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Resilience of rhizosphere microbial predators and their prey communities after an extreme heat event

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Abstract

1. Climate change is known to disrupt above-ground food chains when the various trophic layers respond differently to warming. However, little is known about below-ground food chains involving microbial preys and their predators. Here, we study how climate warming-induced heat shocks influence resistance (change immediately after a disturbance) and resilience (ability to recover back to pre-disturbance levels) in rhizosphere microbial communities.
2. We used three species of rhizosphere protists as microbial predators and six different rhizosphere bacterial communities as their prey. Protist species and bacterial communities were extracted from *Centaurea stoebe*—a range-expanding plant species in the Northern Europe. We then examined the temporal dynamics of protists and bacterial communities after an extreme heat event for several generations with sufficient recovery periods. We hypothesized that bacterial community resistance and resilience after the extreme heat event would be higher particularly when extreme heat effects would negatively affect their predators.
3. Our results show that prey community biomass was strongly reduced after the extreme heat event and persisted with lower biomass throughout the recovery period. Opposite to what was expected, predators showed negligible changes in their active density after the same heat event. However, abundances of the three predators varied markedly in their temporal dynamics independent of the extreme heat event. Extreme heat event further increased the inactive density of predators, whereas one of the predators showed a decline in its body size owing to extreme heat event. Bacterial community resistance and resilience after the extreme heat event were independent of predator presence, although species-specific effects of predators on bacterial community resilience were different in the last week of recovery. Predator resilience (based on active predator density) also varied among the three predators but converged over time.
4. Our results highlight that extreme heat events can be more detrimental to microbial prey communities than microbial predators when microbial predators can exhibit thermal acclimation (e.g. change in body size or become inactive) to

overcome heat stress. Such thermal acclimation may promote predator resilience after extreme heat events.

KEYWORDS
climate extremes, community recovery, ecological resilience, microbiome, soil bacteria, soil protists

1 | INTRODUCTION

Soil micro-organisms are the most abundant and diverse living organisms of terrestrial ecosystems (Bahram et al., 2018; Bardgett & Van Der Putten, 2014; Geisen et al., 2019). They contribute to numerous ecosystem functions mainly *via* their contribution to biogeochemical cycling, plant productivity and disease regulation in both plants and animals (Delgado-Baquerizo et al., 2016; van Elsas et al., 2012; Wagg et al., 2019). The functionality of soil micro-organisms is often tightly linked with biotic interactions within microbial groups (Ho et al., 2016; Wagg et al., 2019). For instance, predator–prey interactions in microbial communities are crucial for their population regulations, diversity maintenance, which have subsequent effects for ecosystem functioning (Thakur & Geisen, 2019; Tveit et al., 2015). Climate warming is likely to alter biotic interactions within soil microbial communities (Classen et al., 2015; Romero-Olivares et al., 2017). Several studies have shown that climate warming differentially affects predators than their prey given that predators are usually more vulnerable to thermal stress than their prey (Estes et al., 2011; Fussmann et al., 2014; Petchey et al., 1999; Zarnetske et al., 2012). However, most of our general understanding of differential effects of climate warming on predator and prey come from macroscopic organisms (Blois et al., 2013; Estes et al., 2011; Harley, 2011), and we in particular know little about the responses of microbial predators and prey under climate extremes, which are becoming more common across the biosphere as a result of anthropogenic climate change (IPCC, 2018).

Climate extremes are pulse events, such as heat waves or prolonged droughts that can impose severe stress on ecological communities even in a small time frame, and thus have the potential to dramatically reduce ecosystem functioning (Harris et al., 2018). A recent synthesis, however, suggested that populations and communities usually return to their pre-extreme event conditions (e.g. population density or community biomass) after extreme events have stopped for some period of time (Hillebrand & Kunze, 2020). The faster recovery of community biomass after climate extremes is indicative of resilient communities (Griffiths & Philippot, 2013). Moreover, the extent to which a community biomass decreases immediately after an extreme event (often known as resistance) plays an important role in determining their recovery periods (Hillebrand & Kunze, 2020). Recent research has shown that resistance and resilience of communities against climate extremes depend on several factors, such as resource availability (Gessler et al., 2017), community diversity (Isbell et al., 2015) and species traits (Gladstone-Gallagher et al., 2019). Studies have rarely considered the roles of predators in determining the resistance and resilience of their prey. Given the intricate interactions between predators and prey, the response and recovery of one is most likely to affect the other. Our aim in this study is to examine the importance of microbial predator–prey interactions in determining microbial resistance and resilience when exposed to an extreme heat event.

