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Dry and warm: a modified open-top chamber for seed ecology research

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Abstract

Several experimental tools allow researchers to manipulate environmental variables to simulate future climate change scenarios during *in situ* seed ecology studies. The most common ones are designed to modify a single environmental variable. For example, open-top chambers (OTCs) increase temperature or rain-out shelters decrease precipitation. However, changes in environmental variables in the future are expected to happen simultaneously, and at present, an understanding of their combined effects in natural environments is limited. Here, we present a passive novel OTC design that simultaneously increases the soil temperature and decreases soil moisture. We assessed the performance of the design during 1 year in a high-mountain environment and reported its effects on the organic and topsoil layers. The modified OTC reduced the soil volumetric water content throughout the study period. Overall, chambers increased the mean day air temperature by 3.3 °C (at 10 cm above the soil surface), the mean day soil surface temperature by 1.35 °C and the mean day below the soil surface temperature by 1.30 °C (at −5 cm) and 1.25 °C (at −10 cm). Remarkably, surface and soil temperatures remained warmer at night (+0.65 at soil surface, +0.41 at −5 cm and +0.24 at −10 cm). We detail the design plans, tools and materials needed for its construction. Furthermore, we recommend on how to use it during seed ecology studies. This tool can help increase our understanding of the potential responses of seeds and seedlings to the combined effects of warming temperatures and a decrease in precipitation.

Introduction

Climate change involves changes in several key environmental drivers that profoundly affect on species reproduction (Walther et al., 2002). The tightly coupled relationships between climate variables, seed dormancy and germination suggest that the expected climatic changes will inevitably affect the ecology of seeds (Ooi, 2012). Field experiments that manipulate environmental variables are a common way to generate crucial data to predict species responses to the effects of climate change (Beier et al., 2012; Knapp et al., 2018; Korell et al., 2019). During field manipulative experiments, researchers use a wide range of experimental tools to modify climatic variables, in order to simulate future climate change scenarios.

Warming temperatures can be created using active (e.g., infrared heaters and fluid-heated pipes) or passive methods (e.g., ground covers, greenhouses or open-top chambers, OTCs). OTCs are the most used tool because of their simple, cost-effective and low-maintenance design (Arft et al., 1999; Welshofer et al., 2018), which also allows natural levels of precipitation, light and gas exchange (Marion et al., 1997). Changes in rainfall can be simulated by watering or restricting precipitation. The most frequently used tools to simulate a decrease in precipitation are rainout shelters. These provide a partial/slatted or full transparent roof to restrict rainfall, reducing the soil moisture within the selected plots (Yahdjian and Sala, 2002; Kundel et al., 2018).

The effects of warming temperatures and changes in precipitation are commonly studied separately during field experiments (Kreyling and Beier, 2013). However, changes in these environmental variables are expected to happen simultaneously, and our current understanding of their combined effects on species responses and ecosystem processes is limited (Kreyling and Beier, 2013; Korell et al., 2019). Some of the existing experiments that have manipulated both rainfall and temperature have used OTCs and rainout shelters simultaneously. However, having two different tools in the same plot can increase the undesired side effects of both designs (Marion et al., 1997; Vogel et al., 2013) and also elevate the costs of research.

Here, we present an OTC designed to increase soil and soil surface temperatures and decrease soil moisture in the organic and topsoil layers. The chamber was designed to be used during seed and seedling manipulative *in situ* experiments (e.g., soil seed banks, seed germination, maternal environmental effects and seedling establishment) in cold, mountainous regions. We tested the chamber design in a high-mountain environment and reported its performance and effects on the soil volumetric water content and the air and soil temperature.

We also provide in detail the tools, materials and design plan needed for its construction. Finally, we give recommendations for its use during field seed ecology studies.

Materials and methods

Study area

We tested the chamber design within the Falls Creek Alpine Resort (36° 51′ S, 147° 15′ E and 1,750 masl), which is located in the Bogong High Plains in south-eastern Australia. The study was carried out in a tall alpine herbfield dominated by *Poa* (Poaceae), *Craspedia* and *Celmisia* (Asteraceae) species. Soils are free-draining, highly acidic alpine humus derived from metamorphic rock or basalt (Costin et al., 2000). The mean annual temperature is 9.5 °C, the mean maximum temperature for the hottest month (January) is 17.9 °C and the mean minimum temperature for the coldest month (July) is −2.9 °C (1990–2021). The mean annual precipitation is 1,307 mm (1990–2021), with most of the precipitation falling during the austral winter as snow, which can persist for around 4–5 months, and the driest period of the year being during late spring and summer (Bureau of Meteorology, 2022).

