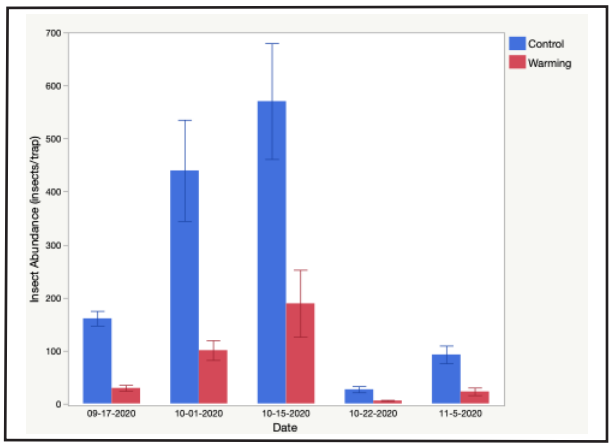

Figure 3. Mean insect abundance (insects per trap) in control and warming plots (n=6). The dates represent when images were taken to collect data, usually in two-week intervals apart. Traps were replaced on October 22nd, 2020, which is why the insect abundance appears to drop so drastically. Error bars represent one standard error from the mean. A MANOVA test found a p-value < 0.05.

Vegetated cover was calculated as percent cover by a group recognized in the Acknowledgements. Standard error bars overlapped frequently between treatments, and no clear relationship could be fit to the data. A t-test comparing a computer's calculation of percent vegetated cover to eye-estimation of percent cover found a p-value of 0.0002. A t-test comparing the means of the control plots and the warming chambers found a p-value of 0.7967. Figure 4a displays the vegetated percent cover values using computer software, and Figure 4b shows the percent cover values of eye-estimation.

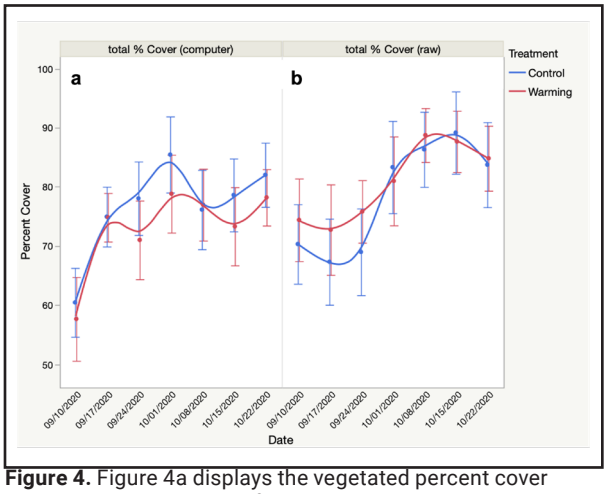

Figure 4. Figure 4a displays the vegetated percent cover values using computer software, and Figure 4b shows the percent cover values of eye-estimation.

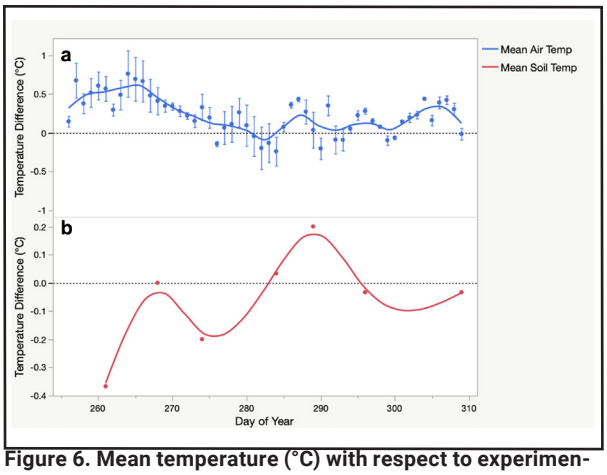

Figure 6. Mean temperature (°C) with respect to experimental plots (n=6) for the duration of the experiment. Data was collected from September 11th to November 4th, 2020. Both temperature differences were calculated as the difference between mean temperature of warmed plots and mean temperature of control plots. Error bars represent one standard error from the mean. There are no error bars for soil temperature because only one measurement per plot was taken per week. A t-test on mean air temperature found a p-value < 0.05.

