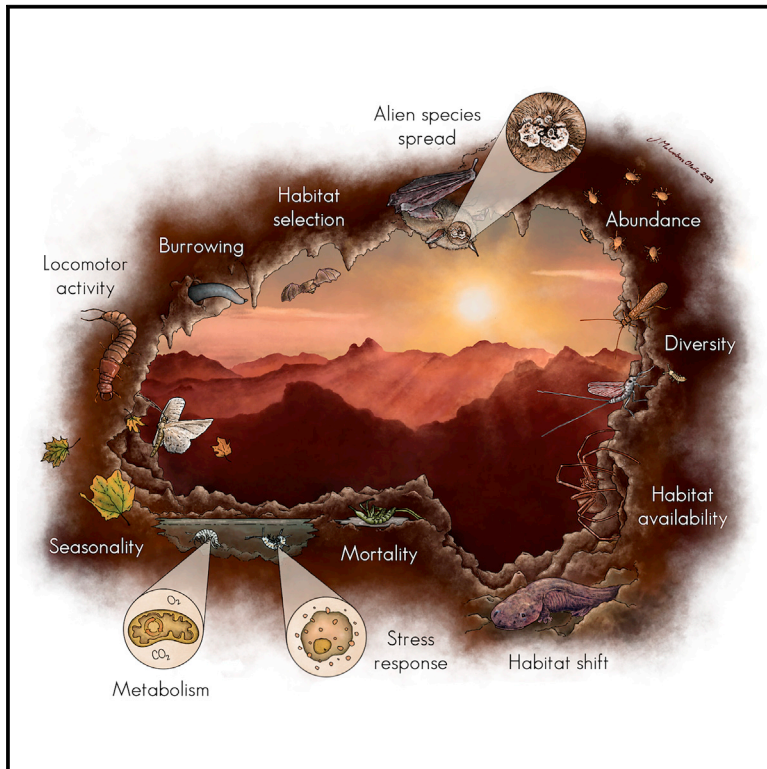


# A global meta-analysis reveals multilevel and context-dependent effects of climate change on subterranean ecosystems

## Graphical abstract



## Highlights

- Climate change impacts on subterranean ecosystems are receiving increasing attention
- Climate change impacts are modulated by habitat, taxa, and subterranean specialization
- Impacts occur at gene to community levels with varying strength and direction
- There is a research disparity toward subterranean arthropods and temperate regions

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## In brief

There is a key impediment that is stalling most attempts to protect subterranean biota: we lack a mechanistic understanding of subterranean species and community response to climate change. Through a meta-analysis of the existing literature, we underscore the multifaceted and intertwined effects of climate change on subterranean organisms, operating at gene to community levels with varying strength and direction depending on habitat, taxa, and ecological specialization. Studying these nuanced responses is critical to ensure the incorporation of neglected subterranean ecosystems in global climate change agendas.

Article

# A global meta-analysis reveals multilevel and context-dependent effects of climate change on subterranean ecosystems

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**SCIENCE FOR SOCIETY** Subterranean environments (e.g., caves, groundwaters) have been overlooked in global climate change agendas. This contrasts with their widespread distribution, high biodiversity, and importance to humans as providers of multiple ecosystem services, including provisioning (e.g., drinking water), regulating (e.g., water quality), supporting (e.g., soil formation), and cultural (e.g., tourism) ones. Human activities at the surface, especially climatic alterations, can trickle down and impact the subterranean levels, compromising this natural capital. By elucidating the biological impacts of climate change on subterranean ecosystems, our findings can inform policy decisions and management strategies. Additionally, our research can contribute to the recognition of subterranean ecosystems in global climate change targets and biodiversity agendas while enhancing public awareness and fostering appreciation for the intrinsic value and ecological importance of subterranean habitats.

## SUMMARY

Subterranean ecosystems (e.g., caves, groundwaters, fissure systems) are often overlooked in global climate change and conservation agendas. This contrasts with their widespread distribution, rich biodiversity, and importance to humans as providers of multiple ecosystem services. Worryingly, evidence is accumulating regarding diverse biological alterations in subterranean ecosystems under climate change exposure. Yet, we lack quantification of the magnitude of these impacts across scales and ecosystem components. Here, we assembled a dataset covering 347 measurements of climate change impact at the organismal physiology, behavior, population/community, and habitat levels. Through a meta-analysis, we showed that climate change effects act at gene to community levels with varying strength and direction depending on habitat, taxa, and degree of subterranean specialization. By building a nuanced understanding of the multi-level impacts of climate change on subterranean ecosystems, our analysis underscores the vulnerability of different ecosystem components, providing a supported rationale for their incorporation into conservation agendas through targeted measures.

## INTRODUCTION

Amid escalating climate and biodiversity crises,<sup>1–4</sup> scientists grapple with a pressing question: how can we anticipate the consequences of global change on ecosystems? Answering this question is fundamental to achieve most Sustainable Development Goals and the ambitious agenda set by the UN Biodiversity Conference (COP 15, December 7–19, 2022, Montreal, Canada). Yet, while this question has been approached in diverse terrestrial and marine contexts,<sup>5–7</sup> it remains largely unanswered when it comes to “hidden” subterranean ecosystems, which are often overlooked in global climate change and biodiversity agendas.<sup>8–11</sup>

Within diverse subterranean environments (caves, aquifers, fissure systems, and the likes) thrive an extensive variety of organisms with varying degrees of specialization to a life in permanent darkness.<sup>12</sup> Some of these species are small-range endemics and display unique phylogenetic histories and adaptations,<sup>13,14</sup> therefore significantly enriching global taxonomic, phylogenetic, and functional diversity. Furthermore, subterranean environments and their biota are functionally connected to most surface ecosystems and contribute critical services,<sup>15–17</sup> from the provisioning of water (for drinking, agricultural, and industrial uses) to the regulation of different ecological processes (e.g., water quality, carbon fixation), along with their cultural value (e.g., recreational caving, tourism, education).

Unfortunately, the natural capital afforded by subterranean environments is under mounting anthropogenic pressure. Human-induced impacts at the surface, especially climatic alterations, trickle down and affect the subterranean levels, directly and indirectly impacting different ecosystem components.<sup>18,19</sup> Ultimately, climate-change-induced biological alterations may compromise diverse ecological processes, although these consequences remain difficult to predict because our understanding of these impacts in subterranean ecosystems is still in its infancy.<sup>18</sup>

Until recently, the accepted narrative depicted caves as sheltered systems characterized by pronounced thermal inertia, substantially unaffected by climatic alterations taking place at the surface. Subterranean-dwelling organisms were long perceived as ancient relics that survived past climatic upheavals<sup>20–22</sup> and thus unlikely to be affected by ongoing anthropogenic climate change. It is only in the past few years that an increase in cave atmosphere temperatures was documented unequivocally,<sup>23,24</sup> confirming theoretical models developed in 2004.<sup>25</sup> Mounting evidence for groundwater systems corroborates these findings,<sup>26–31</sup> solidifying the consensus that climate change is currently driving and will continue to drive numerous ecological alterations in subterranean ecosystems (Figure 1). However, the specifics of these impacts are still scarcely quantified beyond local case studies,<sup>32</sup> and a comparison of the relative importance of different drivers is lacking. Ultimately, the possibility of climate change being a significant global driver of subterranean biodiversity change remains unknown—although expert opinions lean toward its importance.<sup>19</sup>