Extreme heat augments metabolism in both predators and prey with a direct effect on their consumption patterns (Brown et al., 2004; Clarke, 2017). A number of theoretical and empirical studies have shown that higher temperatures induce greater starvation risks in predators owing to their higher metabolic demands than their prey (Fussmann et al., 2014; Gilbert et al., 2014). If so, prey recovery after such an extreme event is likely to be driven by a weakened enemy pressure owing to warming-induced predator extinctions (Petchey et al., 1999). Alternatively, when extreme heat starves predators but does not eliminate their population, they are likely to forage more vigorously on their prey during and after extreme heat event, which could constrain prey recovery (Thakur et al., 2017). Soil micro-organisms can also acclimatize to higher temperatures *via* physiological adjustments to optimize their metabolic rates (Bradford, 2013). Thermal acclimation in micro-organisms are manifested via various responses, such as reduction in microbial respiration rates (Bradford, 2013; Crowther & Bradford, 2013) or via reduction in their size (Gardner et al., 2011; Hessen et al., 2013), which may also reduce their respiration rates. If thermal acclimation further varies between microbial predator and prey, it may decouple their recoveries after extreme heat events. Taken together, the resistance and resilience of microbial predator and prey community during and after extreme heat events can be better understood when considering them together.

Soil bacterial communities were used as the prey and soil protists were used as the predator of bacterial communities in this study. Protists are well-known predators of bacteria in soils (Gao et al., 2018; Thakur & Geisen, 2019). By exposing bacterial communities and protists to an extreme heat event, we expected that extreme heat would negatively affect both bacteria and protists. We tested microbial prey resistance and resilience in terms of bacterial community biomass, whereas microbial predator resistance and resilience were examined using the population of the predators at species level. We hypothesized that bacterial community resistance and resilience after extreme heat event will be determined by protist presence and

negatively so if protists are not excluded by extreme heat event. Finally, for a better understanding of microbial predation effects on bacterial communities, we also measured body size shifts in protist species as a result of extreme heat event to infer to whether there was any thermal acclimation in these microbial predators.

2 | MATERIALS AND METHODS

To examine microbial predator and prey resistance and resilience, we set-up a microcosm experiment with the rhizosphere microbial communities obtained from *Centaurea stoebe* rhizosphere. *Centaurea stoebe* is a range-expanding plant species that has recently established in northern regions of Europe (Ramirez et al., 2019; Wilschut et al., 2019). Recent research has shown that the rhizosphere of *C. stoebe* has a wide variety of micro-organisms including bacteria and protists (Ramirez et al., 2019). We selected three populations of *C. stoebe* in their native range in southern Europe (Slovenia) and three populations of *C. stoebe* in northern Europe (the Netherlands) to extract rhizosphere microbial communities for our experiment.

2.1 | Bacterial and protist strains

In July 2018, soil samples were taken from the rhizosphere of six distinct populations of *Centaurea stoebe* plants, three in the Netherlands and the other three in Slovenia (Table S1). Monoclonal protist cultures were established by manually isolating (Geisen et al., 2014) and growing them in Neff's modified amoebae saline (NMAS) enriched with 0.08% nutrient broth (NB-NMAS) to stimulate slow bacterial growth (Page, 1976).

A diverse bacterial community was isolated from each of the six soils as described in Rosenberg et al. (2009). In short, soils were sieved (1 mm mesh size) and 2.5 g of sieved soils were suspended in 20 ml NMAS and serially filtered up to a mesh size of 1.5 μm to ensure absence of fungal spores, yeasts and protists. A 10 μl fraction of the filtered bacterial suspension was inoculated into Petri dishes containing NB-NMAS for growth. The resulting bacterial communities were routinely inspected for contamination under an inverted microscope (Leica LEITZ DMIRB) at 200× magnification. In the experiment, we used three different protists from the class Heterolobosea isolated from the southern populations of *C. stoebe* rhizosphere soils. These predators were then separately added to six bacterial communities (three southern and three northern *C. stoebe* rhizospheres). Heteroloboseans protists can predate on a wide range of micro-organisms but they primarily feed on bacteria, and can rapidly move towards their prey compared to other protist groups (Pánek et al., 2017).