Chamber design

The chamber is designed to create a drier and warmer microclimate in the organic and topsoil layers (+10 to −10 cm from the soil surface) where seeds and seedlings can be found. The design is based on the traditional cone-shaped OTC (Molau and Per, 1996; Marion et al., 1997), with rain-out structures added to form a partial, water-shedding roof (Fig. 1A–C). The dimensions of the test chambers are 84.6 cm in base diameter, 50 cm open-top diameter, 40 cm in height and 50% of rainfall restriction. As they were designed to be used during seed and seedling experiments, their size is smaller when compared to the traditional designs (Marion et al., 1997). However, the size and the area covered by the rain-out structures can be easily modified (see Supplementary Fig. S1).

Test chambers were made of a single sheet of clear, flexible polycarbonate, 0.8 mm thick (Suntuf, Palram Industries, Ramat Yohanan, Israel), weighing <1 kg. When installed in the field, the rain-out structures are held together using a stainless threaded steel rod (1.2 m long). The rod is positioned vertically in the centre of the chamber and must be pushed into the soil in the middle of the research plot. Two wing nuts above and below the polycarbonate support the top of the chamber roof at a 35° angle. Heavy-duty, clear, weatherproof resistant tape (The Gorilla Glue Company, Cincinnati, OH, USA) was used to hold together the edges of the chamber (alternatively, stainless flathead screws can be used). Standard tent pegs were inserted in each of the four base tabs to secure the chambers to the ground. We left a 2–3 cm gap between the soil surface and the chamber. To hydrologically isolate and prevent sub-surface water flows into and out of the chambered plots, we buried garden edging (rigid but

Figure 1. (A) Chambers used during the study, (B) a chamber after a light snowfall demonstrating how any snow inside the chamber melts faster than ambient conditions, (C) thermal image indicating the temperatures inside and outside the chamber and (D, E) seeds and seedling can be sowed within the chambered plots to understand their response to a warmer and drier microclimate.

Table 1 Established limits for data analysis

Period	Description
Day	From 6:00 to 20:00 hours
Night	From 0:00 to 5:00 and 20:00 to 23:00
Summer	From 21 December 2020 to 28 February 2021and 1 December 2021to 20 December 2021
Autumn	From 1 March 2021 to 31 May 2021
Winter	From 1 June 2021 to 30 August 2021
Spring	From 1 September 2021 to 30 November 2021
Overall	From 21 December 2020 to 20 December 2021

Table 2 Mean soil VWC (m^3/m^3) in control and chambered plots throughout the study period and the mean difference (°C) and its *P*-value significance

Period	Control	Chamber	Effect size (±95% CI)	*P*-value
Spring	0.357	0.337	0.020 ± 0.003	<0.001
Summer	0.291	0.259	0.031 ± 0.001	<0.001
Autumn	0.309	0.271	0.037 ± 0.004	<0.001
Winter	0.353	0.327	0.026 ± 0.002	<0.001
Average	0.327	0.298	0.029 ± 0.002	<0.001
Day	0.328	0.299	0.029 ± 0.003	<0.001
Night	0.326	0.297	0.028 ± 0.003	<0.001

flexible plastic, 1 mm thick and 10 cm high) up to 10 cm below the soil surface around the selected plots but inside the chamber area. To prevent water condensation, we trimmed the vegetation between the garden edging and the chamber and around the chamber (no plants touching the chamber walls). In Supplementary Fig. S1, we show the design plan and specify the materials and tools needed for the construction of the chamber.

The size of the experimental plot covered by the chamber (around 1 m^2) is large enough to carry out experiments with seeds and seedlings simultaneously. Seeds can be buried directly in the ground or inside mesh bags and seedlings can be transplanted or resultant from the buried seeds (Fig. 1D, E).

Experiment design and microenvironment monitoring

We assessed the performance of the chamber design over 1 year from December 2020 to November 2021. Within the experimental study area, we selected three sites with similar environmental and topographic conditions (open areas with <5% slope). At each site, we established four circular plots of 1 m^2 targeting areas predominantly occupied by short graminoids and herbs and devoid of any shrubs or trees that could potentially modify the conditions created by the chamber. Half of the plots were randomly assigned as control plots and the remaining half were covered with chambers. In total, we tested six chambers. However, at the end of the snow-free period, we left only one chamber per site to evaluate if the design would resist the weight of snowpack during winter.