Climate change impacts in subterranean ecosystems should manifest at different biological levels—from genetic responses to entire community dynamics—and vary in magnitude depending on the habitat and the degree of subterranean specialization

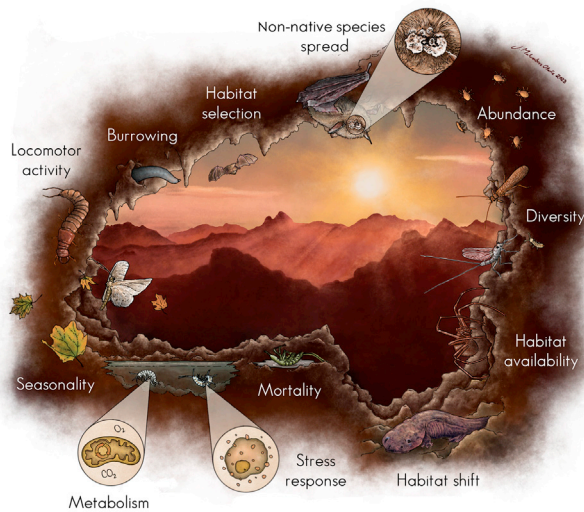
of organisms.<sup>18</sup> Specialized subterranean species should be particularly sensitive to climate change alterations, for a number of reasons. First, the reduced spatial environmental variability of subterranean ecosystems at the local scale prevents species from adapting to environmental changes through behavioral adjustments or habitat shifts.<sup>44</sup> Second, most specialized subterranean species have limited long-range dispersal abilities,<sup>45–48</sup> which makes it difficult for them to track climate change.<sup>48</sup> Third, subterranean species typically have small range sizes and limited habitat plasticity, implying vulnerability to climate change.<sup>49</sup> Finally, subterranean species often have reduced physiological capacity and plasticity to withstand thermal variability because they evolved in thermally constant environments.<sup>50,51</sup> Conversely, less specialized species could be more resilient to climatic alternations, and some could even benefit from subterranean climate change.<sup>18</sup> For example, there is evidence that some species are using subterranean ecosystems as “climate refugia” to escape daily or seasonal unfavorable conditions at the surface.<sup>34–36,52</sup> This may lead to changes in community composition and novel biotic interactions between external and subterranean species.<sup>18</sup>

While there is growing appreciation of the multilevel impacts of climate change in subterranean ecosystems, information is dispersed across a number of disconnected publications, with no consensus of the magnitude, direction, and relative importance of these diverse impacts. Consequently, it is challenging to pinpoint the most vulnerable ecosystem components to climate change, incorporate these systems into regional to global climate change agendas, and ultimately define conservation priorities and management actions.

Here, we aimed to provide a quantitative comparative analysis of the multilevel and context-dependent effects of climate change on subterranean ecosystems. We asked two general questions.

- (1) What is the quantitative evidence of the biological impacts of climate change on subterranean ecosystems?
- (2) How does the frequency and strength of these biological impacts change at different levels of subterranean specialization and across the main subterranean habitats (terrestrial vs. aquatic systems) and taxa?

First, we conducted a systematic review of the existing literature (see Figure S1), selecting 75 peer-reviewed publications that examined the biological effects of climate change on subterranean environments and the associated fauna (accounting for 347 unique estimates). Next, we assigned each individual estimate to one of four major response categories: organismal physiology, behavior, population/community, and habitat (Figure 1). Through a meta-analysis of these data, we underscore the intricate and interconnected impacts of climate change on subterranean organisms. These effects manifest across scales, with varying strength and direction depending on habitat, taxa, and ecological specialization. We found particularly strong responses for specialized subterranean species in terms of physiological stress responses and increased mortality, which may drive substantial changes in community structure. Our analysis is a starting point for comprehending the multifaceted consequences of climate change on subterranean ecosystems, a



**Figure 1. The spectrum of biological impacts of climate change in subterranean ecosystems**

The infographic illustrates the response to exposure to climate change of organismal physiology (metabolism, stress response, mortality), behavior (seasonality, habitat selection, locomotor activity, burrowing), population/community (diversity, abundance, non-native species spread), and habitat (habitat shift, habitat availability). Examples are drawn from the recent literature. Non-native species spread: the spread of white-nose syndrome, a fungal disease causing mass mortality in North American bats.<sup>33</sup> Abundance and diversity: increase in the diversity and abundance of surface-dwelling species in subterranean ecosystems, acting as climate refugia.<sup>34–36</sup> Habitat availability: predicted changes in the availability of habitat for the widespread cave spider *Meta menardi*.<sup>37</sup> Habitat shift: predicted climate-change-induced shifts in the distribution of the cave- and river-dwelling Chinese giant salamander (*Andrias davidianus*).<sup>38</sup> Mortality, stress response, and metabolism: physiological studies on *Proasellus* and *Niphargus*.<sup>39–41</sup> Seasonality: increase in butterflies and moths using caves as daily refugia as climate changes.<sup>34</sup> Burrowing: burrowing behaviors in cave planarians during periods of drought.<sup>42</sup> Habitat selection: bat selection of microclimate within roosting sites to minimize climate change adverse effects.<sup>43</sup> Original illustration by Jagoba Malumbres-Olarte.

pivotal step to advocate for their inclusion within the broader context of global climate change initiatives.

## RESULTS

### Overview of the sampled literature

The assembled dataset covered a wide range of measurements for each of the four response categories (Figure 1), had a wide taxonomic (Figures 2A and 2B) and geographical scope, and included field, laboratory, and modeling studies (Figure 2C). Most of the literature is relatively recent, and the number of papers has grown quickly from 2010 onward (Figure 2D). Most studies were conducted in the Palearctic (45 studies) followed by Nearctic (17 studies) and Australasian (6 studies) biogeographical regions, with a particular focus in Southern Europe, the United States, and Australia. Conversely, we observed research deficits in the Afrotropical (1 study), Indomalayan (1 study), and Neotropical regions (5 studies). The dataset was dominated by physiological (39%), population/community ecology (28%), and habitat measures (23%), yielding both negative

and positive responses. Behavioral measures were the least represented (10%).

### Climate change impacts on subterranean ecosystems

We fitted a first set of meta-analytic linear mixed-effects models, assessing the extent to which climate change affects the response of biological components of subterranean ecosystems. All physiology response variables (stress response, mortality, and metabolism) exhibited statistical significance with the largest effect sizes. Behavioral responses with greater significance were seasonality, locomotor activity (significant reduction), and burrowing (significant increase), while habitat selection was not significant (Figure 3A). None of the response variables associated with the category population/community ecology showed statistically significant results (Figure 3A). Results from the habitat response variables revealed that climate change leads to a substantial reduction in habitat availability, while there was only a weak effect on habitat shift (Figure 3A). Overall, we found no evidence for publication bias in effect sizes based on the Rosenthal statistic (Table S1); for most variables, there was no evidence of funnel plot asymmetry either (Figure S2).

