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Impacts of climate change – simulated flooding and drought events – on terrestrial invertebrates (Enchytraeids and Collembolans)

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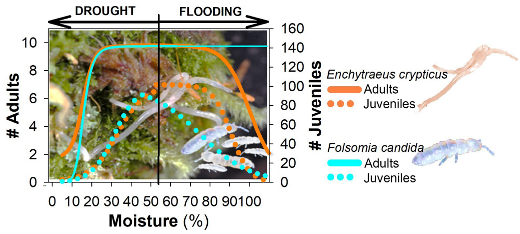
ABSTRACT

- Impacts of soil moisture levels from 10% to 100% on two soil invertebrates.
- *E. crypticus* and *F. candida* survived at extreme scenarios (10% and 100%).
- For both species, reproduction was severely reduced in extreme scenarios.
- Higher adaptative phenotypic plasticity for *F. candida* compared to *E. crypticus*.



Knowledge on impacts of climate change on soil invertebrate communities is scarce. Amongst the biggest challenges are the increase in temperature and arid regions, while at the same time, in other parts of the planet, extreme precipitation events and flooding occur. The aim of the present study was to investigate the impacts of drought and flooding in soil invertebrates. *Enchytraeus crypticus* and *Folsomia candida,* model ecotoxicology test-species (OECD) were used to assess performance (survival, reproduction, size) in LUFA 2.2 soil moistened to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the soil water holding capacity (WHC). Overall, both species had high tolerance for drought and flooding scenarios, with survival threshold for *E. crypticus* being between 10% and 90% moisture and for *F. candida* being between 10% and 100% moisture. Reproduction decreased from moisture ⩽30% and >70% moisture. In drought there was a decrease on adults' size, for both species from ⩽30% moisture. The morphological adaptations observed support evidence of adaptative phenotypic plasticity for both species, but highest for *F. candida*. A redistribution of soil invertebrate species can be expected to occur, this under the present and future climate change scenarios, with new and more tolerant species to prevail in different habitats. This will impact not only soil biodiversity structure, but also its function.

Keywords climate change, soil moisture, phenotypic plasticity, biodiversity, ecosystem services

1 Introduction

One of the biggest impacts of climate change and temperature rise is the increase of global arid regions (European Commission, 2019). Estimates point to an increase of global drylands of about 23% (European Commission, 2019). Predicted hotspots with the shifting towards arid regions identify Amazonia, the Mediterranean, north-western and eastern Asia (Spinoni et al., 2021) as the largest areas. Other major regions are central North America, south-western and south-eastern South America, north-eastern Brazil, and south-western and south-eastern Africa (Spinoni et al., 2021). Simultaneously to increased aridness, in other parts of the planet, the opposite, increase of extreme precipitation events and flooding are predicted (Tabari, 2020). Estimations are that flood intensity events will occur in 75.9% of land area in humid regions (Tabari, 2020). The occurrence and the increase on the frequency of extreme events brings great costs to terrestrial (and aquatic) ecosystems services, and to food production worldwide with serious consequences to human populations (Amundson et al., 2015; Tabari, 2020; Eekhout and de Vente, 2022). The response of how natural populations deal with such climate changes have been investigated mostly in mammals, plants, birds, amphibians, and marine ecosystems (e.g. coral reefs) (Pereira et al., 2012; Ciobanu et al., 2019; Guerra et al., 2021; Yusefi et al., 2021). However, often forgotten or even neglected is the soil fauna, which is a highly important group for key ecosystems' services and goods, e.g., water supply, nutrient cycling, soil formation, food production, flood and erosion control and

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climate regulation (Lavelle et al., 2006; Leal Filho et al., 2023). The protection of soil fauna is essential (Lavelle et al., 2006; Ciobanu et al., 2019; Guerra et al., 2021), as even small changes in soil invertebrate key species can have great impact in ecosystems functions (Barrett et al., 2008). A study with earthworm species (soil macrofauna), showed that drought extreme events decreased earthworm functional diversity and species richness, which of course compromises litter decomposition processes (da Silva et al., 2020). Soil biodiversity is among the best indicators of soil quality in terrestrial ecosystems (Lavelle et al., 2006) and ultimately key for human populations (Guerra et al., 2021). Drought and flooding are both closely associated with soil erosion, compromising natural terrestrial ecosystems services and food production worldwide (Amundson et al., 2015; Eekhout and de Vente, 2022). During soil erosion the loss or reduction of organic matter affects soil water holding capacity and nutrient cycling (Pimentel, 2006; Eekhout and de Vente, 2022). Among soil conservation measures the protection of biodiversity is part of the solution to mitigate soil challenges (Albaladejo et al., 2021; Eekhout and de Vente, 2022).