We recorded hourly abiotic conditions in each site using HOBO (Onset Computer Corporation, Bourne, MA, USA) and iButton (Maxim Integrated, San Jose, CA, USA) data loggers. We did not quantify rain restriction; instead, we measured and

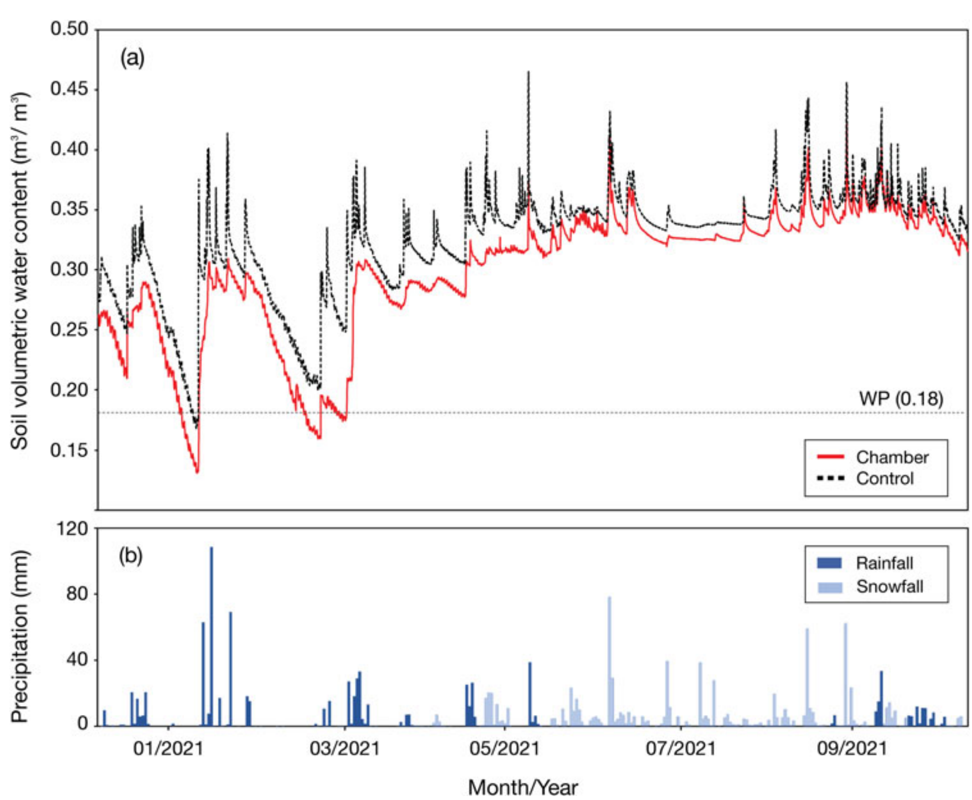

Figure 2. Time series for the study period (December 2020 to November 2021) of (A) mean soil VWC of control and chamber plots at 5 cm below the soil surface and (B) daily precipitation recorded at the Falls Creek weather station (Bureau of Meteorology, 2022). WP, wilting point for the study region (Venn and Morgan, 2009).

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contrasted soil volumetric water between control and chambered plots. To measure soil volumetric water content (VWC), we use three HOBO H21 – USB Micro Station Data Logger with two soil moisture sensors each (HOBO-S-SMD-M005 – large area of influence) that were installed in two randomly selected plots at each site, one control and one chambered ($n = 3$ per condition for the study). We installed temperature loggers in all the experimental plots ($n = 6$ per condition for the study) to measure the air temperature (+10 cm above soil surface), soil surface temperature and soil temperature at 5 and 10 cm below the surface. A radiation shield was used to cover the temperature logger at 10 cm above the soil surface. Sensors and loggers were installed 20–30 cm from the centre of the plots.

We also measured the light intensity for a month with a HOBO logger (HOBO Pendant UA-002-64 Logger) at ground level in one control and one chambered plot at each site ($n = 3$ per condition for the study). In addition, we measured wind velocity 10 cm above the soil surface in one chamber and one control plot at 9:00, 12:00 and 17.00 hours with two Kestrel-1000 wind meters (this measurement was done in another location with similar vegetation conditions). Sensors and data loggers were used under factory default calibration. Finally, we obtained daily rainfall, snowfall and wind velocity data for the complete study period from the Falls Creek Bureau of Meteorology weather station located approximately 1.5 km away from the study area (Bureau of Meteorology, 2022).