The temporal scale of the studies included in the meta-analysis revealed substantial heterogeneity across different response variables but a relatively homogeneous distribution within each variable (Figure 3B). Studies covering over 50 years were predictive models relying on forecasted temperature regimes based on climatic databases (e.g., WordClim 2). As expected, laboratory experiments in the category physiology and field work research in the category population/community had the shortest temporal scale, with most studies lasting from a single day to a few years.

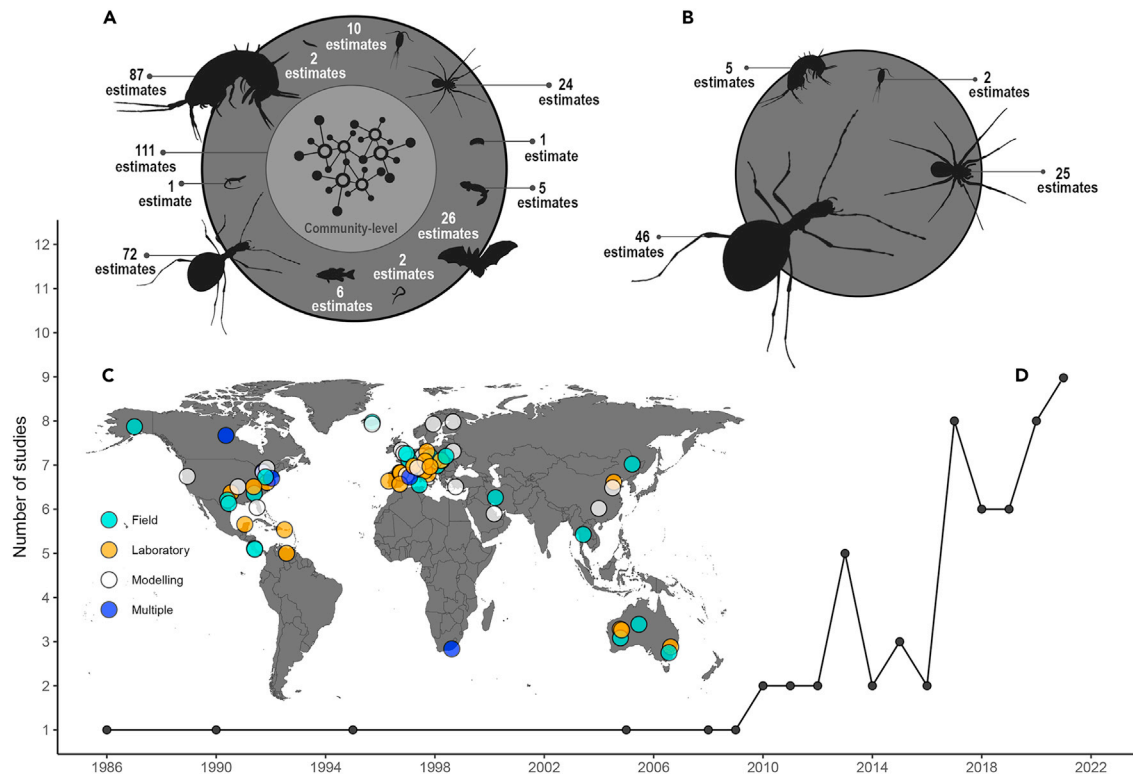
### Modulation of impacts by ecology and habitat

Next, we explored whether the strength and direction of effect sizes vary depending on species ecology and the type of subterranean habitat investigated (Figure 4). There were some divergent responses to climate change depending on both moderators. With respect to physiology, effect sizes for stress response were not significant for subterranean species, which behaved differently than surface species, while mortality showed significant positive effect sizes for both ecological categories (Figure 4A). Also, directionality of metabolism was negative in aquatic and overall positive in terrestrial studies (Figure 4B). Regarding behavior, community-level studies highlighted a positive burrowing effect contrasting the negative effect observed in individual subterranean species. Effect sizes for diversity were overall negative for subterranean species. Effect sizes for abundance were significantly positive for surface species, indicating an increase in the abundance of external, non-specialist species in subterranean habitats with climate change. In terms of habitat-type effects, we observed no significant differences between aquatic and terrestrial habitats except for two responses (Figure 4B). Effect sizes for habitat availability were consistently and markedly reduced across all aquatic studies.

### A focus on physiological tests of lethal temperature

Finally, we extracted 78 estimates of species thermal tolerance from 9 laboratory studies and used a linear mixed model to test for variation in thermal tolerance across taxa, experiment





**Figure 2. Quantitative summary of the dataset**

(A) Number of extracted estimates included in the meta-analysis by species taxonomy (size of silhouettes is proportional to the sample size).

(B) Number of extracted estimates for the analysis of stress response traits ( $\Delta$ LT50 and  $\Delta$ LT100) by species taxonomy (size of silhouettes is proportional to the sample size).

(C and D) Spatial (inset map) and temporal (linechart) distribution of the selected studies. Color coding reflects the classification of the studies in four categories: field (31.5% of studies), laboratory (34.3%), modeling (26%), and multiple (studies combining multiple approaches; 8.2%).

