

## ORIGINAL ARTICLE OPEN ACCESS

# Climate Change Impacts on Diapause Outcomes in *Bombus terrestris* Across an Environmental Gradient

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## ABSTRACT

Most temperate insects survive winter by entering a period of dormancy called diapause. Some of the greatest climate change impacts are likely to occur during this period, as entire populations of some species are represented by a relatively small number of hibernating individuals. Bumblebee populations are particularly vulnerable because only queens overwinter and any decline in the winter survival of queens, or reduced post-diapause fitness, could have significant consequences on population dynamics the following spring. This study investigated the impact of different overwintering conditions on *Bombus terrestris* queens across an altitudinal gradient in the German Alps. Snow pack coverage was manipulated to simulate advanced snow melt and delayed snow melt, and maintained for current/control conditions. We found that changes in snow pack and altitude affect the level and variability of soil temperature across the winter season but that these have no effect on hibernation survival (very high in all cases) or subsequent colony establishment (very low in all cases). However, queen weight loss during diapause did differ across altitudes, with bumblebees hibernating at lower levels and being exposed to higher and more widely fluctuating temperatures, losing significantly more weight. Our results therefore confirm the potential for negative physiological impacts of exposure to higher and more variable winter temperatures during diapause, as is increasingly common under climate change. But we also find that bumblebees have some resilience to these effects, at least in terms of hibernation survival, within the range experienced in our experiments. We conclude that further field studies targeted at the range of conditions likely to be experienced by European insects over winter are necessary to anticipate potential population impacts, but that existing levels of climate change are likely sufficient to affect many of these insects, even if at sub-lethal levels.

## 1 | Introduction

Bumblebees are among the most important pollinators in natural and agricultural ecosystems and are often considered keystone species (Goulson 2010). However, over the last few decades, many bumblebee species have suffered substantial range declines throughout the Northern Hemisphere. Nearly half of the 68 species recorded in Europe have decreasing

population trends (Potts et al. 2015), and 26% of European and North American species are listed as threatened or endangered (Cameron and Sadd 2020). Multiple interacting pressures threaten bumblebees, most significantly land use intensification and human-induced climate change (Goulson et al. 2015; Settele et al. 2016; Prestele et al. 2021). The warming climate is causing bumblebees to experience rapid losses from their southern range limits, as well as lagging range expansions to the north and to

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higher elevations (Kerr et al. 2015; Biella et al. 2017). Local extinctions are occurring near the edges of climatic ranges, and species are losing ground everywhere except their climatic optimums (Williams et al. 2007; Casey et al. 2015). These losses have been linked to the increasing frequency of temperatures that exceed species' upper thermal limits (Soroye et al. 2020; Feuerborn et al. 2023). Continuing climate change in Europe is predicted to have severe consequences for bumblebees, with up to 25 species losing nearly all of their climatically suitable range by 2100 (Rasmont et al. 2015) and 38%–76% of studied species currently classified as 'Least Concern' losing at least 30% of ecologically suitable territory by 2061–2080 (Ghisbain et al. 2023).

Winter months in the Northern Hemisphere are experiencing the most rapid climate change, and this is when many insects, including bumblebees, are going through diapause (Marshall et al. 2020). Despite increasing research into the mechanisms behind the negative impacts of warming temperatures on bumblebees, little is known about the direct impacts on overwintering, which is a key stage in the bumblebee's annual life cycle (Woodard 2017). It is only new queens that survive over winter in diapause and emerge to found new colonies in the spring (Alford 1975), and so the survival and fitness of queens have a disproportionate effect on bumblebee population dynamics and pollination services, as the loss of a single queen during or just after diapause prevents the establishment of an entire colony with up to hundreds of workers and new reproductives. Although it has recently been found that survival rates in the field can be high (Pugsek et al. 2023), diapause is still an extremely important 'pinch-point' in the bumblebee life cycle. It is not yet understood how climate change affects survival during this time (Maebe et al. 2021).

In insects, fat reserves are the most important energy source during diapause (Hahn and Denlinger 2007). In the pre-overwintering period, young bumblebee queens sequester lipids and glycogen from pollen and nectar in the 'fat body' that they then metabolise during diapause (Alford 1969; Votavová et al. 2015). Their survival depends on having good reserves because they can, for example, deplete up to 80% of their fat body lipids during diapause (Alford 1969) and lose on average 28% of body mass (Treanore and Amsalem 2020). It has also been found that survival is positively associated with the pollen and nectar availability and queen weight before the onset of diapause (Beekman et al. 1998; Woodard et al. 2019; Treanore and Amsalem 2020). The metabolism of the fat body occurs faster at higher temperatures and laboratory studies suggest that warmer winter temperatures may reduce the survival of queens during and after diapause as a result (Vesterlund et al. 2014; Vesterlund and Sorvari 2014). However, effects under realistic field conditions are not known and may well be different due to temperature variations, alongside other environmental factors such as humidity and potentially ground flooding (Rondeau and Raine 2024). In fact, fluctuating thermal regimes (FTR) are known to prolong diapause in several species of solitary bee (Colinet et al. 2018). Findings are so far species-specific in bumblebees, with FTR increasing hibernation survival for the commercial *B. impatiens* but the wild species *B. huntii* and *B. vosnesenskii* faring better with constant low temperatures (Lindsay et al. 2024). For many European bumblebees, effects are likely to be determined by the presence, size and variation of