One week before initiating the experiment, protist cultures were established by transferring 100 μl of the original protist suspensions to each of six new 6-cm Petri dishes filled with sterile NB-NMAS to obtain high amounts of actively growing protists. Immediately before starting the experiment, the protist suspension from all the six replicated Petri dishes per protist culture were transferred to 50-ml centrifuge tubes. These suspensions were washed three times by centrifugation (Sigma, 3-16KI) at 800 rpm for 10 min followed by replacing the supernatant with sterile NMAS. In the last round, washed protists were concentrated in 2.5 ml of NMAS, counted and numbers adjusted to the same number per volume by diluting more abundant cultures with NMAS.

Overnight cultures were prepared by adding 200 μl of bacterial suspension into 50-ml tubes filled with 25 ml of 50% NB-NMAS and 50% Tryptic Soy Broth (TSB) to replicate the bacterial communities. The falcon tubes were incubated at constant shaking (Innova 43 incubator shaker) at 37°C before they were washed with NMAS as described above, but at 4,000 rpm for 8 min. Density of all six bacterial cultures was calibrated at a Spectrophotometer (VWR, V-300PC) at an optical density (OD) of 600.

2.2 | Experimental set-up

Protist species and bacterial communities were incubated with a day–night cycle of 20°C (16 hr) and 17°C (8 hr) for 1 week and were then exposed to an extreme heat event of +10°C for 1 week for both day and night temperatures. The control temperature was in the range of monthly mean temperature (July) of the respective countries in 2018 (21°C in the Netherlands and 22°C in Slovenia, source: https://www.timeanddate.com/). We then let the bacterial communities and protist species recover for 4 weeks after the extreme heat week. A temperature of +10°C for a week represents an extreme heat event according to the IPCC prediction for several regions (IPCC, 2018). The experiment was run in 96-well plates. Each well was filled with 180 μl low nutrient 10% NB-NMAS and 10 μl bacterial suspension (one of the six communities) and 10 μl protist suspension. In non-predator controls, 10 μl NMAS was added instead of the protist suspension. Each predator–prey combination for two temperature regimes were replicated eight times for each time point totalling into 288 experimental units (3 protist × 6 bacterial communities × 2 temperature regimes × 8 replicates). Treatments without predators were replicated four times per time point (48 experimental units: 6 bacterial communities × 2 temperature regimes × 4 replicates). Plates were then kept in a separate incubator (Micro Clima-Series, economic lux chamber, Snijders labs) either at ambient temperature or with the ones with extreme heat event. For each temperature regime (ambient and heat shock), we used two incubators each consisting of two plates. Every 2 weeks, 10% of the initial mixture was transferred to wells of new plates filled with sterile 180 μl 10% NB-NMAS to ensure stable nutrient supply. The entire experiment ran for 6 weeks, and measurements of bacterial community biomass and protist density were done once every week throughout the course of the experiment.

Bacterial community biomass was estimated by measuring the OD600 using a microplate reader (BioTek synergy HT) as pre-tests showed that protist OD was negligible. Moreover, microscopic examination confirmed the absence of any notable debris throughout the experiment to confirm that our OD measurements were representative of bacterial densities (Novak et al., 2009). Both active and

encysted (inactive) protists were determined weekly in each well by counting five spots (visual fields under the microscope at a given location) per well at 200× magnification using an inverted microscope. To maintain the population dynamics of bacteria (and protist) over experimental period, we made transfers every 2 weeks during the experimental period. Both bacterial OD and protist counts were then adjusted for the new transfers based on their values from the previous week. For instance, we adjusted bacterial OD in weeks 3 and 4 (after the first transfer) by multiplying these OD values by the ratio of bacterial OD in week 2 to bacterial OD in week 3 (the week of transfer). In a similar way, after the second transfer (at the end of week 4), week 5 and week 6 bacterial OD were adjusted by multiplying the respective OD by the bacterial OD ratio from week 4 and week 5 and also by the ratio of bacterial OD in week 2 to bacterial OD in week 3 (the first transfer). We adjusted protist densities in the same way as done for the bacterial OD. We determined protist lengths and widths under a microscope (Leica DM IRB, Leica, Germany) in week 1 (before the extreme heat event applied in week 2), week 3 (immediately after the extreme heat event applied in week 2) and in week 6 (the last week of the experiment) measuring cell sizes of 20 individuals at 400 x magnification. The measure of protist body length and width provides us with a general idea of their physiological responses, and how they may change with extreme heat to indicate thermal acclimation in predators.