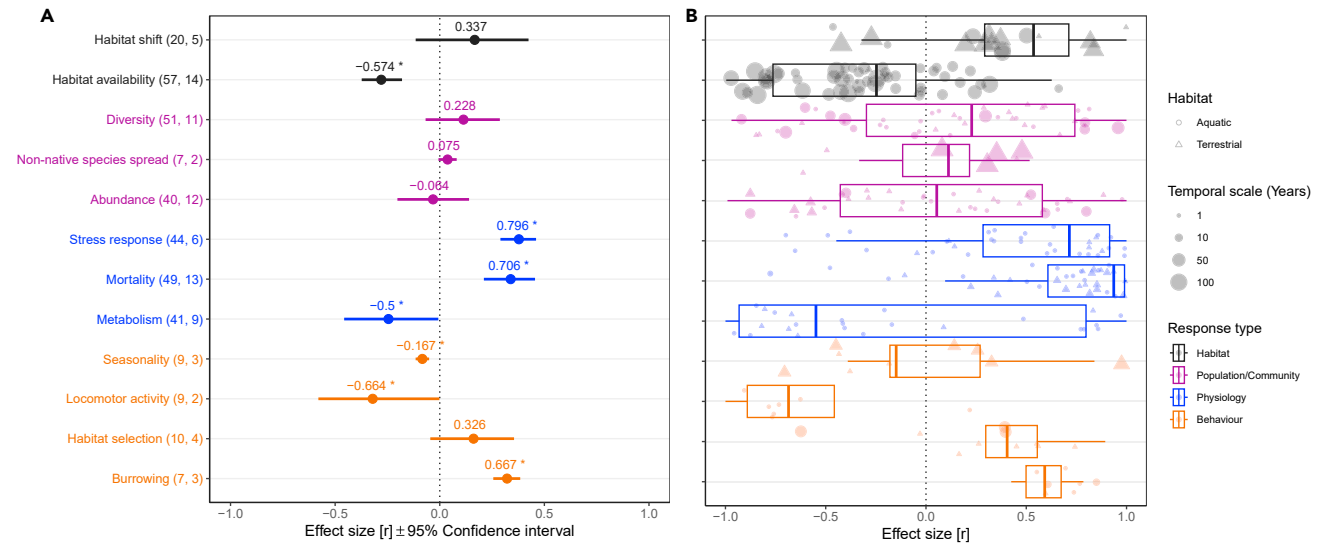
types, and degree of subterranean specialization (Figure 5A). We expressed thermal tolerance as the upper lethal temperatures ( $\Delta$ LTs; see experimental procedures). Insect species had a much higher  $\Delta$ LT50 (estimated  $\beta \pm$  SE:  $16.36 \pm 4.37$ ;  $p = 0.00018$ ) than arachnids, and crustaceans had a slightly higher  $\Delta$ LT50 ( $\beta \pm$  SE:  $2.16 \pm 5.29$ ;  $p = 0.68$ ) than arachnids. Static experiments yielded significantly lower  $\Delta$ LT50 than dynamic experiments ( $\beta \pm$  SE:  $-10.58 \pm 4.22$ ;  $p = 0.012$ ). After accounting for confounding factors, there was no significant effect of subterranean specialization on thermal tolerance ( $\beta \pm$  SE:  $-1.72 \pm 1.43$ ;  $p = 0.227$ ). Results for  $\Delta$ LT100 were qualitatively similar (Figure 5B), although with a more pronounced difference between specialized and non-specialized species. Note, however, that we did not test effects statistically due to the reduced sample size.

## DISCUSSION

Although qualitative reviews of the literature on the biological impacts of climate change in subterranean ecosystems exist,<sup>18,54</sup> there is still no quantitative synthesis on the magnitude, direction, and relative importance of these diverse impacts. Our meta-analysis revealed a mosaic of responses changing from the gene to the community level under climate change exposure

(Figure 3), with some effects varying significantly with subterranean specialization (Figure 4A) and habitat type (Figure 4B). However, this picture is still developing, and considerations and extrapolations based on this literature should be weighed accordingly.

First, long-term datasets are still largely missing in subterranean ecosystems, with most evidence coming from either predictive models or short-term laboratory experiments (Figure 3B). Second, multidisciplinary studies are restricted to the last decade and are far more sparse than studies featuring single approaches (Figure 2). Third, only a handful of studies focused on tropical systems, reflecting a typical pattern in biodiversity research<sup>55</sup> and a delayed appreciation of the presence of specialized subterranean fauna in the tropics.<sup>56</sup> This is a considerable knowledge gap, as tropical climates are typically more stable, and tropical species may therefore be particularly impacted by rising temperatures.<sup>57</sup> Fourth, the quantitative studies included in the meta-analysis focused primarily on metazoan species (Figure 2A), while there was a limited representation of microbiological-oriented studies. This likely reflects a genuine scarcity of quantitative research on subterranean microbial communities. However, this may also be attributed to the limitations of our search string, which may not have effectively captured microbiological studies. This knowledge gap warrants



**Figure 3. Effect sizes for the measures from the main categories**

(A) Effect sizes (Pearson's  $r$ ) with 95% confidence intervals based on results of meta-analytic linear mixed-effects models for each variable from the four main categories (behavior, habitat, physiology, population/community). Exact model estimates are given in Table S1. The numbers in brackets report sample sizes (number of estimates, number of studies), and asterisks (\*) mark significant effects.

(B) Distribution of individual estimates across variables. Jittered points are individual observations, with size proportional to the duration of each study (temporal scale) and shape (dot versus triangle) differentiating studies focusing on aquatic and terrestrial subterranean habitats. Boxplots report median values (thick line), 25<sup>th</sup> and 75<sup>th</sup> percentiles (box), and 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers).

consideration, as recent research suggested that subterranean microbial assemblages are likely to be susceptible to climatic changes,<sup>58–60</sup> with potential impacts on food webs, geochemical processes, and provisioning of ecosystem services.<sup>54,61,62</sup> Last, and on a more technical note, there was some evidence for funnel plot asymmetry for stress response, with a clear overall directional effect size (Figure S2). However, it is probable that this asymmetry is due to true effect heterogeneity related to the moderator ecology rather than being the result of publication bias. Warming is reflected by significant changes in stress molecules (e.g., catecholamines) both in subterranean and surface species.<sup>39</sup> However, variation in stress molecules is less striking in surface species, which are frequently exposed to diurnal and seasonal temperature fluctuations, and the effect may not always be significant.<sup>63,64</sup> The asymmetry in the funnel plot is likely due to the restricted number of studies considering surface species, while the meta-analysis mainly focused on subterranean species, where the warming effect on stress molecules is substantial.

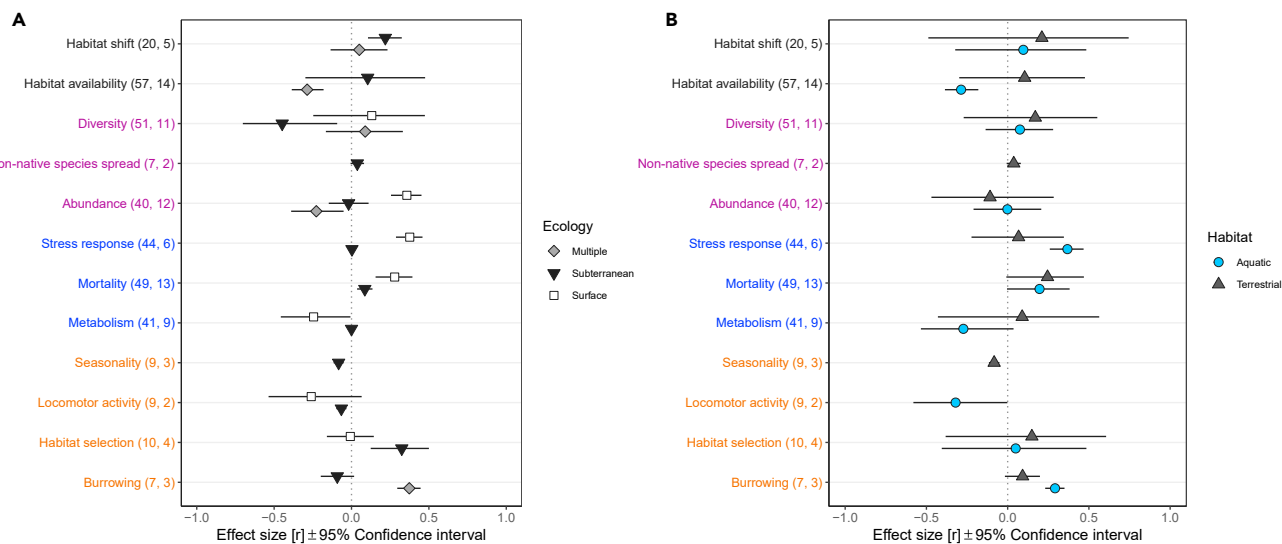
With all these caveats in mind, we discuss below consensus evidence about biological impacts of climate change in subterranean ecosystems.