snow packs over the winter months, as these mediate the effects of air temperature fluctuations on queens overwintering in the soil (Bale and Hayward 2010).

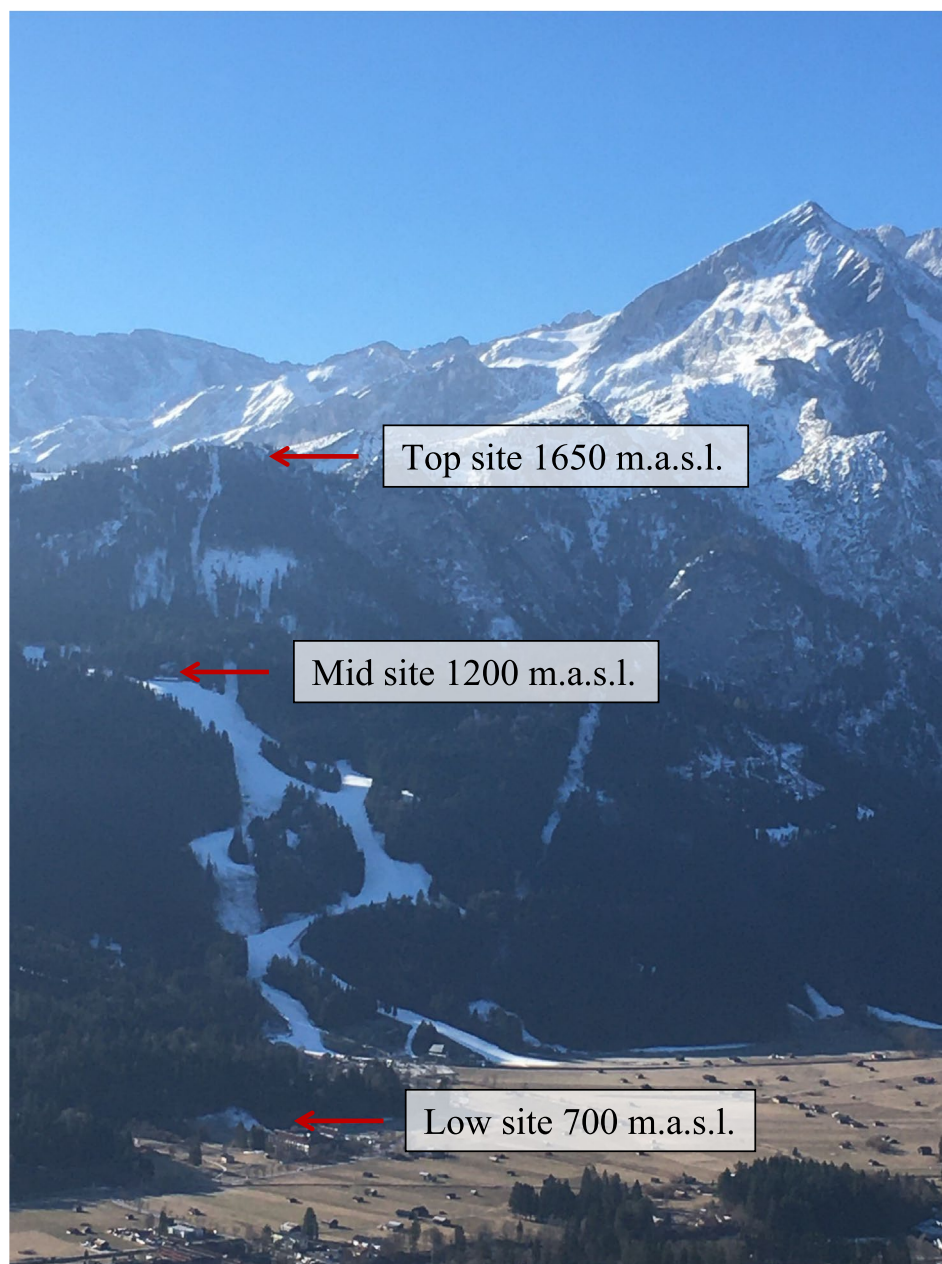
To study the effects of climate change, environmental gradients are widely used as model systems. Altitudinal gradients have advantages over latitudinal gradients for such studies, as they provide variation in abiotic factors within short distances without the confounding effects of, for example, varying biogeography and day length (Hodkinson 2005). Altitudinal gradients can therefore be used to experimentally investigate the interactive direct and indirect effects of climate change, such as occur with variations in snow pack dynamics over a winter season (Hoiss et al. 2015). The Alpine regions of Europe, which provide ideal study locations for altitudinal gradient research, are particularly vulnerable to climate change and are already experiencing higher-than-average temperatures and earlier snowmelt (Gobiet et al. 2014). This is predicted to continue over the following decades and snow cover will be particularly affected by projected climatic changes, with widespread declines except at very high elevations (Kotlarski et al. 2023). Loss of winter snow cover can be costly for overwintering insects as it risks exposure to more extreme air temperatures, increases the frequency of freeze–thaw cycles, and can result in ice encasement (Bale and Hayward 2010; Williams et al. 2015), all processes that are difficult to replicate accurately in the laboratory.

