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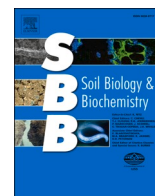
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## Drought shifts soil nematodes to smaller size across biological scales

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### ABSTRACT

Drought events are increasingly affecting the planet's biodiversity. While shrinking body size in response to drought has been observed in many vertebrate animals, whether this rule applies to microscopic animals and the mechanisms during this process remains largely unknown. To address this knowledge gap, we conducted a regional-scale investigation and a microcosm experiment to systematically evaluate the impact of drought stress on the body size of the most abundant soil animals on Earth – nematodes – across various biological scales, including community, population and individual levels. Our results showed that nematode body size declined with drought stress at all biological scales, including a community shift toward smaller-sized species, a smaller body size at the population scale, and a decrease in size-at-age of individuals. Additionally, we designed a Petri dish experiment to examine the reversible plasticity of body size under drought stress using a drought-tolerant nematode species. We found that while nematode body size could not be fully reversed when drought stress was alleviated in the offspring generation, offspring from parents that experienced severe drought conditions could acquire tolerance, leading to a relatively smaller reduction in overall body size compared to those from parents that suffered no or light drought conditions. Overall, our study suggests that the increasing frequency of drought events at the global scale will lead to a reduction in soil nematode body size, potentially causing far-reaching consequences for additional changes in the climate, as well as nutrient cycling in soils.

### 1. Introduction

Global change is causing more intense and frequent drought stress around the world. Drought leads to the loss of biodiversity and a reduction in ecosystem functions (Craine et al., 2013; Bennett et al., 2015). These negative impacts can be explained and predicted by changes in functional traits, among which body size is the most important of these traits (Lindberg and Collins, 2020; Wilkes et al., 2020; de Bello et al., 2021; Green et al., 2022; Guo et al., 2022). Body size is a fundamental trait that relates to an individual's behavior, physiology, ecology, and evolution. It plays a crucial role in understanding ecological processes across multiple biological scales (Díaz et al., 2007; Norkko et al., 2013), because it directly affects an individual's physiological and

behavioral responses to drought stress. These responses can lead to changes in populations, communities and ecosystems due to interactions between individual organisms (Peters, 1983; Allgeier et al., 2020; Wiczyński et al., 2021).

The response of body size to drought has been widely studied across many species, including vertebrates like amphibians (Bendik and Gluesenkamp, 2013), reptiles (Loehr et al., 2007), birds (Cruz-McDonnell and Wolf, 2016), and mammals (Yom-Tov and Geffen, 2006). However, it is still unclear how this response operates at various levels of biological scales (i.e., community, population, individual), despite being well-outlined in the studies examining the effects of global warming on body size (Ohlberger and Fox, 2013). Several hypotheses can be used to predict the potential effect of drought on body size across biological

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scales. Overall, drought often decreases mean body size at the community level (community body size shift hypothesis) (Sheridan and Bickford, 2011). If drought causes a decline in mean body size at the community level, there are three subsequent hypotheses that can possibly explain this shrinkage (Daufresne et al., 2009). First, the decrease in body size at the community level could be due to a greater proportion of small-sized species (composition shift hypothesis) at the population level. Second, it could also be caused by a decrease in mean body size at the population level (population body size shift hypothesis). These body size decreases at the population level could be attributed to the mechanism that individual species' size decreases with increasing drought (size-at-age shift hypothesis). Furthermore, phenotypic plasticity allows species to adjust their morphology in response to environmental change, but this mechanism emphasizes organismal resilience, which allows an individual with small body size to get larger when the growth limiting factor is disappeared (Teplitsky et al., 2008; Ozgul et al., 2009). Understanding plastic changes at the individual level is a mechanistic way to link drought and body size across biological scales, which can also provide better predictions of body size in response to future climate change.

Nematodes, which are the most abundant and functionally diverse animals in the soil ecosystem, are an ideal group of model organisms to test the above hypotheses. First, this group of invertebrates is sensitive to drought stress because their niche is in the aquatic films around soil particles (Meisner et al., 2018; Schimel, 2018; Franco et al., 2022). Second, they vary in size at the individual, population, trophic group and community levels (Bongers and Ferris, 1999; van den Hoogen et al., 2019). Third, their plastic changes to water stress can be conveniently visualized because one of their trophic groups, bacterivores, has a short generation time and can be easily cultured in the laboratory. Additionally, nematodes are critical to the global soil C cycling as they transfer C from primary producers to higher trophic levels (Sohlenius et al., 1988; Filser et al., 2016; van den Hoogen et al., 2019; Vestergård et al., 2019), making them an excellent model to test the consequences of body size changes on ecosystem functions. The metabolic footprint, which evaluates the amount of C that nematode contribute to the soil ecosystem,

provides important estimates for the contribution of these organisms to C cycling (Ferris, 2010; Luo et al., 2021; Franco et al., 2022). However, there is scant empirical evidence to confirm whether a drought-induced change in nematode body size will affect their energy and nutrient flows, such as the C footprint, in affected ecosystems.