### Ecophysiological responses to climatic changes

At the molecular and metabolic levels, laboratory studies documented opposite responses of surface vs. subterranean species in terms of stress response to a temperature increase. The disparity suggests that surface species can mount a more robust molecular defense against thermal stress than subterranean ones.<sup>50,65,66</sup> This is possibly related to the natural patterns of temperature fluctuation experienced in surface habitats,

where climate is generally more variable and unpredictable. The observed disparity suggests a narrower physiological thermal window (i.e., the limited range of body temperatures where molecular, cellular, and systemic processes are optimized) of subterranean species compared with surface ones.<sup>40,53,65,67–69</sup> Such a narrow thermal window likely evolved to minimize maintenance costs in an energy-limited environment,<sup>70</sup> resulting in pronounced functional differences between subterranean and surface species.<sup>71</sup> As subterranean species also appear to mount a weaker molecular defense in the face of other environmental disturbances unrelated to temperature,<sup>66</sup> the combination of climate change and other anthropogenic disturbances has the potential to render subterranean fauna more vulnerable to extinction compared with their surface relatives. For example, it is well known that oxygen levels decrease as water warms, generating a greater metabolic burden on aquatic than on terrestrial species.<sup>72</sup> It follows that groundwater deoxygenation under the predicted climate conditions is expected to further impair the metabolic performance of aquatic subterranean species in the future.

The bulk of laboratory studies on stress responses focused on insects, crustaceans, and arachnids, while we found limited experimental research on vertebrates and microorganisms. Not surprisingly, the effects of climate change appear to be pervasive but with significant variation across taxa (Figure 5). However, the thermal safety range did not seem to differ based on the level of subterranean specialization (Figure 5A). This is because a wide variability in critical temperatures was observed within congeneric species with a similar level of specialization. For example, a large difference in thermal niche breadth was documented in some specialized groundwater isopods in the



**Figure 4. Differential responses based on species ecology and habitat type**

(A) Breakdown of effect sizes based on species ecology (surface, subterranean, multiple). The category multiple is used for community-level studies combining species with different levels of affinity for subterranean conditions. Exact model estimates are given in [Table S2](#).

(B) Breakdown of effect sizes based on the habitat type (terrestrial vs. aquatic). Exact model estimates are given in [Table S3](#).

In (A) and (B), effect sizes are expressed as Pearson's  $r$ , the numbers in brackets report sample size (number of estimates, number of studies), and error bars mark 95% confidence intervals.

genus *Proasellus*, with *P. valdensis* being able to live in groundwaters at temperatures ranging from 3°C to 15°C,<sup>73</sup> and *P. cavaticus* and *P. lusitanicus* being sensitive to small temperature changes ( $\pm 2^\circ\text{C}$  from their habitat temperature<sup>51</sup>). The high thermal tolerance in *P. valdensis* was linked to recent gene flow among populations living in thermally constant habitats with different temperature regimes, providing a possible explanation for the existence of broad thermal tolerance in species that inhabit thermally stable environments.<sup>40</sup> Also, it should be considered that despite the theoretical basis to categorize subterranean specialization as being the same, the methods and specific traits considered may slightly differ across the breadth of studies within our dataset. Indeed, phylogenetically controlled studies focused on specific lineages and using comparable estimates of subterranean specialization across species have shown that this is a key factor driving thermal tolerances of subterranean species.<sup>53,69</sup>

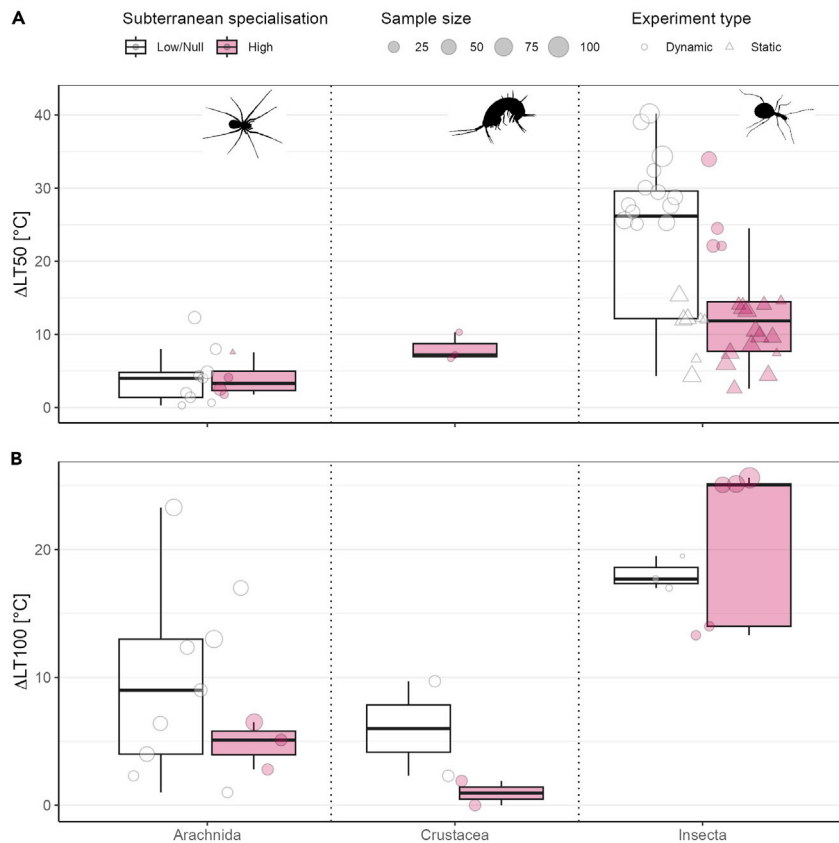
Despite not having sufficient data for statistical comparison, LT100 data also pointed to a trend of reduced thermal tolerance in specialized arachnids and crustacean species (Figure 5B). However, it should be noted that LT100 values provide less accurate information about the thermal tolerance of a population than LT50 or other sublethal responses (see below), as the upper temperature limit for survival under specific conditions is influenced by the tail of the tolerance distribution. Despite these uncertainties, the upper critical temperature remains a useful measure for comparative analysis.