In nature, natural populations deal with such extreme events via processes of adaptative phenotypic plasticity, stimulating evolution, or otherwise face extinction (Schlichting and Wund, 2014; Lalejini et al., 2021). For populations less plastic to certain stressors (e.g., temperature or moisture), phenotypes have lower chance to change under environmental stress, and natural selection will always act to select the most fitted (Lalejini et al., 2021); whereas for more plastic populations the chances for survival are higher. Responses can be of adaptive or non-adaptive plasticity, with advantageous or disadvantageous changes in phenotypes, respectively (Lalejini et al., 2021). Available studies on the impacts of climate change in soil invertebrates show that terrestrial insects and molluscs possess adaptative phenotypic plasticity (Schilthuizen and Kellermann, 2014). Additionally, the ability of a species to cope with climate change events is obviously related with its innate stress tolerance range to abiotic factors, as observed by their capacity to survive and reproduce within a certain stress (Schilthuizen and Kellermann, 2014). Hence, this can be used as a proxy for organisms adaptative phenotypic plasticity (Deutsch et al., 2008; Huey et al., 2009; Sunday et al., 2011).

Phenotypic plasticity refers to changes in gene expression resulting in changes at the phenotype level (Schlichting and Wund, 2014; Lalejini et al., 2021). At the molecular level, genetics and epigenetics mechanisms support phenotypic plasticity (Fusco and Minelli, 2010; Schlichting and Wund, 2014; Duncan et al., 2020). However, this molecular architecture is still poorly known in natural populations (Duncan et al., 2020), but is a key to understand how species will cope with climate change (Guarino et al., 2022). Nevertheless, this topic is virtually unknown for soil invertebrates. Obviously, the lack of studies on such topics is also related to the limitations in molecular tools available for environmental species. Over the last years there has been a lot of progress, e.g., for the enchytraeid (*Enchytraeus crypticus*) and collembolan (*Folsomia candida*), two ecotoxicological models with the whole genome sequenced (Faddeeva-Vakhrusheva et al., 2017; Amorim et al., 2021), and also with optimized methods to evaluate epigenetics (Bicho et al., 2020a, 2020b, 2021).

Enchytraeids and collembolans belonging to the soil mesofauna, are abundant in several ecosystems worldwide, having a global distribution, being essential for several soil ecosystem services, with functions like the decomposition of organic matter and nutrient cycling (Cragg and Bardgett, 2001; Pelosi and Römbke, 2018; Potapov et al., 2020). Enchytraeids can occupy different trophic niches being epigeic, epi-endogeic or endogeic (Korobushkin et al., 2024), although most species are endogeic and live in the first 5 to 10 cm of soil, with similar functions to earthworms (like soil bioturbation) but at different scales (van Vliet et al., 1993; Pelosi and Römbke, 2018), which make them excellent bioindicators of soil quality (Pelosi and Römbke, 2018). Collembolans are either euedaphic, hemidedaphic or hepedaphic (Kærsgaard et al., 2004; Holmstrup et al., 2015). They are particularly important due to grazing activity on fungi with benefits to plants (Lussenhop, 1996; Fountain and Hopkin, 2005). The few available studies with enchytraeids showed impact on survival to mild levels of drought (LD_{50} corresponding to about 32% soil moisture) (Maraldo et al., 2009). Previous field studies showed impacts of changes in soil moisture in enchytraeid populations, also showing that they are sensitive to drought conditions (Abrahamsen, 1971; Briones et al., 1997). For flooding scenarios, a field study with enchytraeids showed population reduction after long periods of flooding, although followed by a rapid recovery (Plum and Filser, 2005). For collembolans (euedaphic, *Folsomia candida*) a laboratory study showed animals survival for 6 days at drought conditions (soil water potential (SWP): −24.7 bar) (Sjursen et al., 2001). For other euedaphic collembolan species (*Protaphorura tricampata*), high level of resistance was observed to extreme drought conditions (SWP: −35 bar) for 22 days (Holmstrup and Bayley, 2013). Available laboratory studies, where other life history traits (reproduction and growth) are evaluated, the effects of different soil moistures are limited to mild ranges (25% to 75% of the soil water holding capacity) (Bandow et al., 2014; Silva et al., 2022; Szabó et al., 2022). Further, for ecological relevant soil fauna, like macrofauna, reviews indicated a limited number of studies on effects on earthworms for extreme soil moisture regimes (e.g., flooding and drought) (Singh et al., 2020; Kaka et al., 2021). Hence, literature shows a knowledge gap on soil invertebrate responses, beyond survival (e.g., reproduction, growth) to extreme climate scenarios and outside sufficient relevance, since organisms experience a wider range of climate events and not mere average conditions (Maraldo and Holmstrup, 2010). Extreme events may pose risk to these communities beyond their threshold survival, with direct impact in their abundance, diversity, and composition (Maraldo and Holmstrup, 2010; Coyle et al., 2017).