This study aims to fill this knowledge gap by investigating the impact of different climatic conditions and associated micro-habitat temperature profiles on overwintering *Bombus terrestris* queens, under field conditions. Overwintering occurred at three different altitudes, and during two winter seasons, using three snow-pack scenarios: (i) 'historic'/reduced snow melt, (ii) 'current'/control conditions and (iii) 'future'/increased snow melt. These are intended to capture the long-term trend of declining snow cover in the Alps, with 'historic' cover being a more persistent snow pack and 'future' cover being a reduced snow pack for shorter durations (Gobiet et al. 2014). Overwintering survival, changes in body mass (second winter only) and post-winter colony establishment were assessed. Survival was hypothesised to be low across all treatments but this was disproved during the first winter, allowing for a longer overwintering period to be assessed during the second winter.

## 2 | Methods

### 2.1 | Field Sites

Field work was conducted on northerly facing slopes in the Wetterstein mountains (47°25'0" N, 11°8'0" E), in the Northern Limestone Alps, close to Garmisch-Partenkirchen in Southern Germany. This area is predicted to be heavily affected by future climate change, with a decline in the volume of snow and snow cover days (Weber et al. 2016). Study sites spanned an altitudinal gradient of 700–1650 m above sea level (m.a.s.l.), with three treatment locations at 700, 1200 and 1650 m.a.s.l., respectively, in mixed woodland (Figure 1). At each elevation, three 2 m x 2 m areas were used to establish different snow melt conditions: increased snowmelt (snow was removed after each snowfall), reduced snowmelt (snow was added after each



**FIGURE 1** | Field sites in the Wetterstein mountains, Garmisch-Partenkirchen.

snowfall) and ambient (snow was left undisturbed) (Table 1). Each treatment area was at least 5 m separate from the others. The same sites were used in two winter field seasons (2020–21 and 2021–22).

## 2.2 | Establishing Diapausing Bumblebee Queens

In October 2020, 14 commercial colonies of *Bombus terrestris dalmatinus* were purchased from Biobest (Belgium). As soon as queens and males emerged, they were removed from the maternal colony and housed in single-sex sibling groups before being mated in large mesh-sided flight cages between 22 and 26 November 2020 (Whitehorn et al. 2009a). After mating, the queens were housed in sister groups of up to three individuals in small plastic boxes with sugar water (Biogluc, Biobest) provided

*ad libitum* (Figure 2A). After 48 h, queens were transferred to hair curlers (25 mm x 63 mm, Haarfrizten, Germany), loosely sealed at each end with cotton wool, and randomly assigned to a treatment group while ensuring siblings were equally distributed among treatments (Figure 2B). Each hair curler was labelled with queen id to ensure a record of the maternal colony of each queen. Queens were then stored for 24 h at 7°C in constant darkness (DD) to allow them to enter diapause ahead of being transferred to field locations.

In October 2021, 330 mated *B. terrestris* queens were purchased directly from Biobest. The queens were dispatched immediately after mating and arrived in ten boxes, supplied with nectar and pollen *ad libitum*, with 33 sibling queens in each box. After arrival, the queens were stored for 24 h in a cool room at 7°C, after which they were weighed, placed in hair curlers and randomly



**TABLE 1** | Experimental design showing the altitude and snow conditions of the treatments and the number of bumblebee queens in each. Within each altitudinal group, the 'control snow' treatment was left undisturbed, whilst the 'advanced' treatment had snow removed after each snowfall (future climate scenario), which was then transferred to the 'delayed' (historic climate scenario) treatment.