Data analysis and management

In order to ensure that the data from the soil moisture or temperature loggers were accurate, we inspected all the records, and we decided to include or exclude them from the analysis following the next criteria: (i) when we found an evident failure in the records extracted from the loggers (e.g., not real temperatures like 888 °C or negative values in soil moisture), we removed the records of the particular logger from the database for the entire day when the malfunctioning was recorded, and the analysis was done using the data from the rest of the established loggers, and (ii) when all the established loggers in one position (−10, −5, 0 +10 cm) or condition (chambered or control plots) failed simultaneously, we excluded those dates from the final analysis. We detail the dates and records that were removed/excluded from the final analysis and database in Supplementary Table S1 and Database S1.

We conducted an exploratory analysis, plotting the data from all sites for the following periods: spring, summer, autumn, winter, the entire study period, and day and night (see Table 1). The visualization of the data helped us determine that the effect of the chambers on soil moisture and soil and air temperature was constant across sites. The overall values within treatments were similar, suggesting no site effect (i.e., all chambered plots had lower soil moisture and warmer temperature than control plots). For this reason, and the lack of data for some sites during significant periods due to logger failures, we decided to pool the data from all sites. We then compared the mean hourly soil VWC and the mean hourly air and soil temperatures of the control and chamber plots for the periods shown in Table 1 using two-tailed unpaired t-tests or Mann–Whitney U tests where the assumption of normality was not met. We also calculated and plotted the effect sizes (mean difference or median difference) and their 95% confidence intervals (Ho et al., 2019). To compare the performance of the test chamber on temperature and soil moisture with that of the commonly used OTC and rain-out shelters, we conducted a thorough search for published and unpublished data. Analyses were done in R (R Core Team, 2022), and figures were constructed in Adobe Illustrator.

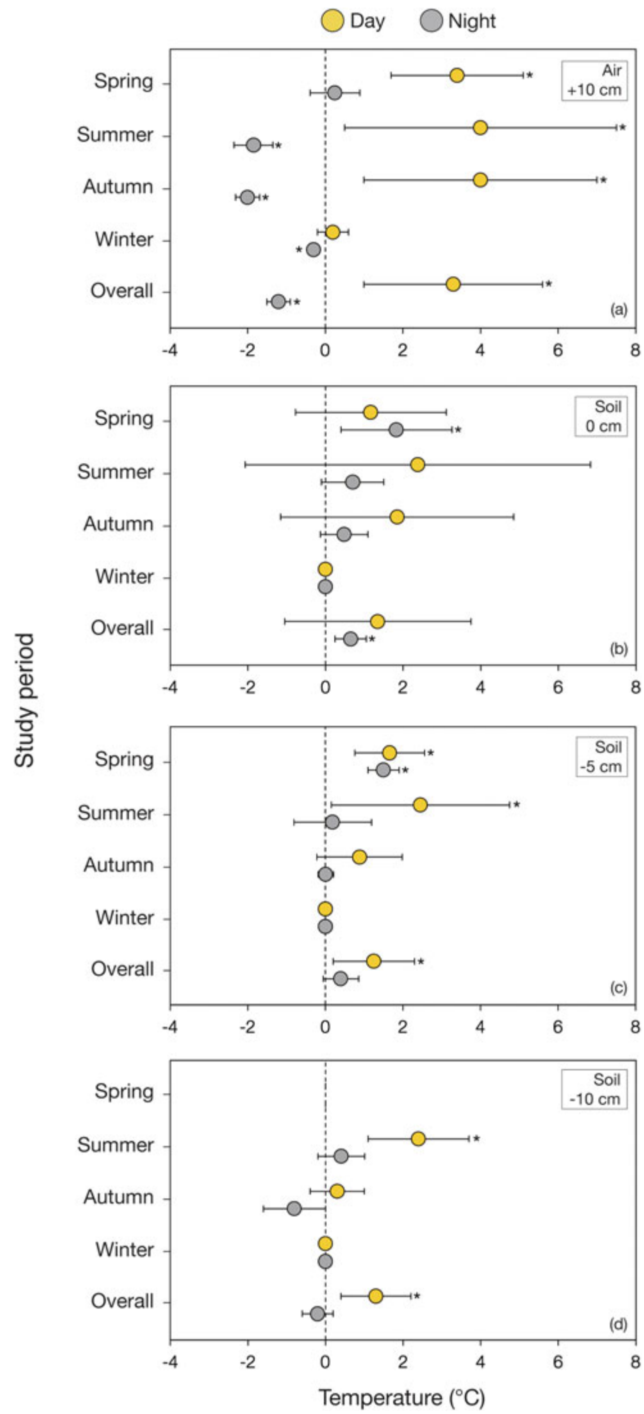

Figure 3. Mean difference (±95% CI) between chambered and control plots for (A) air temperature at 10 cm above ground, (B) soil surface temperature, (C) soil temperature at 5 cm below the soil surface and (D) soil temperature at 10 cm below the soil surface during the study period. Significant differences are pointed out with (*).