2.3 | Resistance and resilience of bacterial OD and predator densities

Bacterial resistance and resilience were calculated using the commonly used approach of comparing changes in bacterial OD in extreme heat treatments relative to control treatments (ambient temperature) after the period of extreme heat event (week 2 onwards in our experiment; Griffiths & Philippot, 2013; Kaufman, 1982).

$$\text{Bacterial resistance} = \frac{\text{Bacterial OD in extreme heat treatments in week 3}}{\text{Bacterial OD in control treatments in week 3}},$$

$$\text{Bacterial resilience} = \frac{\text{Bacterial OD in extreme heat treatments in week 4 or 5 or 6}}{\text{Bacterial OD in control treatments in week 3}}.$$

In this way, we were able to track bacterial resilience at weeks 4, 5 and 6 of the experiment. We calculated predator resistance and resilience using the same formula above by replacing bacterial OD with protist densities resulting into predator resilience for three predatory species. The resilience in week 4 can be considered as a measure of short-term recovery, whereas week 6 resilience represents a relatively long-term recovery of bacteria and protists given their shorter generation times. The bacterial OD and predator densities used in resistance and resilience calculations were averaged for each time points per predator treatment (i.e. six replicates for each predator level).

2.4 | Statistical analysis

The variations in bacterial community biomass were analysed using linear mixed-effects models with predator treatments (no predator, predators 1, 2 and 3) and the extreme heat event (ambient or control temperature vs. heat wave or heat shock) as two fixed effects. We used time points and bacterial community identity (three northern population + three southern population) as random intercepts in mixed-effects models. The linearity assumptions of mixed-effects models were visually inspected by looking at the homogeneity of variance and normality of residuals. We log-transformed bacterial community biomass (bacterial OD) for meeting the linearity model assumptions of mixed-effects models. Variations in protist density were also analysed using mixed-effects models but with negative binomial error structure given that protist populations were obtained as count data. We tested the effects of extreme heat event and predator identity (predators 1, 2 and 3) on both active protist density and inactive protist density also with time points and bacterial community identity as random intercepts. Predator identity effects on prey biomass and predator densities were further examined using post hoc Tukey tests. Protist body length and width were analysed with the linear mixed-effects model structure (bacterial community identity as random intercepts) with Gaussian error terms.

Predator treatment effects on variations in bacterial community resistance and resilience were examined using a linear model followed by a Tukey's HSD test for multi-group comparisons. Predator resistance and resilience were also analysed using predator identity as the fixed effect in a linear model followed by a Tukey's HSD test. Both bacterial and predator resilience analyses were carried out separately for resilience in weeks 4, 5 and 6. All statistical models were run in the R statistical software (R Core Team, 2018). Linear mixed-effects models were run in the LME4 package (Bates et al., 2015). The *F*-values and degrees of freedom of mixed-effects models were obtained using the Kenwood–Roger method from the PBKRTEST package (Halekoh & Hojsgaard, 2014). Linearity assumptions were tested with the DHARMA package (Hartig, 2017), and also visualized using the PERFORMANCE package (Ludecke et al., 2020). Tukey's HSD test was run with the MULTCOMP package (Hothorn et al., 2008).

3 | RESULTS

Our results from mixed-effects models show that bacterial OD reduced in the presence of protist predators (Table 1). Moreover, bacterial OD was strongly reduced by the extreme heat event (Figure 1; Table 1). We found, however, no interactions between the two treatments on bacterial OD over the experimental period (Table 1). Among the predators, we found that predators 1 and 2 particularly suppressed the bacterial biomass over the experimental period (Figure S1). The density of active protists differed among three species over the experimental duration, whereas the effects of extreme heat event on them were negligible (Figure 2; Table 1). Among the three predators, predator 1 had the highest

TABLE 1 Results from mixed-effects model for the bacterial and protist responses. The denominator degrees of freedom reported are based on the Kenwood–Roger method, which were only obtained for linear mixed-effects models with Gaussian error terms. The bold values are statistically significant (p-value < 0.05). Please note that for predator responses, we used predator identity treatments only with three levels (predators 1, 2 and 3) without using predator-free controls