We conducted three studies to provide a comprehensive understanding of how drought affects nematode body size at different biological scales, including community, population and individual levels. First, we re-analyzed nematode community data from 30 grassland ecosystems along three precipitation transects in Northern China: Inner Mongolia, Loess Plateau, and Tibetan Plateau (unpublished, Fig. 1a). We tested the 'community body size shift hypothesis' and 'composition shift hypothesis' using the relative abundance of each nematode genera and their corresponding mean body size estimated from the "Nemaplex" database ([http://nemaplex.ucdavis.edu/Ecology/nematode\\_weights.htm](http://nemaplex.ucdavis.edu/Ecology/nematode_weights.htm)). We expected that drought would decrease the community body size of nematodes and shift nematodes to a community with a greater proportion of small-sized species. Second, we conducted a microcosm experiment with different drought levels to test the 'population body size shift hypothesis' and 'size-at-age shift hypothesis' (Fig. 1b), using the relative abundance of each nematode genera and their actual measured body size. This experiment eliminated the potential impact of other environmental factors such as temperature and soil properties, and the measured nematode data could also be used to confirm the two hypotheses in the regional-scale study. We expected that drought would decrease the community body size of nematodes by decreasing the mean body size of several resistant nematode populations and the size of a drought-tolerant species (*Acrobeloides* sp.) within the same age. Here, we only considered adult nematodes due to the difficulty in identifying juveniles at the genus level in a soil nematode community under a microscope. Third, we cultivated the drought-tolerant species (*Acrobeloides* sp.) in the group of bacterivores, and conducted a Petri dish experiment mimicking various drought levels to examine the phenotypic plasticity of their body size (Fig. 1c). We expected that nematodes would exhibit a reversible response when drought stress was alleviated or aggravated.

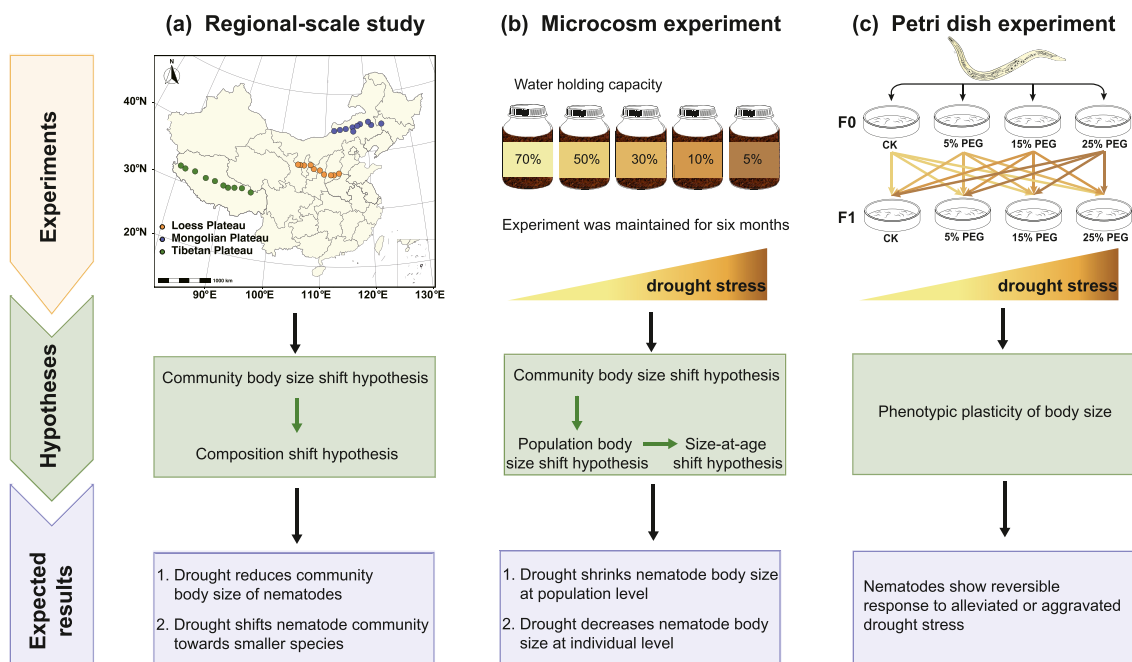


Fig. 1. Schematic framework describing the three conducted experimental studies. (a) Regional-scale study shows the response of nematode body size to geographic aridity gradients along three precipitation transects in Northern China. (b) Microcosm experiment shows how nematode body size responds to decreasing soil moisture levels. (c) Petri dish experiment shows the reversible plasticity of body size in the offspring generation of soil nematodes at the individual level.