Hence, in the current study the main aim was to investigate the innate stress tolerance range to wide soil moisture range, representative of extreme scenario events, drought and flooding (10% to 100% moisture) to investigate threshold survival limits and other life history traits (reproduction and growth). Two standard soil model species were selected, *Enchytraeus crypticus* (Enchytraeidae) and *Folsomia candida* (Collembola) (OECD, 2016a, 2016b), due to their extensive use in ecotoxicological assays, and because they represent different ecological functions within the soil mesofauna. *E. crypticus* and *F. candida* were exposed in LUFA 2.2 soil for 21 and 28 days, respectively, to assess survival, reproduction, and size.

2 Materials and methods

2.1 Test organisms

The test species *Enchytraeus crypticus* (Oligochaeta: Enchytraeidae) and *Folsomia candida* (Collembola) were used. *E. crypticus* cultures are kept in agar, consisting of sterile Bacti-Agar medium (Oxoid, Agar No. 1) and a mixture of four different salt solutions at the final concentrations of 2 mM $CaCl_2{\cdot}2H_2O$, 1 mM $MgSO_4$, 0.08 mM KCl, and 0.75 mM $NaHCO_3$, under controlled conditions of temperature (20±1) °C and photoperiod (16:8 hours light:dark). The cultures were fed with ground autoclaved oats twice per week. Adults with well-developed clitellum were used.

F. candida cultures are kept on a moist substrate of plaster of Paris and activated charcoal (8:1 ratio), at (20 ± 1) °C, under a photoperiod of 16:8 (hours, light:dark). Food consists of dried baker's yeast (*Saccharomyces cerevisae*) provided weekly. Age-synchronized juveniles (10–12 days) were used for the test.

2.2 Test soil and spiking procedures

The standard LUFA 2.2 natural soil (Speyer, Germany) was used. The main characteristics are summarised as follows: pH (0.01 M $CaCl_2$) of 5.6, 1.61% organic matter, 8.5 meq/100 g CEC (cation exchange capacity), 44.48% maximum WHC (water holding capacity), grain size distribution of 8.9% clay, 13.9% silt, and 77.2% sand.

In the standard guideline for enchytraeids and collembolans (OECD, 2016a, 2016b) the recommendation is to have 40%−60% of the soil maxWHC. The aim was to cover as wider range of moisture variation as possible to assess tolerance ranges. To obtain all soil test moisture regimes (flooding to drought) deionised water was added to obtain the following concentrations: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the soil WHC (corresponding to the following volumes (mL) of water added to each 100g of soil: 4.45, 8.90, 13.34, 17.79, 22.24, 26.69, 31.14, 35.58, 40.03, 44.48 mL). For each soil moisture regime, water added to soil was homogeneously mixed, split onto test vessels, and immediately covered with parafilm to avoid water evaporation. Soil was equilibrated for 1 day prior to test start. At test start, small holes were made in the parafilm for aeration, and water was replenished weekly to maintain the corresponding start soil moisture regime.