Response variable	Fixed effects						Random effects	
	Predator identity (P)		Extreme heat (E)		P × E		Time points	Bacterial identity
	F-value$_{df}$	p-value	F-value$_{df}$	p-value	F-value$_{df}$	p-value	Variance (*SD*)	Variance (*SD*)
Bacterial OD	**$3.31_{3,1997}$**	**0.01**	**$329.4_{1,1997}$**	**<0.001**	$0.88_{3,1997}$	0.44	0.01 (0.12)	<0.01 (0.02)
Protist density (active)	**73.70**	**<0.001**	2.48	0.10	0.26	0.77	1.33 (1.15)	<0.01 (<0.01)
Protist density (inactive)	**154.83**	**<0.001**	**118.57**	**<0.001**	**7.71**	**<0.001**	0.44 (0.66)	<0.01 (0.04)
Protist length (active)	**$7.21_{2,707.32}$**	**<0.001**	$1.56_{1,709.08}$	0.21	**$8.13_{2,707.32}$**	**<0.001**	0.04 (0.21)	0.04 (0.21)
Protist width (active)	**$12.80_{2,706.18}$**	**<0.001**	$0.50_{1,707.38}$	0.47	**$18.81_{2,706.16}$**	**<0.001**	0.14 (0.37)	0.02 (0.16)

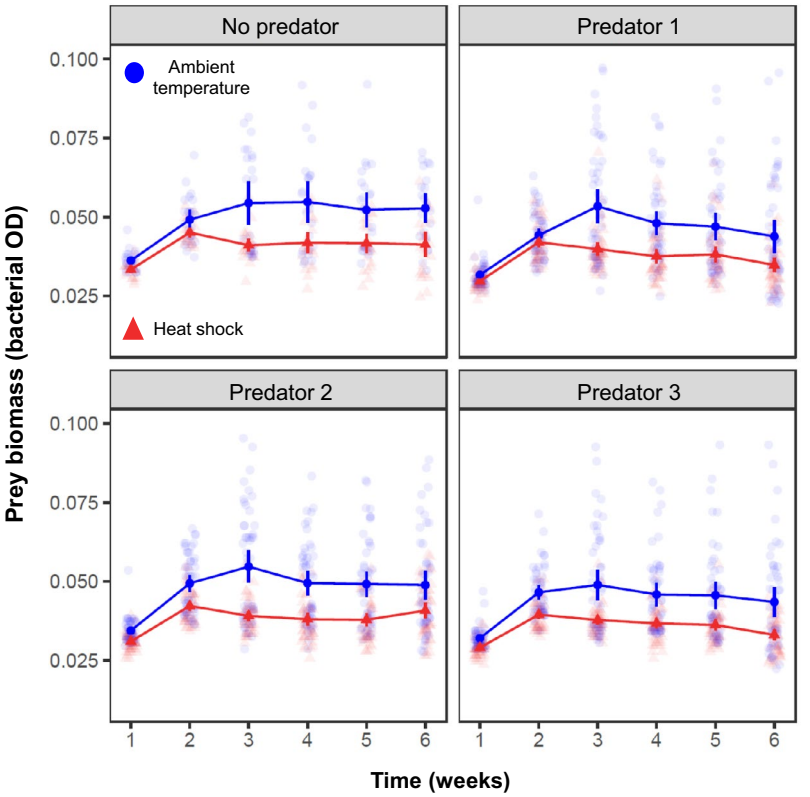

FIGURE 1 Effects of extreme heat event (heat shock) and predation by protists on the dynamics of bacterial optical density (OD, log-transformed) over the experimental period. In the figure, darker points are mean (± standard error vertical lines) of the raw data shown in light colour. The extreme heat event took place in week 2 of the experiment

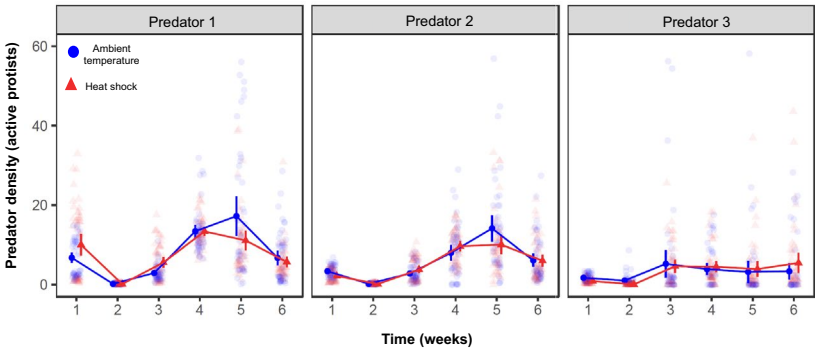

FIGURE 2 Effects of extreme heat event (heat shock) on (active) protist density over the experimental period. In the figure, darker points are mean (± standard error vertical lines) of the raw data shown in light colour. The extreme heat event took place in week 2 of the experiment

active density over the experimental duration independent of extreme heat (Figure S2). We also did not detect any significant interaction between predator identity and extreme heat event affecting the variations in active protist densities (Table 1). The density of inactive protists significantly increased with extreme heat events (Table 1), and overall inactive density was also higher for predator 1 (Table 1; Figure S3).