## 2. Materials and methods

### 2.1. Regional-scale study

#### 2.1.1. Site description

We conducted field sampling along three precipitation transects in Northern China: Inner Mongolia, Loess Plateau and Tibetan Plateau (80.15–123.51°E, 31.38–45.11°N). Average annual temperature ranges from –4 to 12 °C, with warmest conditions in the Loess Plateau, followed by the Inner Mongolia and Tibetan Plateau. The average precipitation ranges from 150 to 600 mm. Together, the three transects covered 4000 km, with 900 km in Inner Mongolia, 800 km in Loess Plateau, and 1500 km in Tibetan Plateau. Each transect includes three typical habitat types: meadow grassland, typical grassland, and desert steppe (Table S1). Detailed information on dominant plant species and soil properties in each site is available on previously published works (Xu et al., 2021; Xu et al., 2022).

#### 2.1.2. Soil sampling

We collected soil samples in the growing season (July to August) in 2018. In each transect, ten sampling sites without human disturbance were chosen. In each site, eight quadrats (1 m × 1 m) were established in a 100 m × 100 m area, and the intervals between adjacent quadrats were at least 10 m for random sampling. In each quadrat, five random soil cores (0–10 cm in depth and 5 cm in diameter) were pooled as a composite sample. This resulted in a total of 240 samples collected from the three transects. Soil samples were bagged in coolers for transportation to laboratory and stored at 4 °C.

#### 2.1.3. Nematode data

Within 7 d of sampling, nematodes were extracted from 100 g fresh soil samples using a centrifugation method (Liu et al., 2008). Nematode abundance was counted under a stereomicroscope (Motic SMZ-168), and all live specimens in each sample were identified to genus level under a microscope (Olympus BX50) at 400–1000 × magnification (Bongers and Verening, 1988). Identified genera were allocated to five functional groups: bacterivores, fungivores, herbivores, omnivores, and predators based on morphology (Yeates et al., 1993).

The abundance of total nematode and five functional groups was expressed as individuals per 100 g dry soil. Then, the relative abundance (%) of each genus and trophic group in the community was calculated. The estimated mean fresh body size of each nematode genus was retrieved from the “Nemaplex” database developed and maintained by Howard Ferris, University of California ([http://nemaplex.ucdavis.edu/Ecology/nematode\\_weights.htm](http://nemaplex.ucdavis.edu/Ecology/nematode_weights.htm)). We analyzed the CWM body size, a trait-based indicator integrating mean body size with relative abundance (Laliberté and Legendre, 2010; Liu et al., 2015; Andriuzzi et al., 2019), to examine nematode body size shifts at a community level. The CWM body size in all samples was calculated using R package FD (Laliberté et al., 2014). CWMs were calculated for the total nematode community and each functional group. In addition, we calculated the metabolic footprint (MF) of soil nematodes to estimate their contribution to the influx of C and energy in the soil food web, using the measured abundance and estimated mean body size from the “Nemaplex” database (Ferris, 2010). The MF was calculated using the formula:

$$MF = \sum (N_t \times (0.1 \times (W_t / m_t) + 0.273(W_t^{0.75}))),$$

where  $W_t$  and  $m_t$  represent the body weight and the colonizer-persister (cp) class of genus  $t$ , respectively, and  $N_t$  represents the number of individual nematodes of genus  $t$  (Ferris, 2010).

### 2.2. Microcosm experiment

#### 2.2.1. Experimental design

We collected a typical soil from the top layer (0–20 cm) of an abandoned field (34°6'N, 119°51'E). The soil was classified as Fluvisols (fluvo-aquic soil) with a sandy clay texture. Initially, this soil had 4.2 g organic C kg<sup>-1</sup>, 20 mg available N kg<sup>-1</sup>, 3.5 mg available P kg<sup>-1</sup> and 82 mg available K kg<sup>-1</sup>, with pH (H<sub>2</sub>O) of 8.4 and soil gravimetric moisture content of 30%. The collected soil was transferred to microcosms (plastic plots, 25 cm diameter, 10 cm tall) in a greenhouse. Five drought levels with four replicates were arranged, which resulted in 20 plastic pots. Each pot was filled with 2 kg soil (dry weight basis). Pots were air-dried until the soil moisture was 70%, 50%, 30%, 10% or 5% of field capacity, which were the five drought gradients in the experiment. The incubation period was about six months, and soil samples were collected when the target field capacity reached each of the five drought gradients. For each drought gradient, four soil cores from 0 to 10 cm were collected randomly and mixed together as a composite sample for the measurement of nematode communities and body size.

#### 2.2.2. Nematode data

Nematodes were extracted from 100 g soil using the centrifugation method (Liu et al., 2008). The nematode abundance was counted under a stereomicroscope (Motic SMZ-168), and all live specimens in each sample were identified to the genus level using a microscope (Olympus BX50) at 400–1000 × magnification (Bongers and Verening, 1988). The identified genera were assigned to one of the following functional groups: bacterivores, fungivores, herbivores, omnivores, and predators based on their morphological traits (Yeates et al., 1993). Omnivores and predators were then combined into one group due to their low abundance. All the identified genera were measured for their body length ( $L$ ) and the greatest diameter width ( $D$ ).