Air and Soil Temperature

Overall, the data showed a significant difference in air temperature between the warming and the control plots ($p<0.05$). There was about a 1˚C increase seen in the warming treatment plots during the day (Figure 5). Air temperature is shown in Figure 6a to be higher in warming plots by an average of 0.2 ˚C, and a t-test on average air temperature found a p-value $<$ 0.05. Soil temperature was slightly warmer in the control plots than it was in the warming chamber but not by a significant amount after performing a t-test on average plot temperatures ($p=0.86$). Figure 6b shows the difference between warming plots and control plots, and soil temperature was not higher in warming plots than control plots.

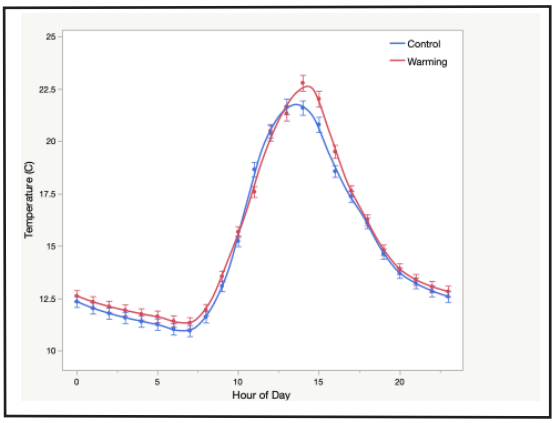

Figure 5. Mean air temperature (°C) at different hours of the day for warming and control plots (n=6). Data was collected from September 10th to November 4th, 2020. Error bars represent one standard error from the mean. A MANOVA test found a p-value < 0.05.

Discussion

We sought to test and answer three questions regarding the treatment effects and artifacts imposed by open-top warming chambers. Previous studies have suggested warming chambers influence insect abundance more than warming temperatures (10–13). To disentangle warming vs. barrier effects, we collected flying insect abundance data using sticky traps. Then, we compared our insect abundance data with other data collected on percent vegetated cover in control and warmed plots. Lastly, we predicted soil and air temperature to increase in warming chamber plots.

Insect abundance in the control plots was consistently higher than the warmed plots, even though there was a significant increase in temperature in the warming chamber plots (Figures 3, 5, 6). This result remained consistent even after the traps were changed (Figure 3). We rejected the null hypothesis for hypothesis (a). The physical barrier of the warming chamber most likely caused this trend because previous studies have found increased insect activity in higher temperatures in more urban areas (15,16). After replacing the sticky traps on October 22nd, the trend of higher insects in control plots vs. warming plots held consistent, which further supports that insect reductions in warming plots most likely come from the chamber artifact. This finding is also consistent with previous literature that suggest warming chambers decrease insect abundance (12). However, our study did not completely disentangle warming vs. barrier effects because a more specific study on the impacts on

insect abundance produced by our levels of warming would examine warming separately from the barrier. It is possible for both effects to be at work – warming increasing insect abundance with the chamber barrier having an overshadowing influence. We argue that, although this is possible, the impact the chambers have on flying insect abundance is the most important factor because it overshadows other influences on insect abundance. Future research should explore the influences of warming temperatures without chamber barriers on insect abundance separately.

The implications of this finding are important in many ways. First, this chamber artifact is one that potentially has major scientific implications for other global change experiments. If warming chambers influence insect abundance this drastically, then insect interactions are likely reduced in all experiments that use similar warming chambers (4, 6–8, 11). Ecosystem responses to these warming chambers should be interpreted with this artifact in mind. If a response like plant biomass, which is correlated with insect abundance because many flying insects are herbivorous, increases in warming plots, then the absence of insects could cause a large overestimation in the plant biomass response (Mentor personal communication, 2020). The broader significance of this finding is that further global change experiments using warming chambers must recognize and account for this artifact when measuring ecosystem responses and creating conclusions for how ecosystems will respond to climate change. Multi-trophic ecosystem and organism responses need to be considered in mitigating climate change impacts (14).