Importantly, physiological evidence is largely based on the study of two specific variables: survival (lethal thermal limits) and thermal acclimation capacity, which are generally measured in relatively short periods of exposure (from days to weeks). The only study that explored sublethal responses (oxidative stress) in

a subterranean species suggests that an increase in temperature, within the tolerance range of the species, could have negative physiological effects on several other metabolic pathways.<sup>50</sup> Also notably, most physiological studies have focused on a single life cycle stage (mostly adults), which is not necessarily the one most sensitive to climate change. Exploring ontogenetic responses therefore represents an important undertaking because different ontogenetic stages often express different levels of tolerance to warming.<sup>74</sup> Accordingly, it seems essential to broaden the study of sublethal responses to obtain realistic estimates of the temperature ranges that could be physiologically more favorable for subterranean species. Likewise, other components of fitness and long-term effects of thermal stress remain poorly explored (e.g., growth, reproduction). Finally, experiment type can exert an impact on the results. Dynamic methods (thermal tolerances estimated from ramping temperature experiments) were almost exclusively used for arachnids and crustaceans. These often provide higher estimates of thermal tolerances than static experimental setups, which can be related to general biochemical or physiological constraints, in particular the denaturation temperature of most proteins.<sup>75</sup>

### Behavioral responses to climatic changes

Behavioral studies were scarcely represented in our database. Our analysis suggests that climate change will impact subterranean species due to the reduced seasonal timing of activities (seasonality for terrestrial species) and by limiting the capacity of behavioral thermoregulation through microhabitat selection (impairment of locomotor activity). Concurrently, behaviors such as burrowing (through which surface ectotherm species exploit cooler and moisture-rich microhabitats or shallow subterranean habitats) could compensate for the loss of efficiency of



**Figure 5. Variation in upper lethal temperature (LT) values for subterranean arachnids, crustaceans, and insects with varying degrees of subterranean specialization (high vs. low/null)**

To ensure comparability across studies, we expressed LTs as a thermal safety range, the delta between the LT measured in the laboratory and the temperature of the natural habitat of each specimen.<sup>53</sup>

(A)  $\Delta LT_{50}$ , namely the thermal safety range estimated from the temperature at which 50% of mortality was reached (note that some studies expressed mortality as total paralysis).

(B)  $\Delta LT_{100}$ , namely the thermal safety range estimated from the temperature at which 100% of mortality was reached.

the first line of defense against drought or heat stress, as seen in planarians<sup>42</sup> and aquatic crustaceans.<sup>76</sup>

### Community and habitat responses to climatic changes

At the population/community level, the diversity of subterranean species is predicted to decrease under climate change exposure, which can be linked to both physiological constraints (see above) and habitat loss (see below), leading to potential local extinctions. Conversely, some studies found that the abundance of surface-dwelling species is increasing in different subterranean habitats.<sup>34,35</sup> This can be linked to a refugium effect whereby subterranean habitats act as climatically stable and sheltered refugia for surface species facing an increasingly unpredictable aboveground climate. In the case of aquatic species, the role of local hydrology in creating hydrologic microrefugia warrants further exploration, particularly given that observations of species distributional shifts appear to be governed by water availability rather than by temperature trends.<sup>52,77</sup> The combination of local disappearance of specialized subterranean species and invasion by surface species will affect community composition, leading to novel biotic interactions that are as yet poorly recognized, with potential fallout effects on community assembly processes and the provisioning of ecosystem services.

At the habitat level, our analysis reveals a reduction in available habitat for most specialized subterranean species, paralleled by limited habitat shift. Subterranean habitats are indeed often patchily distributed and present limited subterranean connectivity, hindering long-range dispersal.<sup>78</sup> Expansion of habitat under climate change exposure was predicted exclusively for

poorly specialized species able to migrate through surface habitats, for example in cave spiders of the genus *Meta*.<sup>37</sup> Aquatic subterranean species appear to be more prone to habitat loss than terrestrial ones. Beyond changing temperature conditions,<sup>30,79</sup> aquatic subterranean habitats are exposed to a drastic decline in water availability due to drought and salinization in coastal aquifers.<sup>27,80,81</sup> Although an extinction of subterranean species under climate change exposure was not

observed yet, it was hypothesized that the reduction in habitat suitability, paralleled by low dispersal ability and the presence of dispersal barriers (e.g., hydrogeological discontinuities), may drive the extinction of local populations for most specialized species.<sup>38,82</sup> Given the high rate of short-range endemics found among subterranean species, the disappearance of a local population may often coincide with a global extinction. Importantly, the loss of suitable habitat may lead to an impoverishment of genetic and functional diversity—for example, it was recently estimated that more than 10% of global genetic diversity was lost because of shrinking habitat.<sup>83</sup> Hence, the reduction of subterranean habitats due to climate change may spiral into a virtually never-recovering genetic loss, exacerbated by the slow pace of natural mutagenesis in subterranean communities owing to the low reproduction rates of most species.<sup>44</sup>

### Conclusions

While individual studies reported evidence of physiological, phenological, behavioral, and habitat shifts in subterranean-dwelling species, our dataset indicates that different categories documented positive, negative, and neutral effect sizes across species in subterranean habitats. We highlight the need for multifaceted approaches to investigate the cumulative effects of climate change, as current studies have only focused on a single or a few predictors and methodologies. With climate change expected to impact simultaneously on different ecosystem components, investigating multiple predictors simultaneously would provide insights into cumulative, synergistic, or antagonistic biological responses. Furthermore, our results demonstrate the



### Box 1. An agenda for climate change research in subterranean ecosystems

Stemming from our assessment of the literature, we identified research gaps warranting further exploration.

- Implement experiments comparing congeneric species differing in key traits (e.g., habitat specialization, dispersal capacity), as well as different ontogenetic stages of the same species, to gain further insights into their role in determining the response to climate change.
- Implement experimental studies combining multiple stressors together to check for cumulative, synergistic, or antagonistic effects.
- Complement physiological studies with responses other than survival, exploring thermally sensitive processes at different levels of biological organization, long-term responses, and different fitness components to assess the potential for adaptation in subterranean species to climate change, including the role of genetic diversity and evolutionary processes.
- Increase the studies focusing on behavioral responses to understand whether adaptive behavioral traits conferring climate change resilience are exclusively genetically determined or may be the result of trait plasticity.
- Develop mechanistic species distribution models (instead of correlative approaches, which have multiple limitations for poor dispersal species), for example by combining physiological and distribution data to obtain more accurate predictions of species response to climate change.<sup>85,86</sup>
- Implement predictive models beyond the species level (e.g., joint species distribution models), and include phylogenetic and trait information when possible, to predict how communities will change and how biotic interactions will be affected by climate change.
- Extend analyses on those subterranean habitats facing higher risk (e.g., glacier caves,<sup>87</sup> subarctic caves<sup>58</sup>), as well as in poorly studied regions (e.g., the tropics) and organisms (e.g., microbial communities), to evaluate the extent to which the ecosystem services they provide will be affected.
- Explore the potential for management interventions, such as habitat restoration and conservation, to mitigate the impacts of climate change on subterranean ecosystems.

challenge of developing effective conservation strategies to mitigate the effects of climate change on subterranean ecosystems. We found that among the studies analyzed, virtually none provided concrete conservation strategies for climate change mitigation, highlighting a critical knowledge gap that requires immediate attention.

In conclusion, our meta-analysis provides a valuable starting point for understanding biological impacts of climate change in subterranean ecosystems but also emphasizes how considerable knowledge gaps still linger (Box 1). With the current pressures of climate change hastening the need for preserving subterranean biodiversity, bridging these knowledge gaps is essential to ensure the integration of subterranean ecosystems in climate change targets and the design of conservation and management actions.<sup>8,84</sup>

### EXPERIMENTAL PROCEDURES

#### Resource availability

#### Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Stefano Mammola ([stefano.mammola@cnr.it](mailto:stefano.mammola@cnr.it)).