Our linear models revealed that bacterial community resistance was not affected by protist predators (Table 2). Bacterial community resilience in week 4 (2 weeks after the extreme heat event) and week 5 (3 weeks after the extreme heat event) were also not affected by predator treatments. But we found a significant predator effect on bacterial community resilience in week 6 (4 weeks after the extreme heat event; Table 2). This effect of predator treatments on bacterial resilience was mainly owing to predator-specific differences as revealed by post hoc tests (Figure 3). Predator resistance (active predators) also did not vary among three predators after the extreme heat event (Table 2). We found that predator resilience in weeks 4 and 5 varied among predators, whereas this difference disappeared in week 6 (Figure 4). Predator 3 had the lowest resilience in weeks 4 and 5, whereas by week 6, we did not detect any difference in resilience among the three predators (Figure 4).

Both protist length and width were reduced in only one of the protist species (predator 3) due to extreme heat event (Figure 5), resulting into a significant interaction between predator identity and extreme heat treatment (Table 1). Predator 3 had the bigger size (mainly in the plates that were exposed to extreme heat before the extreme heat week indicating a large variation among individuals) than other two predators before the extreme heat week, which reduced after the extreme heat week (week 3) and remained similar in the last week of the experiment (week 6; Figure 5).

TABLE 2 Protist (Predator) identity effects on the resistance and resilience of bacterial community and protists after extreme heat event. Results are based on linear models. The bold values are statistically significant (p-value < 0.05)

	Bacteria (prey)		Protists (predators)	
	F-value$_{df}$	p-value	F-value$_{df}$	p-value
Resistance	$0.65_{3,20}$	0.58	$0.43_{2,15}$	0.65
Resilience (week 4)	$0.85_{3,20}$	0.48	$\mathbf{10.10_{2,15}}$	**<0.01**
Resilience (week 5)	$0.81_{3,20}$	0.50	$\mathbf{16.69_{2,15}}$	**<0.001**
Resilience (week 6)	$\mathbf{4.32_{3,20}}$	**0.01**	$0.27_{2,15}$	0.76

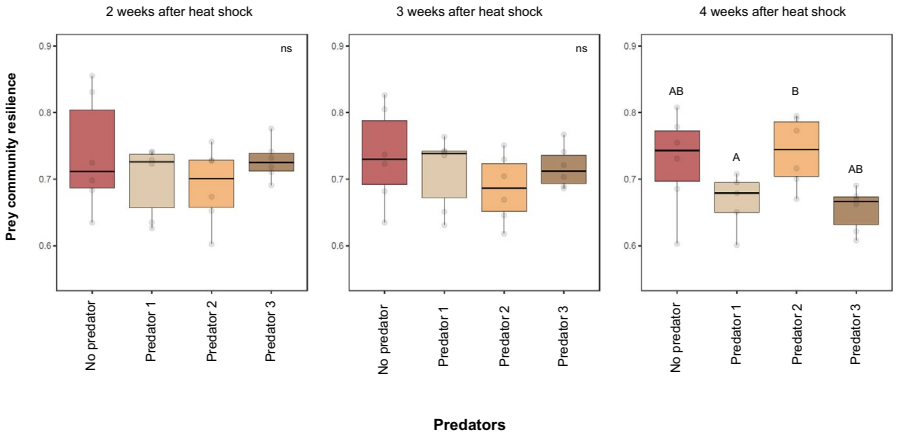

FIGURE 3 Prey (Bacteria) community resilience after the extreme heat event across predator treatments. The letters above boxplots are based on post hoc Tukey's HSD test. ns stands for statistically not significant ($p > 0.05$)