Altitude	Snow treatment	Location	Number of bumblebees	
			Winter 20/21	Winter 21/22
Control	N/A	Indoors	33	30
	Advanced	700 m.a.s.l.	33	33
Low	Control	700 m.a.s.l.	33	33
	Delayed	700 m.a.s.l.	33	33
	Advanced	1200 m.a.s.l.	33	33
Mid	Control	1200 m.a.s.l.	33	33
	Delayed	1200 m.a.s.l.	33	33
	Advanced	1650 m.a.s.l.	33	33
Top	Control	1650 m.a.s.l.	33	33
	Delayed	1650 m.a.s.l.	33	33

assigned to a treatment group as before, ensuring siblings were equally distributed among treatments. Again, each was labelled to ensure a record of the maternal colony of each queen.

## 2.3 | Hibernation

Once queens had been assigned to a treatment group, with 33 queens in each group, they were placed together in a wooden box (34×25.3×5.3 cm, plywood thickness 3 mm, Creative Deco, UK) for hibernation (Figure 2B). The wooden boxes had been previously treated with beeswax (a light coating on outer surfaces) and lined with a mix of dampened sand and compost. These boxes were then closed and transferred to the different altitudes and snow treatments (Table 1), with one box remaining indoors as a control. This control was stored in the cool room at 7°C and constant darkness for the first winter, and in the second winter the control box was transferred to a fridge after 2 weeks in the cool room, and thereafter maintained at 4°C in constant darkness. The boxes for the nine other treatments were transferred to the field sites on 1 December 2020 and on 30 October 2021. At each location, the boxes were dug into the ground, with the top of the box 10 cm below the surface of the soil, and then re-covered with soil and leaf litter. A temperature data logger (HOBO Pendant MX Temp, Onset, US) was placed next to each box in the soil, which recorded the temperature every hour over the hibernation period.

Sites were checked weekly and accessed by foot and ski (made substantially easier in the second winter when COVID-19 restrictions ended and nearby cable cars were in operation). Snow depth was measured with a metre ruler and, if any additional snow had fallen, it was removed from the 'advanced snowmelt' sites and added to the 'delayed snowmelt' sites (methods adapted from Hoiss

et al. 2015). Snow was dug from a 2 m x 2 m square above where the box was buried and the 'advanced snowmelt' sites were left with no snow covering. The control sites were left undisturbed.

## 2.4 | Post-Hibernation

The first overwinter period ended on 17 February 2021 after 78 days and the second overwinter period ended on 18 March 2022 after 145 days. Both diapause durations are within the natural range for *Bombus terrestris*, with the second period being more realistic, particularly for this Alpine region (Rasmont et al. 2008). The boxes of queens were then dug up (Figure 2C,D) and queens were placed in individual plastic boxes and maintained under laboratory conditions (25°C, 50%–60% relative humidity, DD). Additionally, in 2022, the queens were weighed again post-hibernation to provide a measure of weight change. Hibernation survival was recorded as queens that were still mobile 72 h post-diapause, as those that were not were unlikely to have survived under natural conditions (Gerloff and Schmid-Hempel 2005). Queens were supplied with sugar water (Biogluc, Biobest) provided *ad libitum* and pollen balls (ground fresh frozen organic pollen (ImkerPur, Germany) mixed with Biogluc) three times a week. Colony foundation was recorded as queens that were able to successfully rear at least one offspring to adulthood.

## 2.5 | Statistical Analysis

Data were analysed in R, version 4.2.2 (2014 The R Foundation for Statistical Computing). Preliminary analyses showed that hibernation survival was not affected by the different treatments (it was universally high) and so no further analysis was carried out on this variable. A generalised linear model with binomial distribution was used to analyse colony foundation success, with each overwintering period analysed separately. Snow treatment was nested within altitude, and maternal colony was an additional factor. Pairwise comparisons of estimated marginal means among significant treatments were made using the *emmeans* package (Lenth, 2021), fitting the p-values with Tukey's test. Visual inspection of residual plots did not reveal any deviations from normality.

The change (loss) in weight of queens before and after the second winter was analysed with a nested ANOVA, with snow treatment nested within altitude and maternal colony as an additional factor. Visual inspection of residual plots did not reveal any deviations from normality. Pairwise differences between factor means were investigated using Tukey's post hoc tests.

## 3 | Results

### 3.1 | Environmental Data

During the first winter of 2020/2021, the snow treatments resulted in distinct but overlapping temperature regimes for the hibernating queens (Figure 3A). The 'advanced' snow treatments, where snow cover was removed, resulted in lower and more variable temperatures in the soil for the queens hibernating at the top and middle sites (Table 2, Figure 3A). However, the



**FIGURE 2** | (A) Bumblebee queens post-mating, pre-hibernation; (B) Queens in hair curlers ready for hibernation; (C) Post-hibernation; (D) Queen emerging from hibernation.

same ‘advanced’ treatment resulted in higher temperatures for the queens hibernating at the lowest site (Table 2, Figure 3A).