There was no significant decrease of percent vegetated cover in warmed plots over control ones in the time frame of 6 weeks from September to October (Figure 4) which led us to fail to reject the null hypothesis of hypothesis (b). The significant difference between the computer-generated values of vegetated percent cover and eye-estimation is an important finding for methodological practices in the future for percent cover data collection and analysis (Figure 4). A four-fold decrease of insect abundance did not appear to increase vegetated percent cover in warming chambers like we expected if the insect-plant percent cover relationship was a direct one. A 5-year study found insect grazing to significantly decrease plant percent cover of a specific species and increase percent cover of other plant species (17). Compared to this study, our experimental period might not have been long enough or large enough to see warming chambers decrease insect abundance to a level that clearly impacts vegetated percent cover. We suggest more long-term ecological research studies that examine the tropic relationship between insects and plants when treatments like increased temperature and chambers are applied along with the best metrics to measure how changes in one affect another. Satellite imagery data is promising for efforts like these, and research should pursue the combination of field data for insects with satellite imagery for vegetation indexes (18).

There was no significant difference in soil temperature between warming plots and control plots (Figure 6), and we fail to reject the null hypothesis of hypothesis (c). However, we found that the shadiest plot (plot 1, Figure 1) had a noticeably lower soil temperature throughout the course of the experiment. Thus, we concluded that the amount of sunlight the plot received had a larger effect on the temperature of the ground than the air temperature directly above the plot. The chambers primarily warm the atmosphere in the plots and have a negligible effect on the heat balance of the soil (24), although shading from chambers may actually cool the soil underneath even while warming the air within the chamber (8).

We predicted the air temperature would increase around 2 ˚C due to the warming treatment because of convection within the chamber (7–9). We found a 1 ˚C higher average plot air temperature in warming chambers during the day (Figure 5), confirming that our experimental treatment of warming influenced the plots and leading us to reject the null hypothesis of hypothesis (d). We believe the short period of time in which the difference was largest (around hour 15) can be attributed to increased direct sunlight around the middle of the day for all plots. Peaks and valleys in the temperature were observed when comparing days to one another without a clear pattern, which is why we decided to take the average temperature difference between warmed and control plots and found an average increase of 0.2 ˚C in chambered plots (Figure 6). Figure 6 shows overall air temperature difference was strongest in early fall with more direct sunlight and steadily decreased from late September to mid-October (day of the year 260-280). We attribute this fluctuation to changes in the degree of direct sunlight due to cloud cover variation. We did not record the weather of each day, so this is not accurately confirmed. An experimental error that could have altered some of the data is that the warming chambers were accidentally left off the plots from October 22nd to October 27th. This may have caused a reduction to the average warming effect, and we removed the air temperature data from our average analysis for this period. Overall, there was a significant difference in air temperature between the warmed and controlled plots throughout the experiment (Figure 5).

In conclusion, there was a significant decrease

in insect abundance in the warming plots compared to the control plots due to the physical barrier of the chamber, but this decrease did not have an impact on vegetated percent cover. The warming chambers did not alter the soil temperature significantly. There was a significant difference in air temperature between warmed plots and controlled plots over the course of the experiment with a 1 ˚C increase in the warmed plots. The results from our experiment are meaningful because they highlight the artifact the physical barrier of a warming chamber presents. To adequately study how global warming will affect an ecosystem, insect abundance is a variable that must be considered. Future experiments should study the effect the chambers have on plant biomass while monitoring insect abundance. Plants store carbon, and accurately imitating the impacts warming will have on the local, regional, and global natural carbon cycle includes understanding multi-trophic interactions.

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Kevin Voigt is a member of the Villanova University Class of 2022 from Massapequa, New York. He is pursuing a major in Biology. Kevin is also a member of the 2021 Big East Champion and Sweet 16 participant Villanova Men's Basketball team. He was able to contribute to the research team largely from quarantine during the Fall of 2020 COVID protocols. He looks forward to completing his degree in May of 2022.

Mentor

Dr. Adam Langley

Dr. Langley uses long-term field experiments to learn how ecosystems respond to perturbations with particular emphasis on the feedbacks that will determine the severity of future global change. He teaches Ecology and Microbiology in Villanova's biology department. The work presented here arose from a Global Change Ecology class project in which students built experimental warming chambers on Villanova campus and to assess how our local ecosystems will handle future climatic warming.