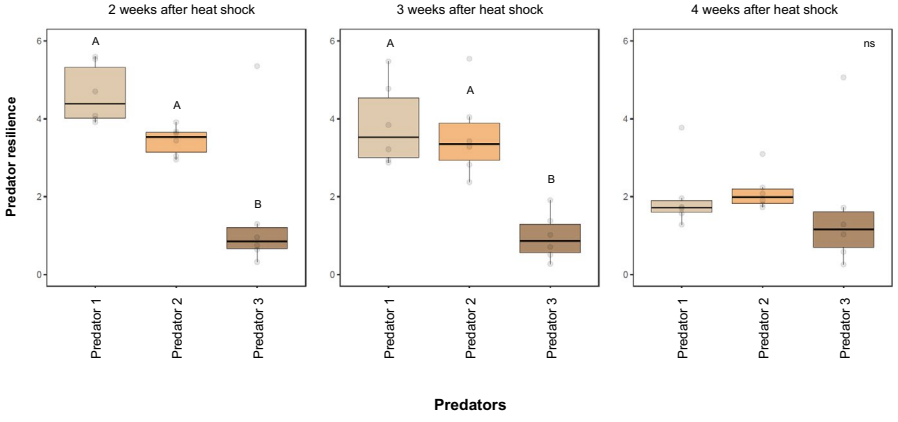

FIGURE 4 Resilience of three predators (protists) after the extreme heat event. ns stands for statistically not significant ($p > 0.05$). The letters above boxplots are based on post hoc Tukey's HSD test. ns stands for statistically not significant (p-value > 0.05)

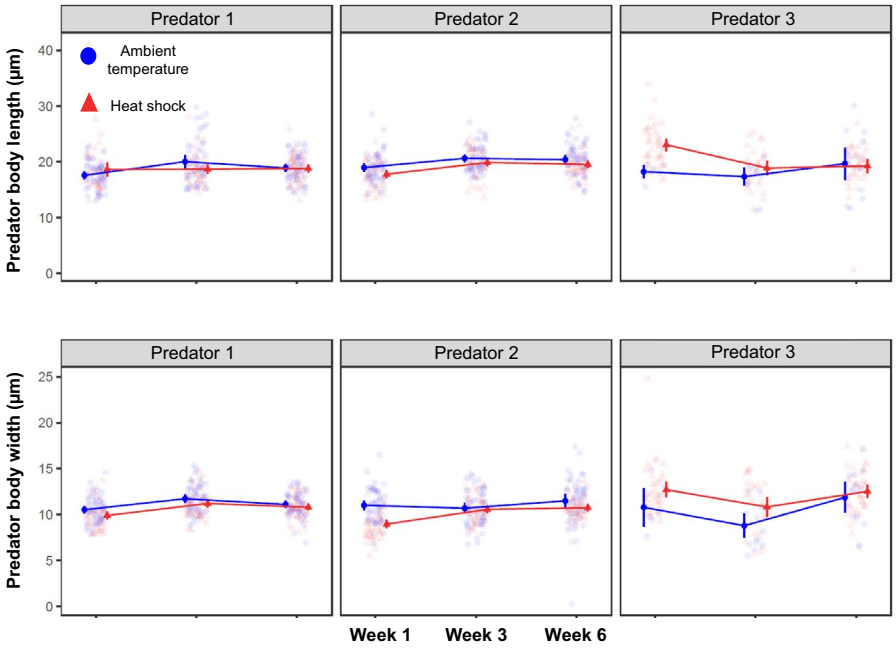

FIGURE 5 Effects of extreme heat event (heat shock) on body length and body width of three protist species used as predator in the experiment at multiple time points of the experiment (weeks 1, 3 and 6). In the figure, darker points are mean (± standard error vertical lines) of the raw data shown in light colour

4 | DISCUSSION

Understanding the recovery of ecological communities after climate extremes is crucial for advancing climate change ecology (Harris et al., 2018). Our experimental results suggest that microbial predatory species and their prey communities when exposed to an extreme heat event may show divergent recovery patterns. The negligible negative effect of extreme heat event on the active population of three protist species in contrast to strong negative effects of heat event on their prey communities (bacteria) led to this divergent pattern. The persistent lower biomass of bacterial communities post extreme heat event indicates a potential shift of bacterial communities and biotic interactions therein. Moreover, bacterial resilience only varied among predators in the last week of recovery also when predator-specific resilience differences disappeared. This temporal mismatch in bacterial and protist resilience highlights the importance of time-dependent differential responses in predator–prey interactions (Karakoç et al., 2020; Thakur, 2020). Finally, our results indicate thermal acclimation in one (predator 3) among three predator species in terms of their size reduction. The greater number of inactive predators in response to thermal stress further suggests a thermally adaptive response in such microbial predators.