During the second winter of 2021/2022, the snow treatments resulted in varying temperature regimes that followed a similar pattern to the previous year (Figure 3B). The advanced treatment at the mid and top sites had the lowest temperatures and the advanced treatment at the bottom site had the warmest temperatures (Table 2). However, the temperature range experienced was broader, and the low-control site was particularly variable.

There was a consistent pattern of soil microhabitat temperatures across the altitudinal gradient studied, with the low-altitude sites consistently experiencing higher mean soil temperatures and a greater range of temperatures, with higher maximums than high-altitude sites.

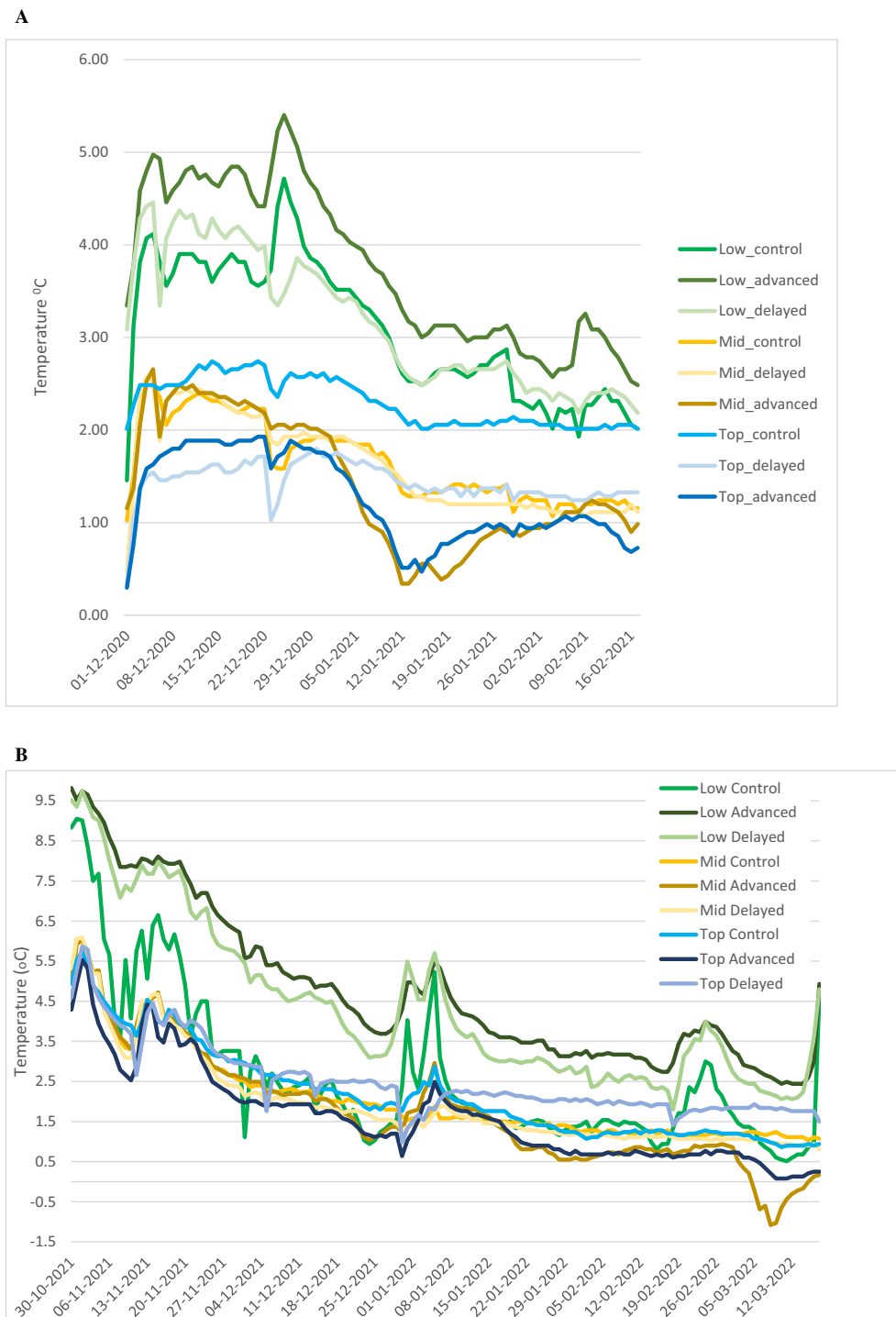
### 3.2 | Survival

During the first winter of 2020/2021, despite the varying temperatures, there was a survival rate of 90%, with a total of 300 out of the 330 queens surviving hibernation, and there was no difference in this survival rate among treatments (Table 3).