#### Materials availability

This study did not generate new unique materials.

#### Data and code availability

Data supporting this study are deposited in OSF (<https://doi.org/10.17605/OSF.IO/2EAD5>). R code to reproduce the analysis is available in GitHub ([https://github.com/StefanoMammola/Meta\\_Analysis\\_Subterranean\\_Climate\\_Change](https://github.com/StefanoMammola/Meta_Analysis_Subterranean_Climate_Change)).

#### Preregistration of the study

The study methodology, hypotheses, sampling strategy, and analytical approach were preregistered prior to the start of the research (<https://doi.org/10.17605/OSF.IO/MRQ3U>). With respect to the initial preregistration plan, we could not test differences between temperate and tropical regions

because the sample of studies was heavily skewed toward temperate regions (Figure 2C). Furthermore, given data availability, we restricted the stress response trait analysis to a limited number of traits (see section [stress response traits](#)).

#### Focus of the study

We focused the meta-analysis on subterranean ecosystems. Following the recent function-based classification for Earth's ecosystems,<sup>88</sup> we included all habitats within the categories “subterranean” (S) (including “subterranean lithic” [S1] and “anthropogenic subterranean voids” [S2] biomes) and “subterranean-freshwater” (SF) (including “subterranean freshwater” [SF1] and “anthropogenic subterranean freshwater” [SF2] biomes). Conversely, we only considered anchialine systems among the “subterranean tidal” (SM1) biome.<sup>88</sup>

#### Systematic literature search

To ensure a systematic and comprehensive literature search, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow,<sup>89,90</sup> along with its recent extension to the fields of ecology and evolution.<sup>91</sup> We did our primary literature search on the Web of Science platform. Initially, we conducted a scoping exercise by running searches with various terms and assessing the relevance of the first 100 references to refine our search procedure. We developed a research question using a modified version of the PICO (Population, Intervention, Comparator, Outcomes) framework,<sup>92</sup> adapted for our study by focusing only on the PIO components.<sup>93</sup> Our inclusion criteria consisted of studies that assessed the biological impacts of climate change on subterranean ecosystem components. After preliminary testing with different search terms in March 2022, we reached a consensus on a broad search string (in Web of Science notation):

TS = (anchialine OR MSS OR mesocavern\* OR “mesovoid shallow stratum” OR “Milieu Souterrain Superficiel” OR “shallow subterranean habitat” OR “superficial subterranean habitat” OR aquifer\* OR caves OR cave OR karst OR hyporheic OR “lava tube” OR groundwater\* OR hypogea\* OR subterranean OR underground OR subsurface OR fissural OR talus OR scree OR rocky debris OR boulder field OR cavern\* OR roost OR hibernacul\*) AND TS = (animal\* OR organism\* OR species OR fauna OR endemic OR obligate OR facultative OR genes OR protein\* OR “biological community\*” OR ecosystem\* OR habitat\* OR stygo\* OR troglo\* OR eutroglo\* OR eustygo\* OR

invertebrat\* OR vertebrat\*) AND TS = ("climate change" OR "global warming" OR "climate warming" OR "global change" OR "temperature increase" OR "temperature rise" OR "relative humidity change" OR "relative humidity drop" OR "salinity increase" OR "salinity change" OR "relative humidity decrease" OR "sea level change" OR "sea-level change" OR "extreme event" OR "stochastic event" OR "tipping point" OR drought OR aridity) AND TS = ("thermal tolerance" OR "thermal limits" OR CTmax OR CTmin OR LTmax OR LTmin OR "thermal stress" OR "thermal shock" OR acclimat\* OR physiol\* OR ecophysiology\* OR survival OR mortality OR fitness OR metabol\* OR locomot\* OR growth OR development\* OR size OR "gene expression" OR genetic OR enzym\* OR proteomic OR hormonal OR oxidative OR behavior\* OR reproduct\* OR life cycle OR immune OR distribution\* OR dispersal OR range OR population OR community OR "species composition" OR occurrence OR habitat\* OR extinction OR decline OR migration OR refugium OR refugia OR shelter OR abundance OR presence OR richness OR competition OR interaction OR dispersal OR phenolog\* OR season\* OR circadian OR "heat shock" OR heat-shock OR adaptation OR drift OR "alien species" OR invasion OR "species turnover" OR thermophile OR stenothermal OR warm-dwelling OR sensitivity OR "thermal niche" OR pathogen\* OR parasite\* OR virus\* OR viral OR "white-nose syndrome" OR "white nose syndrome" OR "*Pseudogymnoascus destructans*" OR "*Geomyces destructans*" OR swarming).

The initial search on April 4, 2022, returned 4,746 articles (Data S1). We screened these articles for eligibility (see [extracting relevant reference from the initial search](#)). We also set up a Web of Science alert so that new articles meeting the search criteria were sent to us by e-mail. The extraction of additional literature through the Web of Science alert continued until the end of 2022.

### Criteria for inclusion of studies

We included studies that met the following criteria: (1) focused on subterranean environments, as defined in the section [focus of the study](#); (2) quantified the effect of temperature increase or other climatic alterations on subterranean environments and biota; (3) quantified climate change impacts, whether direct or indirect, on subterranean ecosystems and biota; (4) investigated impacts on any level of organization, from genes to ecosystem processes; and (5) field and laboratory studies, as well as distribution modeling exercises. We excluded studies that (1) focused on marine caves; (2) focused on soil fauna; (3) lacked quantitative estimates (e.g., opinion pieces and literature reviews); (4) focused on past climate change, such as biogeographic studies on the influence of past climatic events on the fauna; and (5) lacked control or reference groups in experimental studies.

To assess the effectiveness of our inclusion and exclusion criteria, we ran inter-rater agreement tests between two authors (I.V. and R.C.) who independently scored 200 studies using the specified criteria. We calculated the agreement level with Cohen's kappa,<sup>94</sup> which yielded an excellent level of repeatability (Cohen's kappa: 0.89; 95% confidence interval [CI]: 0.82–0.95).

### Extracting relevant references from the initial search

We initially screened all titles and abstracts to exclude clearly inappropriate references based on the inclusion criteria (see [criteria for inclusion of studies](#)). Following the screening phase, we selected 586 papers. We read the full text of each paper and, for those relevant, extracted meta-data, predictors, and response variables (see [meta-data extraction](#)). To ensure maximum coverage of the literature, we also inspected the bibliography of each eligible article to extract additional relevant papers. We coupled the Web of Science search with an unstandardized search for gray literature,<sup>95</sup> including articles not in English<sup>96</sup> and those known to us that were not captured by previous searches.