Results

All test chambers were able to withstand the harsh alpine environmental conditions such as −12 °C ambient air temperatures, the maximum wind gust speed of 67 km/h and a winter snowpack of up to 1.3 m of depth with minor damages in their structure. Chambers did not cause changes in their surrounding area (e.g., create a channel in the soil around them and cause any mortality of nearby plants). We found no evidence of small or large mammals (rodents, rabbits, possums and macropods) using the chambers during the experimental period.

Chambers significantly reduced the soil VWC throughout the study period, with a greater effect recorded during autumn and a smaller effect during spring (Table 2). No differences in the effect of the chamber on soil VWC during day and night were detected. During the whole experiment period, the soil VWC values were under the soil wilting point for 12.5 days in chambered plots compared to just 1 day in control plots (Fig. 2).

The effect of the chambers on temperature varied throughout the study period (Fig. 3). The chambers caused more extreme peaks in air and soil surface temperatures as they fluctuated during the day (24 h), with peak temperatures around solar noon (Fig. 4). The effect of the chambers also varied from day to day as a consequence of local weather conditions; on sunny days, temperatures were significantly higher in the chambers compared with control plots; however, this effect was not as strong on overcast, cloudy and rainy days. During snow events, chambers restricted the amount of precipitated snow that accumulated inside, which then melted faster due to the warmer temperatures inside the chambers and led to an increase in the frequency of freeze/thaw soil cycles (Fig. 5).

The chamber design significantly reduced the wind velocity near the soil surface (+10 cm) at 9:00 ($t(4) = 9.67$, $P < 0.001$), 14:00 ($t(4) = 10.93$, $P = 0.002$) and 19:00 h. ($t(4) = 13.05$, $P < 0.001$). There was also a reduction in the relative light levels (lumens/m^2) inside the chambered plots, but the difference was not statistically significant (27,600 ± 22,938 *vs* 35,123 ± 25,688, mean ± s.d.; $t(28) = 2.04$, $P = 0.4$). We report the obtained values for wind velocity and relative light levels in Supplementary Database S1.

Discussion

The chambers created a drier and warmer microclimate in the organic and topsoil layers, which are the conditions projected for mountain regions such as the Australian Alps (Sánchez-Bayo and Green, 2013), the Mediterranean mountains (Giorgi and Lionello, 2008) and the Andes (Masiokas et al., 2020). Average soil surface temperatures inside the chambers are within the threshold of mid-century low and intermediate greenhouse gas concentrations and global warming predictions (Representative Concentration Pathways, RCPs 2.6 and 4.5) for high-mountain areas (Hock et al., 2019) and a high concentration (RCP8.5) for global surface (IPCC, 2021).

Overall, the chambers reduced soil VWC in a similar manner reported for traditional rain-out shelters (Yahdjian and Sala, 2002; Kundel et al., 2018; Alon and Sternberg, 2019) but provided a greater reduction in VWC than a hexagonal OTC in the same study region (Table 3). Importantly, VWC in our chambered plots was always below that of the ambient control plots, even after rain events and during the peaks of the dry and wet seasons (Fig. 2). This constant effect on soil moisture in chambered plots could be explained by the combined effect of less precipitation (rain-out structures) and greater evaporation (warmer temperatures) inside the chambers.

The daily and seasonal fluctuations in mean air temperature inside the chambers were similar to those reported for cone-shaped OTCs in the Arctic (Marion et al., 1997) and other OTCs in similar environments (Schmidt et al., 2002; Tercero-Bucardo et al., 2007; Grau et al., 2013; Bernareggi et al., 2015; Welshofer et al., 2018). The colder night air temperatures inside the chamber are a common feature of these instruments (Marion et al., 1997; Hollister et al., 2022). The observed variations in warming, influenced by sky conditions and weather, coincide with those reported for chambers utilized by the ITEX network (Hollister et al., 2022). It has been suggested that this variability might provide a more accurate representation of future climate change compared to methods that apply a constant temperature increase (Hollister et al., 2022).