During the second winter of 2021/2022, treatment had no effect at all on hibernation survival, as 100% of queens survived in the field. However, five out of the 30 queens hibernating indoors did not survive (Table 3).

### 3.3 | Colony Foundation

Unfortunately, colony foundation success was low, with only 23 queens across both years rearing at least one offspring until adulthood (14 in the first winter and 9 in the second; Table 3). In the first winter, the snow treatment and maternal colony had no effect on the number of queens founding colonies ( $\chi^2=10.49$ ,  $df=6$ ,  $p\text{-value}=0.11$  and  $\chi^2=15.63$ ,  $df=10$ ,  $p\text{-value}=0.11$  respectively). Altitude had a marginally significant effect on colony foundation ( $\chi^2=6.09$ ,  $df=2$ ,  $p\text{-value}=0.048$ ), with those hibernating at the mid-altitude more likely to find a colony, but pairwise comparisons indicated that this was not significantly different to the low and high altitude treatments ( $p=0.116$  and  $p=0.204$  respectively). In the second winter, no factor had a significant effect on colony foundation success ( $p\text{-value}=0.283$  for maternal colony,  $p\text{-value}=0.350$  for snow treatment and  $p\text{-value}=0.098$  for altitude, respectively).



**FIGURE 3** | (A) Soil temperatures experienced by hibernating queens at each of the field sites during the first winter of 2020–2021. Temperatures are those collected at hourly intervals by HOBO Pendant data loggers. (B) Soil temperatures experienced by hibernating queens at each of the field sites during the second winter of 2021–2022. Temperatures are those collected at hourly intervals by HOBO Pendant data loggers.

### 3.4 | Queen Weight Change

The change in mass of queens during diapause in the second winter (2021–2022) was not affected by the snow treatment within each altitudinal site ( $F_{6,279} = 1.152$ ,  $p = 0.332$ ), but it was significantly affected by altitude and maternal colony ( $F_{2,285} = 29.368$ ,  $p < 0.001$  and  $F_{9,285} = 9.656$ ,  $p < 0.001$  respectively). A Tukey post

hoc test revealed that the queens hibernated at the lowest altitude lost on average 0.033 g (95% CI 0.021–0.044 g) and 0.032 g (95% CI 0.020–0.043 g) more weight than the middle and top sites respectively (Figure 4). Mean winter soil temperature at the low site was 3.86°C (range 0.38°C–9.82°C) compared to 1.91 and 2.10 (–1.55–6.09 and 0.00°C–5.96°C) at the middle and top sites, respectively.

**TABLE 2** | Mean and range of soil temperatures (°C) at each location during both winters.

	Winter 2020/2021		Winter 2021/2022	
	Mean temp	Range (min–max)	Mean temp	Range (min–max)
Low control	3.10	1.41–4.76	2.55	0.38–9.18
Low advanced	3.76	2.44–5.40	4.74	2.36–9.82
Low delayed	3.17	2.10–4.50	4.30	1.67–9.78
Mid control	1.67	0.94–2.57	2.07	1.03–6.05
Mid advanced	1.42	0.25–2.66	1.74	–1.55–6.09
Mid delayed	1.65	0.47–2.61	1.93	0.81–6.09
Top control	2.30	1.97–2.79	2.18	0.86–5.83
Top advanced	1.26	0.30–1.93	1.61	0.00–5.57
Top delayed	1.45	0.34–1.80	2.51	0.81–5.96