The abundance of total nematodes and four functional groups were expressed as individuals per 100 g dry soil. The relative abundance (%) of each genus and trophic group in the community was then calculated. The body size of each identified individual was determined by calculating the body length ( $L$ ) and the greatest width of diameter ( $D$ ) according to  $W = (L \times D^2)/(1.6 \times 10^6)$ , where 1.6 is an empirically determined constant (Ferris, 2010). Measured body size was used to calculate the body size at individual, population and community levels. Additionally, we calculated the metabolic footprint (MF) of soil nematodes by integrating their abundance and measured body size to estimate their contribution to C cycling (as detailed in the ‘Nematode data’ section of the ‘Regional-scale study’).

### 2.3. Petri dish experiment

#### 2.3.1. Nematode preparation

Because the bacterivore *Acrobeloides* is a drought-tolerant genus, based on results from the microcosm experiment, we further designed a Petri dish experiment to examine the reversible plasticity of this genus. We isolated *Acrobeloides* through morphological analysis from the soils of the microcosm experiment using a modified Baermann method (Liu et al., 2008). The nematodes were cultivated on nematode growth medium (NGM) inoculated with *E. coli* OP50 at 20 °C, and transferred to new culture plates until a single nematode genus was observed in the NGM. Finally, individuals of the isolated soil-dwelling *Acrobeloides* were subjected to 18S rRNA sequencing and molecular data analysis using BLAST in the NCBI database, followed by phylogenetic analysis with the Neighbor-Joining method in MEGA software. The molecular analyses confirmed that the selected taxon was *Acrobeloides* (NCBI Accession Number: OR000730), which was designated as *Acrobeloides* sp. NJ (Fig. S1).

#### 2.3.2. Experimental design

Four drought gradients were created by applying different

concentrations of polyethylene glycol (PEG) 6000 solution (PEG-6000, Aladdin Industrial Corporation, Shanghai, China), which has been used to create drought stress in laboratory studies with plant-parasitic and entomopathogenic nematodes (Michel and Kaufmann, 1973; Glazer and Salame, 2000; Verslues et al., 2006; Strauch et al., 2010). PEG-6000 was added to nematode growth medium (NGM) to simulate three levels of drought: 5, 15 and 25% (w/v), with unmodified NGM as the control. Therefore, this experiment had four treatments: CK, 5%, 15% and 25%, and each treatment had 10 NGM dishes for replicates. These concentrations were designed to create a certain degree of drought stress while ensuring a certain amount of reproduction. Briefly, 0g, 5g, 15 g and 25g solid PEG 6000 were respectively added to 100 ml deionized water to make a series solution of PEG 6000: 0%, 5%, 15% and 25%.

Approximately 200 age-synchronized nematode eggs were transferred onto the corresponding plates of four drought treatments to examine the effect of drought on the parental generation (F0) (Chen et al., 2018). On the eighth day, 50 pregnant F0 nematodes were picked using a sterile platinum wire needle and transferred onto new plates to lay eggs for 6 h, generating the offspring generation (F1) (Li et al., 2020). Each Petri dish was supplemented daily with 100  $\mu$ L *E. coli* OP50 to ensure adequate food resources for the nematodes.

Nematode eggs (F1) under the four drought treatments experienced by the parental generation (F0) were collected to arrange for a full factorial design. This design considered all possible combinations of the four drought levels (four F0 drought levels and four F1 drought levels, giving 16 combinations) (Fig. 1c). Each combination had 10 petri dishes for replicates, giving a total of 160 petri dishes. For each Petri dish, approximately 50 nematode eggs (F1) derived from the corresponding treatment were added onto the plates. Besides, 100  $\mu$ L *E. coli* OP50 were added onto each plate and the same amount of food was supplied daily to provide sufficient food resources for the nematodes.

### 2.3.3. Nematode data

For the above experiment, 50 live adult individuals in each generation or combination were randomly selected from 10 replicates to measure their body length ( $\mu$ m) and width ( $\mu$ m). Adult nematodes were identified based on the criterion of having laid their first egg. Nematode body size was calculated for each individual using the formula based on body length and greatest diameter width (as detailed in the 'Nematode data' section of the 'Microcosm experiment') (Ferris, 2010).

## 2.4. Statistical analysis

### 2.4.1. Regional-scale study

To test community body size shift hypothesis (i.e., drought decreases mean body size at the community level), the relationships between CWM body size and aridity were firstly examined using linear regression for each plateau separately. Aridity index was obtained from Global Aridity Index and Potential Evapo-Transpiration (ET0) Climate Database (Trabucco and Zomer, 2018) at a resolution of 30 arc-seconds. Aridity was represented as  $1 - \text{aridity index}$  to match the general expectation that sites with higher aridity values are more arid. Besides aridity, we recognized that soil samples from the three plateaus also vary in temperature and soil nutrients, which may distort the effect of aridity on CWM body size. Therefore, we also used partial regression to tease apart the effects of annual mean temperature (extracted from WorldClim database at a 30 arc-seconds resolution) and soil organic carbon (SOC) obtained from (Xu et al., 2021). Other potential variables were not considered here as the three plateaus are limited more by temperature and soil nutrients than other environmental factors (Ren et al., 2021).