The chambers increased soil surface and soil temperatures similarly to a tall hexagonal OTC deployed in an alpine region (Wang et al., 2018) but increased soil temperatures more than a hexagonal OTC in the study region (Camac et al., 2017) and a tetragonal OTC in a similar environment (Bernareggi et al., 2016). It is important to mention that, in our study, the soil surface and soil temperature remained significantly warmer during the night inside the chambers for most of our study period, in

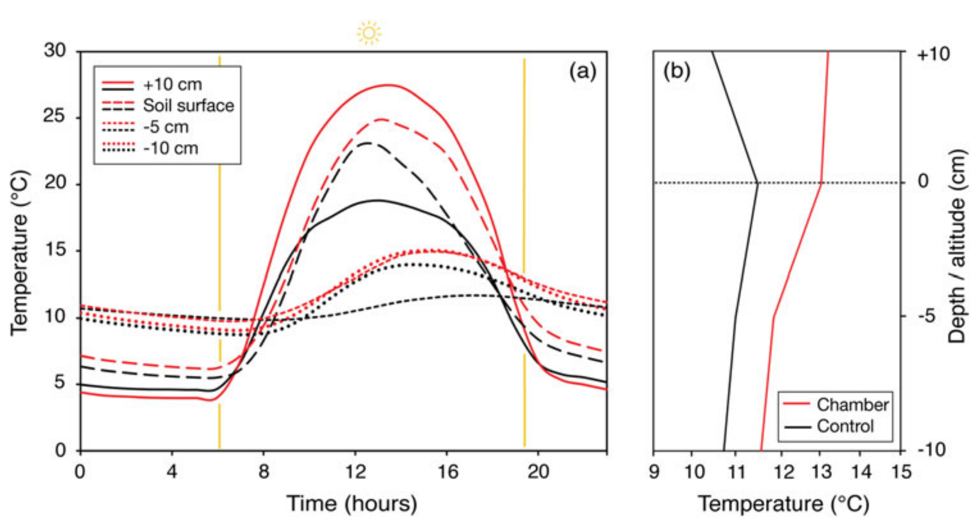

Figure 4. Daily fluctuations in the mean hourly air and soil temperatures (A) and mean temperature profile (B) for chambered and control plots during spring–summer–autumn.

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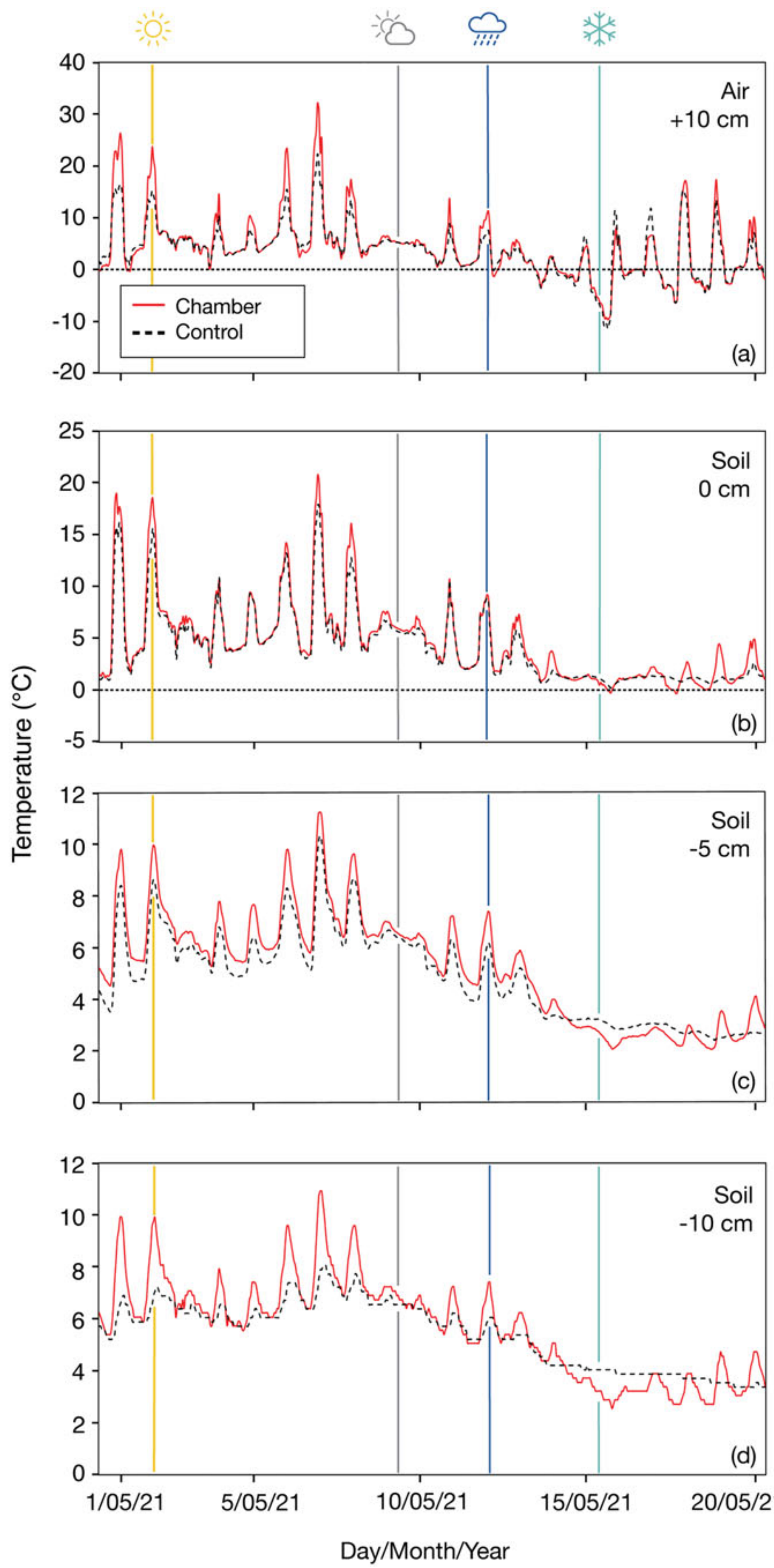