**TABLE 3** | Numbers of queens in each treatment, that survived hibernation and that founded a colony.

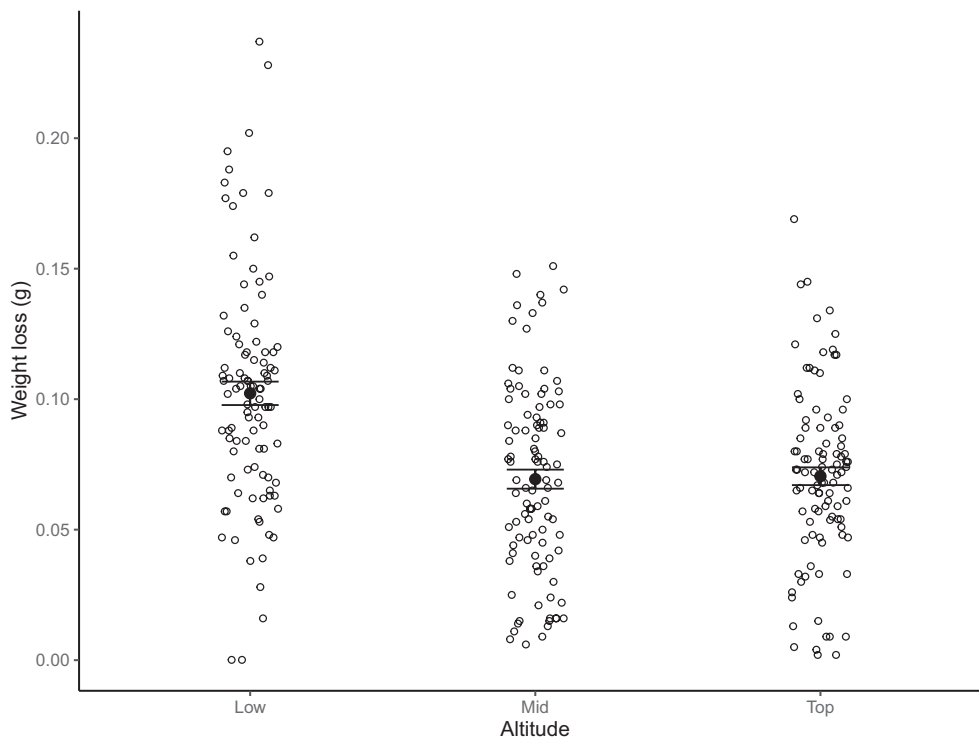
Altitude	Snow treatment	Winter	Total	Survived hibernation	Colony foundation
Control	N/A	20/21	33	29	0
	Advanced	20/21	33	29	2
Low	Control	20/21	33	30	0
	Delayed	20/21	33	31	0
	Advanced	20/21	33	31	5
Mid	Control	20/21	33	28	3
	Delayed	20/21	33	30	1
	Advanced	20/21	33	30	1
Top	Control	20/21	33	29	2
	Delayed	20/21	33	33	0
Control	N/A	21/22	30	25	0
	Advanced	21/22	33	33	2
Low	Control	21/22	33	33	0
	Delayed	21/22	33	33	0
	Advanced	21/22	33	33	2
Mid	Control	21/22	33	33	2
	Delayed	21/22	33	33	2
	Advanced	21/22	33	33	0
Top	Control	21/22	33	33	1
	Delayed	21/22	33	33	0
			657	622	23

## 4 | Discussion

Winter in temperate ecosystems imposes strong selection pressure on many organisms, determining fitness and

shaping evolution (Williams et al. 2015). Climate change is occurring relatively fast during the winter months, altering the mean and variability of air temperatures and reducing the extent and persistence of the snow cover that would otherwise





**FIGURE 4** | Weight (g) lost by hibernating queens during the 2021–2022 winter. Queens hibernating at the low altitude site lost significantly more weight than those hibernating at the middle and top sites ( $F_{2,285} = 29.368$ ,  $p < 0.001$ ).

insulate overwintering insects from temperature fluctuations. These abiotic variables are key drivers of biological responses to winter, so understanding how they impact the fitness of different species is key to anticipating climate impacts on species survival and potential evolutionary responses (Marshall et al. 2020).

This research offers insight into the potential mechanism through which winter climate change could impact the fitness of bumblebees during and after overwintering. Although the different treatments had no significant impact on survival, we found that the queens hibernating at the lowest altitude, with warmer and more variable soil temperatures, lost more weight than the queens hibernating at higher altitudes, with colder and more stable temperatures. Previous research has found that an increase in energy expenditure during diapause causes a decrease in fat body, body weight and hence winter survival in solitary bees (Bosch and Kemp 2004; Sgolastra et al. 2011; CaraDonna et al. 2018). Our results are in partial agreement with these findings and suggest that warmer environmental conditions during diapause can negatively impact a queen's condition, which then may have knock-on effects on a colony establishment and daughter queen production (Yoon et al. 2004).

Nevertheless, the survival rates of queens in our experiment were notably high, which is in concordance with the study of Pugesek et al. (2023), who found high survival of *Bombus impatiens* queens hibernating under field conditions in the US. Field mortality only occurred in the first year of our experiment, when bees were subject to a more dramatic temperature change at the start of hibernation. However, our finding contrasts with many laboratory studies that have found survival rates of queens to be very low (e.g., Gosterit and Gurel 2009), and indeed, the only

mortality we recorded in the second winter was in laboratory conditions, perhaps due to lower humidity. Our findings might therefore suggest an advantage to overwintering under field conditions, and the method used in this experiment for overwintering could therefore be used to enhance the captive rearing of bumblebees for the purposes of research or conservation. The materials required are inexpensive and readily available online or from high street stores.