To test the community shift hypothesis (i.e., whether the proportion of small-sized species increases along the aridity gradient), we estimated the size spectrum exponent in our regional-scale study. Size-spectrum exponent is a parameter of a power-law function that characterizes the relationship between abundance and body size within a community, with a generally negative value indicating that abundance tends to

decrease as body size increases (White et al., 2007). A larger absolute value of the exponent indicates a higher proportion of individuals in smaller size classes. Here, we used the maximum likelihood method to estimate the exponent for each community (Edwards et al., 2017). The body size data was binned into size intervals with equal widths on a log2 scale, and then the maximum likelihood estimate of the parameter (i.e., exponent) was calculated based on the binned data using the bounded power-law distribution model (Edwards et al., 2017). The relationship between the exponent and aridity was examined using linear regression for each plateau separately. In addition, the 95% confidence interval for the exponent was determined using the profile likelihood-ratio test (Edwards et al., 2017).

### 2.4.2. Microcosm experiment

To confirm the community body size shift hypothesis, linear regression was used to assess the relationship between CWM body size (body size was represented as the mean value of all treatments of each genus) and soil moisture level. Additionally, to further confirm the community shift hypothesis, we estimated the exponent of size spectrum for each community. Linear regression was also used to examine the relationships between the exponent and aridity, and the 95% confidence interval for the exponent was determined using the profile likelihood-ratio test (Edwards et al., 2017). To improve estimation accuracy, he measured individuals from four replicates were combined to estimate the exponent for each treatment.

To test the population body size shift hypothesis (i.e., drought induces a decrease in mean body size at the population level), we used the mean body size values of six dominant and resistant genera from the microcosm experiment. A linear mixed model was adopted to assess the effect of soil moisture level (fixed factor) on mean body size, with the identity of the genera treated as a random factor. The random intercept model was chosen over the random slope model based on AIC values as it provided a better fit to the data.

For the size-at-age shift hypothesis (i.e., individual species' size decrease with increasing drought), we performed a linear regression analysis on the body size of bacterivore *Acrobeloides*, a dominant genus taxon in all microcosm experiments, at the individual level, with soil moisture level as the independent variable.

### 2.4.3. Petri dish experiment

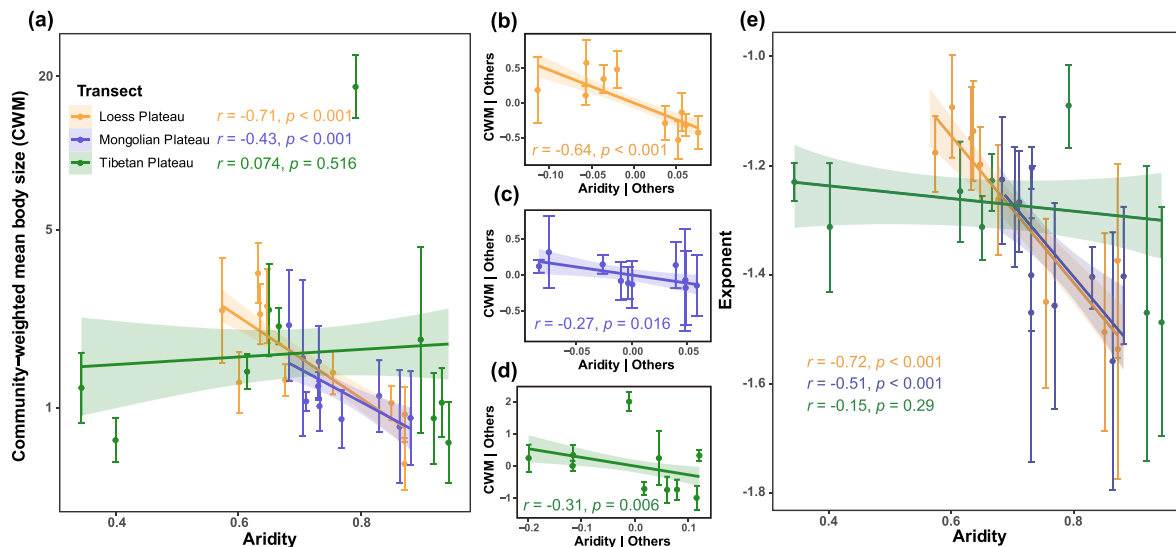
To assess the phenotypic plasticity of body size in *Acrobeloides*, the parental body size (F0) in the Petri dish experiment was used, and one-way ANOVA with Tukey's post-hoc tests were adopted to assess the variation of body size among treatments.

To examine how nematode body size responds to drought across generations, we also compared the offspring body size (F1) of *Acrobeloides* under different combinations of parental and current (offspring) drought treatments in the Petri dish experiment. Differences in body size among treatments were determined using ANOVA tests, and Tukey's post-hoc test was used for pairwise differences. In addition, the relative importance of parental and current drought treatments on the body size of offspring (F1) was determined using *relaimpo* package, with the recommended *lmg* metric (Grömping, 2007). All the analyses were conducted using R 4.0.3 (R Core Team, 2021).