Figure 5. Mean hourly temperatures at (A) +10 cm in the air, (B) soil surface, (C) −5 cm below the soil surface and (D) −10 cm below the soil surface for control and chambered plots in late autumn (1–20th of May 2021) at the study site. Weather conditions for the days displayed are indicated by vertical-coloured lines. Yellow, sunny days; grey, overcast days; blue, rainfall; green, snowfall.

Table 3 Effects of the tested OTC, a traditional ITEX hexagonal OTC and rain-out shelter on the soil volumetric water content, air temperature (+10 cm) and soil temperature (−5 cm) in the same study region (Bogong High Plains, Victoria, Australia)

Experimental tool	Mean effect on volumetric water content at −10 cm (m^3/m^3)	Mean effect on air temperature at +10 cm (°C)	Mean effect on soil temperature at −5 cm (°C)
Modified OTC[a]	−0.029	+1.6	+0.9
Rain-out shelter (60% restriction)[b]	−0.027	–	+0.3
Hexagonal OTC[c]	−0.01	+0.9	+0.9

[a]This study (11/2020 to 10/2021).
[b]Unpublished data Australian Mountain Research Facility: www.amrf.org.au (01/2022 to 05/2022).
[c]Camac et al. (2017) (03/2010 to 05/2016).

contrast with the mentioned studies, where the soil night temperatures were commonly colder than control plots. This is a valuable feature of the presented design because night temperatures are also expected to increase as a consequence of climate change, which will have particular effects on plant life cycles (e.g., Hänninen and Tanino, 2011).

To conclude, our modified OTC worked well during *in situ* seed and seedling ecology studies in cold mountain regions. The design increased air and soil temperatures and decreased soil VWC, with the drier and warmer microclimate created by the chambers matching future climate projections for several mountain regions around the globe. The design presented is simple, inexpensive, requires minimal maintenance and provides an effective experimental tool to help increase our understanding of the potential response of seeds and seedlings to the combined effects of warming temperatures and decreasing precipitation.

Table 4 Recommendations for the use of the presented OTC design during seed ecology studies