A strong decline of bacterial OD after the extreme heat event in our experiment could relate to a number of factors (Figure 1). Usually, bacterial populations in experimental microcosms rapidly increase at higher temperatures when heat stress eliminate their protist predators (Fussmann et al., 2014). Since extreme heat did not eliminate active predators, we suspect that protists might have increased their foraging on bacterial prey given the greater likelihood of their increased starvation during the extreme heat week. Further, a lack of bacterial community recovery over the experimental period could potentially relate to an increased mortality of bacterial species and likely stronger competitive interactions under limited nutrient availability (culture medium in our microcosms were nutrient limited in general) after the week of extreme heat event. Limited nutrient availability in our microcosms could further have lowered the thermal tolerance in bacterial populations (Bestion et al., 2018). These explanations encourage future studies to examine whether nutrient availability and predators can interactively regulate bacterial community resilience.

Our results revealed weaker effects of extreme heat on protists, particularly on their active density response (Figure 2). Moreover, there were differences in temporal population dynamics among the predators independent of heat treatments (Figure 2; Table 1). While these three predators were from the same class (Heterolobosea), it seems from their temporal dynamics that predators 1 and 2 showed a similar growth pattern (Figure 2), whereas predator 3 showed a slightly different growth trajectory (i.e. their densities did not peak in recovery weeks as much as predators 1 and 2 although again irrespective of warming). Such differences in their growth could also relate to their ability to differentially suppress bacterial prey (Figure S1).

While the effects of extreme heat on active density of protists were marginal, we did find a consistent increase in inactive density of protist in response to extreme heat (Figure S3). We speculate that the ability of these microbial predators to remain inactive during stressed conditions may have contributed to their lesser vulnerability to extreme heat (Buckley & Huey, 2016). Moreover, among the three predators, predator 3 showed a higher plasticity to heat stress by reducing its length and width (Figure 5), which indicates their morphological plasticity to overcome thermally adverse conditions. Such morphological plasticity in predator 3 could relate to their slower growth than other two predators specifically in terms of their active density. Indeed, a study with invertebrate predators and prey had shown that thermally acclimated predators had lower foraging rates of their prey in low resource environments than non-acclimated

predators (Sentis et al., 2015). The moderate increase in the density of predator 3 relative to predators 1 and 2 accordingly indicates their lower foraging of bacteria at least in weeks 4 and 5 (Figure 2). The distinct temporal dynamics of three predators, however, had a lesser conspicuous effect on bacterial recovery mainly in weeks 4 and 5 (Figure 3).

Abrupt decline in populations owing to extreme heat stress has often been observed in experiments (Bestion et al., 2020; Thakur et al., 2017). Our results show that such a pattern was more evident for bacterial communities than their protist predators during and after the extreme heat event. These results indicate that lower trophic groups could also become more vulnerable to climate change than their predators, and it is likely that thermal mismatch across trophic levels depends on both biotic and abiotic contexts (Franken et al., 2018; Thakur et al., 2018). Predator's strategies to minimize thermal stress, such as by remaining inactive or decrease their size in our study may have contributed them with an advantage over prey communities. However, if the prey community collapses, their predators are bound to collapse. It is interesting that even lower prey availability allowed predators to exhibit with strategies to overcome thermal stress. The persistence of lower prey community biomass after extreme event merits further investigations, such as how biotic interactions within prey communities unfold after extreme heat events on top of predation pressure.

We conclude that rhizosphere bacterial community may become more vulnerable to extreme heat than their protist predators possibly owing to competitive interactions among bacterial species. The weaker response of predators (active density) to extreme heat could relate to their potential thermal acclimation (e.g. increase in inactive density and reduction in body size). Moreover, microbial predator and prey resilience might show divergent patterns, which further indicate that biotic interactions within trophic groups could weaken the interdependence of ecological resilience between trophic groups.

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AUTHORS' CONTRIBUTIONS

M.P.T. conceived the idea; M.P.T. and S.G. designed the experiment; B.V. conducted the field work; F.A. and E.A. conducted the experiment under the supervision of S.G. and M.P.T.; M.P.T. analysed the data and wrote the manuscript with substantial contributions from W.H.v.d.P. and S.G. All the authors contributed to revisions.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.hdr7sqvgb (Thakur et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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