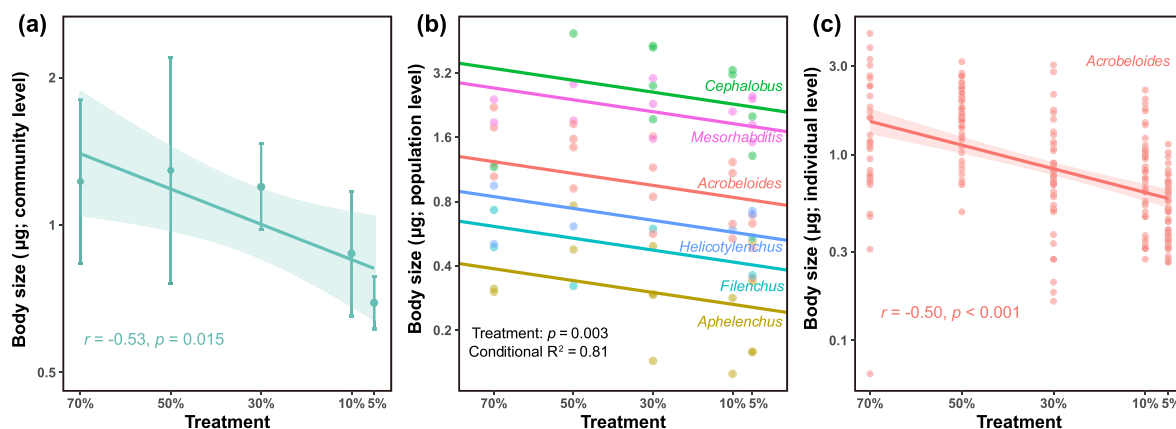
## 3. Results

### 3.1. Declining body size in response to drought occurs at multiple biological scales

Generally, in both the regional-scale study and microcosm experiment, the abundance of soil nematodes decreased in drier environments (Figs. S2 and S3), and their body size was reduced at all biological scales ( $p < 0.05$ , Figs. 2 and 3). The regional-scale study showed that aridity decreased the CWM body size of nematodes in the Loess Plateau and Mongolian Plateau transects but not in the Tibetan Plateau transect



**Fig. 2.** Community-weighted mean (CWM) body size of soil nematodes and estimated exponent of size spectrum for each community along an aridity gradient in three plateaus. (a) Relationship between CWM body size and aridity based on simple linear regression. (b–d) Relationship between CWM body size and aridity, accounting for the effects of annual mean temperature and soil organic carbon. (e) Relationship between estimated exponent of size spectrum and aridity. Size-spectrum exponent is a parameter describing the relationship between abundance and body size in a community, with a larger absolute value indicating a higher proportion of individuals in smaller size classes. Error bars represent standard deviation.



**Fig. 3.** Relationships between nematode body size and soil moisture level at community (a), population (b) and individual (c) levels in the microcosm experiment. Linear mixed model was used to assess the relationships at the population level, with genus identity as a random factor. Conditional  $R^2$  is provided in the plot.

(Fig. 2a). However, when controlled confounding factors such as soil nutrients and temperature statistically in each transect, CWM body size declined by 59–64% with increasing aridity in all transects ( $p < 0.05$ , Fig. 2b–d). The microcosm experiment also confirmed that CWM body size decreased by 46% (70% vs. 5% of field capacity) in drier soils ( $p < 0.05$ , Fig. 3a). Results from both the regional-scale study and microcosm experiment support the community body size shift hypothesis.

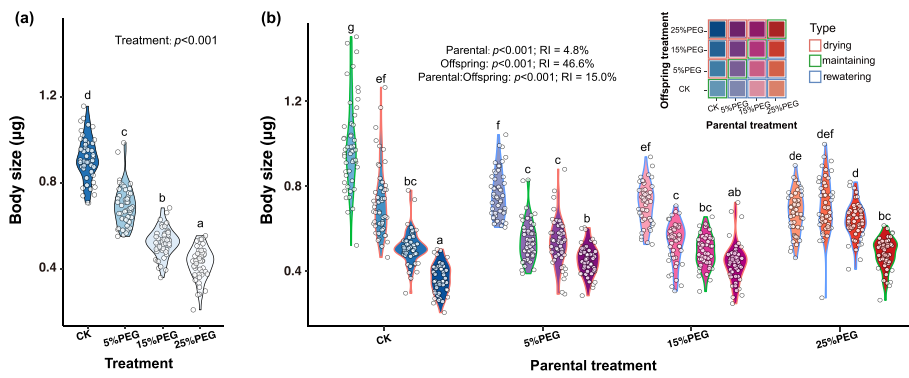
The composition shift hypothesis received support from two transects (Loess and Mongolian Plateau) in the regional-scale study. In these transects, the proportion of small-sized species (bacterivores and fungivores) increased, while the proportion of large-sized species (omnivores and predators) generally decreased as aridity increased (Fig. 2 and S4). Besides the interspecific pattern, the microcosm experiment confirmed the population body size shift hypothesis, as the mean body size of six genera that survived in all treatments decreased with increasing drought ( $p < 0.01$ , Fig. 3b).