Research step	Recommendation
Artifact	
Construction	Step-by-step instructions are given in Supplementary Fig. S1
Transporting	*Vehicle*: flattened chambers are easier to transport *In the field*: chambers can be rolled up and tied with rope
Installation	Recommendations for installing the chamber in the field can be found in Supplementary Fig. S1. Critical issues to consider during installation are as follows: • *Water lateral flow*: trench around the plot or bury an impermeable barrier, like a garden edging, to hydrologically isolate the plot. • *Condensation*: trim the vegetation inside the chamber but outside the experimental plot (no plants touching the chamber walls) to avoid condensation. • *Air circulation*: leave 2–3 cm between the chamber and the soil surface to increase air circulation.
Maintenance	The tested chambers were used for over 2 years and remained in good condition. The lifetime of the chambers will depend on local weather conditions and the quality of material used for their construction.
Experimental design	
Sites and plots	When choosing the location of experimental plots and sites, consider factors such as soil type, light availability and natural moisture levels that may affect the OTC performance, and thus, seeds and seedlings.
Randomization	Randomly assign treatments to the established plots (e.g., which ones will have a chamber).
Replication	Avoid pseudo-replication. You can bury several seeds of the same species within each chambered plot, but each chamber is a replicate. You will need at least three chambers per site. Perform a power analysis.
Control	Establish appropriate control plots to account for natural variation in the response variable.
Adding factors	Not only temperature and water availability are changing. Multiple environmental factors can be tested in combination with the chambers (e.g., fire and nutrient availability).
Environmental monitoring	
Air temperature	If the experiment only involves seeds, measuring air temperatures may not be necessary and is difficult to measure correctly without expensive equipment. If investigating seedling responses, use thermocouples attached to the seedling leaves.
Soil temperature	Measure during the whole study period as chambers directly affect soil temperatures. Ideally, measure at the soil surface and the same depth that seeds have been buried. Waterproof data loggers (such as iButtons) by wrapping them in parafilm, self-fusing silicone tape and using air-sealed bags.
Soil moisture	As for soil temperature, measure soil moisture during the complete study period. Try to measure parameters that show the available water to plants. Otherwise, soil moisture (%) or volumetric water content (m/m) can be measured, but results must be carefully interpreted and preferably calibrated against gravimetric methods.
Other variables	Consider that any artifact, including the OTCs, may affect other environmental factors which might influence seed germination or seedling establishment (e.g., wind, light and nutrient availability). Try to measure these.

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General recommendations for using the design during seed ecology experiments

General recommendations for the use of the OTC design here presented are summarized in Table 4. Furthermore, we recommend reading about the advantages and disadvantages of using passive warming methods (Marion et al., 1997; Hollister et al., 2022), the challenges and complexity of manipulative experiments to study global warming (Beier et al., 2004; Knapp et al., 2018; Korell et al., 2019) and how the use of OTC can be complemented with other types of methods to better understand the responses of seeds and seedlings to climate change (Yang et al., 2018). Changes in the final proportion of germinated seeds and germination time can be expected as an effect of the drier and warmer microclimate created by the chamber.

Limitations of the study and OTC design

As the presented chamber is designed to be used during seed and seedling ecology manipulative studies, we focused on the effects of the design on the organic and topsoil layers where temperature and moisture are relevant for seeds and seedlings. We did not measure the temperature or humidity/moisture above or below 10 cm from the soil surface, where the effects of the chamber could be enhanced (air) or diminished (soil). If researchers wish to establish a long-term study to understand the impacts of climate change on plant communities (e.g., diversity or composition), we suggest using the traditional hexagonal OTC or rain-out shelters, which are commonly used in global monitoring efforts such as ITEX or Drought-Net.

Regarding the edge effect reported for other OTC designs (Marion et al., 1997), thermal images from our chambered plots show an even effect on the soil surface temperature, probably due to the small size of the chamber and the uniformity of the ground cover at our study site (Supplementary Fig. S2). However, we did not measure if the chamber created an edge effect on the soil VWC and this should be considered when using the presented design or any other OTC during seed ecology experiments.

Finally, the performance of the chambers appears to be ideal for use in cold-climate regions, where mean maximum ambient temperatures do not exceed 25 °C. Temperatures higher than this will likely overheat the air inside the chambers around noon, one of the major undesirable effects of passive temperature-enhancing instruments (Marion et al., 1997). Should researchers wish to use our chamber design in more benign, temperate ecosystems, the chambers could be suspended higher off the ground (using the threaded rod in the middle to elevate them and securing them to the ground with large pegs) or adding holes to increase air circulation inside the chamber and allow the excess heat to dissipate. However, it is important to mention that we did not test how these modifications may affect our design's overall performance.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S096025852400014X.

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Author contributions. J.V.-R. and S.E.V. conceived the idea and designed the methods. J.V.-R. and S.E.V. established the field experiment. J.V.-R. collected the data. J.V.-R. analysed the data. J.V.-R. and S.E.V. wrote the manuscript. Both authors contributed critically to the drafts and gave final approval for publication.

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Competing interests. The authors declare no conflict of interest.

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