### Meta-data extraction

For all articles included in the meta-analysis, we extracted the type of publication, year of study, geographic and taxonomic scope, including family and genus, type of subterranean habitat, and type of climate change impact and biological component impacted (see [categorization of effect sizes](#)). Furthermore, we collected all associated statistical measures to conduct a meta-analysis. Specifically, we collected all statistical tests used to measure the impact of climate change on subterranean ecosystems, their test statistic, degrees of freedom, number of observations, p value, the direction of effect, and sample size. When studies presented partial statistics, we contacted the correspond-

ing authors of these studies asking for missing information ( $n = 11$ ; response rate: 72%). Using standard conversion formulas,<sup>97</sup> we converted all test statistics that describe the effect of climate change impact on a given biological component of the subterranean ecosystem to Pearson's  $r$ . This is a common measure of the effect size (ranging continuously between  $-1$  and  $1$ ), expressing the strength of a given linear association between the predictor and the response variable. We calculated  $r$  for any test within a given study, such as when multiple impacts were tested by the author(s).

### Categorization of effect sizes

We categorized the effect size measures into four main groups: response to exposure to climate change of (1) organismal physiology, (2) behavior, (3) population/community, and (4) habitat. During data collection, we also listed a fifth group, life history, but later excluded it from analyses because it contained only two estimates. These groups broadly overlap with the categorization by Sanders et al.<sup>98</sup> Furthermore, we selected subcategories within each of the four major groups that we thought described the dataset best.

We categorized studies examining the impact of climate change on organismal physiology into three subgroups: metabolism, stress response, and mortality. Metabolic studies mostly focused on the ability of species to acclimate to temperature rise. Studies classified under the stress response subcategory measured cellular and nervous system stress caused by experimental temperature increases, mostly through oxidative stress and other molecular biomarkers. The mortality subcategory included studies focusing on experimental survival trials under temperature increases, using different proxy variables to measure critical thermal limits or organismal performance.

We categorized studies examining the impact of climate change on organismal behavior into four subgroups: seasonality, habitat selection, locomotor activity, and burrowing. Changes in the seasonality of species often result from phenological mismatches and changes in the seasonal timing of events, such as resource inputs from external environments. Changes in habitat selection and locomotor activity patterns emerge as species actively select optimal microhabitats or readjust their niches to enhance survival. Burrowing is a behavior that has been explored in several studies whereby different species exploit cooler and moisture-rich microhabitats or shallow subterranean habitats (e.g., sheltering during drought periods).

We categorized studies examining the impact of climate change on populations and communities into three broad subgroups: diversity, abundance, and non-native species spread. Several studies have examined changes in population- and community-level metrics under climate change exposure or differential thermal regimes. Additional studies have investigated the spread of non-native species in subterranean environments, often because of climate change.

Finally, in the habitat category, we included estimates of future habitat shift and changes in habitat availability under climate change exposures, mostly inferred through predictive models.

### Meta-analysis

We conducted the meta-analysis with R,<sup>99</sup> using the R package "metafor" v.3.0.2.<sup>100</sup> We interpret model-derived estimates of Pearson's  $r$  as the strength of the standardized effect, which we considered significant when the 95% CIs did not overlap zero. Prior to model fitting, we converted Pearson's  $r$  to Fisher's  $z$  to approximate normality.<sup>101</sup>

We fitted a first set of meta-analytic linear mixed-effects models, assessing the extent to which climate change affects the response of biological components of subterranean ecosystems. In all models, we specified a publication-level nesting factor to account for study-level non-independence due to multiple measurements per study. Next, we tested whether species-level response to climate change varies depending on species ecology and the type of subterranean habitats investigated. For this, we fitted two sets of meta-analytic models testing the effect of (1) a moderator (ecology) comprising three levels: subterranean (species that depend on subterranean environments to complete at least one stage of their life cycle), surface (surface-dwelling species occasionally using subterranean ecosystems), and multiple (for community-level studies), and (2) a moderator (habitat) comprising two levels: terrestrial and aquatic. In all these models, we used the same publication-level nesting factor to account for study-level non-independence,

given that a single study can report multiple estimates (number of estimates per paper [mean  $\pm$  SE]: 5.03  $\pm$  0.56).

### Publication bias

For all variables included in the initial set of meta-analytical models, we examined evidence of publication bias. Publication bias occurs when studies with low effect sizes (or those that report negative results) are less likely to be published compared with those that find supportive evidence. To detect publication bias, we employed the fail-safe number, which we calculated using Rosenthal's method<sup>102,103</sup> with the "metafor" function *fsn*. This method determines the number of studies reporting negative results that would need to be added to the existing set of response variables to reduce the combined significance level to a target alpha level of 0.05. We also visually explored publication bias using asymmetry in funnel plots of meta-analytic residuals (Figure S2).

### Stress response traits

For a subset of the reviewed articles (mostly experimental ones), we also extracted stress response data to test how species' response to climate change varies across different groups and to what extent they are mediated by species traits related to the degree of subterranean adaptation of each species ("high" vs. "low/null," which we largely based on the information available in each paper). The data gathered comprised lethal (upper thermal tolerance limits measured with different methodologies) and sublethal responses (metabolic rates, acclimation capacity, locomotor activity, immune defense, gene expression, behavioral responses, and molecular stress biomarkers such as antioxidant enzymes activity and hormones). Except for one study analyzing responses to pH variation, all studies measured responses to temperature changes. For each trait, we extracted the type of response (lethal or sublethal), the specific response variable measured (e.g., survival time, oxygen consumption rate), baseline values, measured at control or reference non-stressful conditions, trait values at stressful conditions, and information about the experimental design and methodology applied. Regarding the latter, we indicated if the method was static (measurement of knockdown time at a constant temperature) or dynamic (ramping method in which a critical temperature is measured while the organism is gradually heated)<sup>104,105</sup> and the ramping rate (for dynamic methods).

We only obtained sufficient sample size to analyze data of upper thermal tolerance limits (LT50 or LT100; i.e., the temperature at which 50% or 100% of the tested organisms died). To ensure comparability across studies, we expressed LTs as a thermal safety range, that is, the delta between the LT measured in the laboratory and the temperature of the natural habitat of each specimen.<sup>53</sup> We tested whether  $\Delta$ LT values differed between taxa (Arachnida, Crustacea, Insecta), degree of subterranean specialization (high vs. low/null), and experiment type (static vs. dynamic) by fitting a linear mixed model to the data. We incorporated a study-level random structure to account for non-independence due to multiple measurements per study. We fitted the model using the R package "glmmTMB" v.1.1.5<sup>106</sup> and validated it with the R package "performance" v.0.9.0.6.<sup>107</sup>

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.oneear.2023.09.001>.

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### AUTHOR CONTRIBUTIONS

Conceptualization, S.M.; methodology, D.M.P.G., D.S.-F., E.P., I.V., M.D.C., R.C., S.M., and S.P.; literature survey and screening, I.V., R.C., and S.M.; extraction of standardized estimates, D.S.-F., E.P., I.V., M.B.M., M.D.C., R.C., S.M., S.P., and T.D.L.; data analysis, S.M.; visualizations, I.V. and S.M.; writing – original draft, S.M.; writing – review & editing, all authors.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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