Finally, the microcosm experiment that focused on the small-sized bacterivore *Acrobeloides* revealed a significant decrease in body size up to 64% (70% vs. 5% of field capacity) ( $p < 0.001$ , Fig. 3c), confirming the size-at-age hypothesis. Similarly, the Petri dish experiment also

detected a decrease in body size of *Acrobeloides* by 24–53% under dehydrating growth conditions ( $p < 0.001$ , Fig. 4a).

### 3.2. Body size change is partially reversible under drought conditions

To evaluate the reversible plasticity of individual nematodes under drought stress, we conducted a Petri dish experiment involving *Acrobeloides* extracted from the microcosm experiment. We cultured this species on the Petri dish and exposed two generations (parent: F0, offspring: F1) to a range of water stress levels. Our results showed that both the current and parental drought stress, as well as their interactions, significantly reduced the body size of F1 by 22–59% ( $p < 0.001$ , Fig. 4b). Moreover, even when the current environment was fully alleviated, the body size of F1 did not revert to the initial body size of F0 (Fig. 4b). For example, the body size of F1 only reversed by 40% and 36% when they were derived from parents that experienced drought stress of 15% and 25% PEG, respectively (Fig. 4b). Furthermore, offspring born to parents that endured heavy drought environments (15% and 25% PEG) exhibited greater acclimation capacity and showed a relatively smaller reduction in overall body size compared to the



**Fig. 4.** Parental (a) and offspring (b) body size of nematodes under different drought levels in the Petri dish experiment. The filled colors in (b) represent different combinations of drought treatments in parental and offspring generations, while the border colors indicate whether the offspring experienced rewetting, drying or a constant drought level compared to the parental generation. Different lowercase letters indicate significant differences between treatments (Tukey’s honest significant difference test,  $p < 0.05$ ). RI indicates the relative importance of parental environment, current environment and their interactive effect on the body size of offspring.

offspring from parents that suffered from no or light drought environments (CK and 5% PEG) (Fig. 4b).

### 3.3. Drought-induced size-shift reduces metabolic footprint of soil nematodes

Drought-induced body size declined at the community level was accompanied by 80–95% in the nematode metabolic footprint across three transects in the regional-scale study ( $p < 0.001$ , Fig. 5a), as well as in the microcosm experiment ( $p < 0.001$ , Fig. 5b). Specifically, the decline in nematode C footprint was more pronounced in bacterivorous nematodes on the Tibetan Plateau (Fig. S5), as well as in bacterivorous nematodes in the microcosm experiment (Fig. S6).

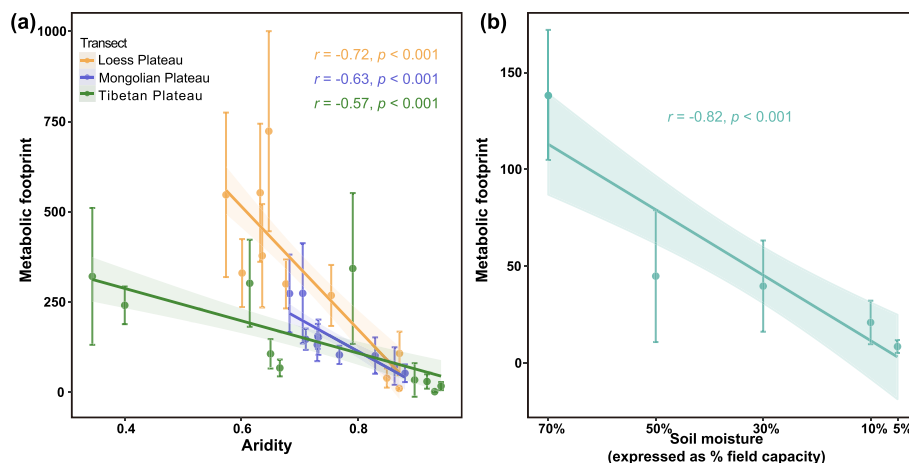
## 4. Discussion

Our study combining three studies at different scales provides robust empirical evidence that drought-induced shrinking body size is an ecological response that occurs across multiple biological scales of soil nematodes. The observed patterns are consistent with our four hypotheses and suggest that drought leads to significant changes in the size structure of soil communities. Given that the biota examined in this study are diverse in the soil food web, and the ecosystems are complex, various potential factors such as microhabitat loss, food limitation and plastic change may impact their body size structure.