The high survival could also be explained, however, by the relatively high weight of all queens entering diapause; the mean weight of queens prior to diapause in the second winter was 1.09 g (range 0.79–1.32 g). The queens were fed ad libitum pre-diapause and were therefore in good condition, and all exceeded the minimum weight of 0.6 g that other studies have found necessary to survive diapause (Beekman et al. 1998). Pre-diapause diet is known to be an important factor, with pollen and nectar limitations reducing survival (Woodard et al. 2019). Additionally, the queens in the second winter had access to pollen as well as nectar, which could explain the even higher survival rate compared to the first winter, when the queens were only fed nectar prior to diapause. The pollen would have provided protein, and a high protein-to-lipid ratio has been shown to significantly improve survival (Treanore et al. 2023).

It is important to note that the wooden boxes used for containing the overwintering queens could have buffered against the most extreme temperature fluctuations. The thin plywood would not have provided significant insulation but could have created a microclimate within the box that was not as extreme as the surrounding soil temperatures. If this method is replicated in the future, it would be valuable to place a temperature data logger within the box, as well as in the adjacent soil.



The low colony foundation success was possibly due to suboptimal laboratory-rearing conditions. Post-hibernation, queens were housed individually in plastic boxes and kept in constant darkness in a rearing room following methods previously found successful (Whitehorn et al. 2009b). Unfortunately, Covid restrictions made the management of this rearing room challenging and humidity was more variable than desired, and this likely had an impact on the queen colony foundation.

The effects of warmer overwintering temperatures found during the course of this experiment may be amplified in other bumblebee species that are more adapted to even colder climates. *Bombus terrestris* is a widespread species across Europe, with a distribution extending from temperate to Mediterranean regions (Rasmont et al. 2008). The subspecies used in this experiment was *Bombus terrestris dalmatinus*, which originates from the eastern Mediterranean region and has been used extensively for commercial rearing (Velthuis and Van Doorn 2006), but this species is known to have comparable cold tolerance to the more temperate subspecies *B. t. audax* in the United Kingdom (Owen et al. 2013, 2016). Research has shown that the males of *Bombus terrestris* are relatively tolerant of heat stress compared to other species, which is likely because of their evolution in warmer climates (Martinet et al. 2021). Other bumblebee species, particularly those adapted to montane, boreal and polar or tundra climates, such as *Bombus polaris*, *Bombus jonellus* and *Bombus hyperboreus*, are less tolerant of heat stress (Martinet et al. 2021) and it is probable that hibernating queens of these species will also be more vulnerable to higher temperatures.

Climate change is likely to have increasingly negative impacts on bumblebees. In the face of this ongoing change, we can help our wild bumblebees by ensuring a good supply of forage into late summer and early autumn, so that queens enter diapause in as good nutritional condition as possible and have a better chance of survival. Queens spend their early life feeding within the natal nest, and this is an important period for nutrient sequestration (Woodard et al. 2019). The pollen and nectar that they consume are collected by their sister workers, so sufficient floral resources within the 9–10 km foraging range around nests are important for the long-term survival of queens (Woodard et al. 2015, 2019). This can be achieved by increasing late summer pollen and nectar availability, particularly in agricultural landscapes, with late-flowering cover crops, such as red clover, and mowing regimes that delay flowering of field margins until September (Timberlake et al. 2021). Other autumn flowering plants, such as ivy, *Hedera helix*, can also play an important role (Lam et al. 2024). Further research is required to establish whether this increased resource provision would directly enhance hibernation survival through improved nutrition and fat reserves.

In conclusion, our results confirm the potential for negative physiological impacts of exposure to higher and more variable winter temperatures during diapause, as is increasingly common under climate change. But we also find that bumblebees have some resilience to these effects, at least in terms of overwintering survival, within the range experienced in our experiments. This research contributes to the knowledge base combining physiological and ecological knowledge to provide meaningful contributions to wild bee conservation-based research (Leroy et al. 2023; Woodard 2017). We conclude that

further field studies to investigate a wider range of conditions likely to be experienced by European insects in diapause are necessary to anticipate potential population impacts, but that existing levels of climate change are likely sufficient to affect many of these insects, even if at sub-lethal levels.

## Author Contributions

**Penelope R. Whitehorn:** conceptualization, methodology, investigation, formal analysis, funding acquisition, writing – original draft, writing – review and editing. **Romy Rehschuh:** investigation, writing – review and editing. **Stephanie Rehschuh:** investigation, writing – review and editing. **Scott A. L. Hayward:** conceptualization, writing – review and editing. **Calum Brown:** investigation, writing – review and editing.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data is available on Dryad: <https://doi.org/10.5061/dryad.5mkkw7h7fx>.

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