Precipitation-induced reduction of soil moisture is a direct physical effect that decreases nematode body size at the community level. This could be attributed to the significant shifts in community composition at the population level (composition shift hypothesis), as confirmed by the regional-scale study that showed a shift in the size spectrum from large-

to small-bodied species in response to reduced precipitation (Fig. 2). Water availability in microhabitats directly affects nematode activity by limiting the soil water films on which nematodes depend (Freckman et al., 1987). Generally, large-bodied organisms belonging to K-strategists are more vulnerable to water limitation (Ledger et al., 2013; Franco et al., 2019). They depend more on thicker water films around soil particles for movement and activity (Wallace, 1968; Franco et al., 2019), and are thus more limited in accessing refuges (e.g., water-filled space in soil) compared to smaller r-strategists (Aspin et al., 2019). However, small-bodied nematodes are expected to be more tolerant of extreme drought conditions, because most of them belong to lower trophic groups (bacterivores and fungivores) that may rely on less water and smaller aggregate fractions (Andriuzzi et al., 2019).

Besides the interspecific patterns, a reduction in soil moisture content can also lead to a decrease in mean body size at the population (population body size shift hypothesis) and individual (size-at-age hypothesis) levels, which can contribute to a decrease in size at the community level. This is supported by evidence from microcosm and Petri dish experiments showing that populations of six genera and one bacterivorous species, *Acrobeloides*, shrink their body size in response to drought (Figs. 3 and 4). One of the most important factors limiting species’ body size is the lack of food resources resulting from water limitations, especially for large-bodied species with relatively high individual metabolic demands (Aspin et al., 2019). For example, a decrease in aboveground biomass, decaying organic matter, and associated microbes during drought periods led to a small-sized community, as evidenced by soil collembola and mites (Xu et al., 2012). Our study also observed a negative relationship between aboveground biomass and community body size in the two transects of the regional-scale study (Fig. S7). These results support the idea that body size shrinkage could



**Fig. 5.** Metabolic footprint of nematode communities along aridity gradients in the regional-scale study (a) and soil moisture gradients in the microcosm experiment (b).

be an adaptive strategy to cope with a period of food shortage (Bendik and Gluesenkamp, 2013), which may be attributed to a decrease in their energy expenditure and metabolic rate (Alonso-Alvarez and Tella, 2001; Zhang et al., 2018).

Plastic changes in response to drought intensification appear to underlie the changes in species' body size, as shown by the Petri dish experiment where the size of *Acrobeloides* was reduced by up to 53%. This body size change is attributed to both the environmental conditions experienced by the current generation (47% relative importance) and the environmental conditions experienced by their parents (5% relative importance). Drought stress can influence the physiology of life-history trade-offs, leading to epigenetic self-regulation that is transmitted to successive generations, resulting in phenotypic modifications in the offspring (Lancaster et al., 2017). Many studies on plants and animals have indicated that stress-induced responses can be inherited through transgenerational stress memory (Boyko et al., 2010; Shama et al., 2014; Beaman et al., 2016). This memory enables species to develop adaptive mechanisms and acquire tolerance to survive drought over generations. This agrees well with our finding that offspring born to parents who experienced severe drought conditions exhibited a relatively smaller reduction in overall body size compared to offspring from parents that suffered no or light drought conditions. Furthermore, our Petri dish experiment observed a partial reversibility of body size when drought stress was partially or fully alleviated in the offspring generation. Although statistical analysis indicates that both current and parental drought conditions explain the size variation, the underlying physiological and genetic mechanisms are still unclear and require further study.

Finally, we found that drought-induced declines in body size at the community level weaken the overall contribution of soil nematodes to C cycling, as measured by metabolic footprint in both the regional-scale study and microcosm experiment (Fig. 5). Since the measurement of metabolic footprint considers both absolute abundance and body size, lower abundance and a smaller body size may both lead to a reduced metabolic footprint. However, the strong evidence of size reduction across biological scales highlights the importance of body size in predicting the effect of drought on ecosystem functioning. Larger species typically have higher absolute energy consumption (Cardillo et al., 2005), thus the community composition shift towards smaller individuals predicts the importance of losing larger taxa for ecosystem functions related to C cycling, such as decreases in decomposition and C turnover. These changes in metabolic footprint of soil nematodes are likely to have implications for global C cycling, given that the amount of C respired by soil nematodes globally is roughly equivalent to 15% of C emissions from fossil fuel use (van den Hoogen et al., 2019).

Our study suggests that drought could reduce the community body size of soil nematodes by shifting towards smaller body sizes within the community. Thus, we predict that large-bodied animals occupying higher trophic levels in the soil food web may be more vulnerable to future droughts. However, there is some cause for optimism as keystone species that are tolerant to drought can partially reverse the effects of drought through rewetting and their offspring can resist shrinkage in drier conditions through transgenerational effects. This plastic change links drought and body size across different biological scales and supports the general rule that shrinking body size is an ecological adaptation to climate change. By examining how body size changes with drought stress from community to individual levels, and how it affects ecosystem functions, this study enhances our understanding of ecosystem structuring and may improve our ability to predict the impacts of extreme dry and wet conditions on biota in the future.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2023.109099>.

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