2.3 Test procedures

2.3.1 *Enchytraeids*

Test procedures followed the standard guideline (OECD, 2016a) with adaptations. In short, 10 organisms from cultures were placed in each test container (∅4 cm) with 20 g of moist soil (moisture was adjusted as mentioned above), and food supply (24±1 mg, autoclaved rolled oats, grinded). Food (12±1 mg) and water were replenished weekly. Exposure duration was 21 days (standard) at 20 ºC and 16:8 h photoperiod. Four replicates per treatment/sampling were done, plus one without organisms for measurements of pH and moisture content. Endpoints included survival, reproduction and adults' size (number of adults, number of juveniles, and adult´s length). At test end, organisms were fixed with 96% ethanol and Bengal rose (1% solution in ethanol) to colour and facilitate counting. Samples were then sieved through three meshes (1.6, 0.5, 0.2 mm) to separate individuals from most of the soil and to facilitate counting using a stereo microscope. To measure adults' size (length), animals were transferred to a petri-dish with a millimetre paper, photographed and analysed using the software ImageJ (Schneider et al., 2012).

2.3.2 *Collembolans*

Test procedures followed the standard guideline (OECD, 2016b) with adaptations. In short, 10 organisms were introduced into each test container (∅5.5 cm), with 30 g of moist soil (moisture was adjusted as mentioned above) and food supply (7–10 mg of granulated dry yeast). Exposure duration was 28 days (standard) at 20 ºC and a photoperiod of 16:8h (light:dark). Food (7–10 mg) and water were replenished weekly. Four replicates per treatment/sampling were done, plus one without organisms for measurements of pH and moisture content. Endpoints included survival, reproduction and size (number and length of adults and juveniles). At test end, vessels were flooded with water, the content was transferred to a crystallizer, stirred gently with a spatula, and the surface was photographed for automatic counting and measuring of adults and juveniles using the software ImageJ (Schneider et al., 2012).

2.4 Data analysis

One-way analysis of variance (ANOVA) followed by Dunnett's comparison post-hoc test ($p \leqslant 0.05$) was used to assess differences between controls and treatments (SigmaPlot, 1997). Effect Concentrations (ECx) estimates were done modelling data to logistic 2 parameters regression model, using the Toxicity Relationship Analysis Program (TRAP v1.3) software. Since dose-response was overall bell-shape, for the section with increased response data was inverted to fit to model. Dose response data concerning drought, 10%−50%, was inverted to use the dose-response regression models to estimate ECx values. In short, data was transformed to percentage of the maximum response (average at 50% for both species (40% moisture for size in *F. candida*)). Zero values were replaced by 0.0001 to avoid no data point in logarithmic scale.

3 Results

The validity criteria were fulfilled as within the standard OECD guideline (OECD, 2016a, 2016b) in controls at 40%−60% moisture. For *Enchytraeus crypticus*, adult mortality was <20% and the number of juveniles >50 per replicate, with a coefficient of variation <50%. For *Folsomia candida*, adult mortality was <20% and the number of juveniles >100 per replicate, with a coefficient of variation <30%.

For *E. crypticus* (Fig. 1A) survival was affected only at extreme levels of 10% and 100% moisture, with LC_{90s} of 16% and 99% moisture (i.e., each side of the optimum curve) for drought and flooding scenarios, respectively. Reproduction showed an optimum between 50%−70%, outside which (<50% and >70%) it decreases.

Under flooding the *E. crypticus* EC_{50} was estimated to be 85% moisture and in drought scenarios the EC_{50} was of 33% moisture (Table 1). In terms of size (Fig. 1B) there was an impact of drought on adults' length, starting from $\leqslant$ 30% moisture (EC_{50}=23%).

For *F. candida* (Fig. 1C) survival was not impacted, except at extreme drought conditions $\leqslant$10% moisture. Reproduction showed best performance around 50% moisture, outside which there was a decrease, with EC_{50} of 78% under flooding and 39% moisture in drought conditions (Table 1). Size (Fig. 1D) was mostly impacted in drought, with smaller adults from $\leqslant$30% moisture (EC_{50}=18.5%).

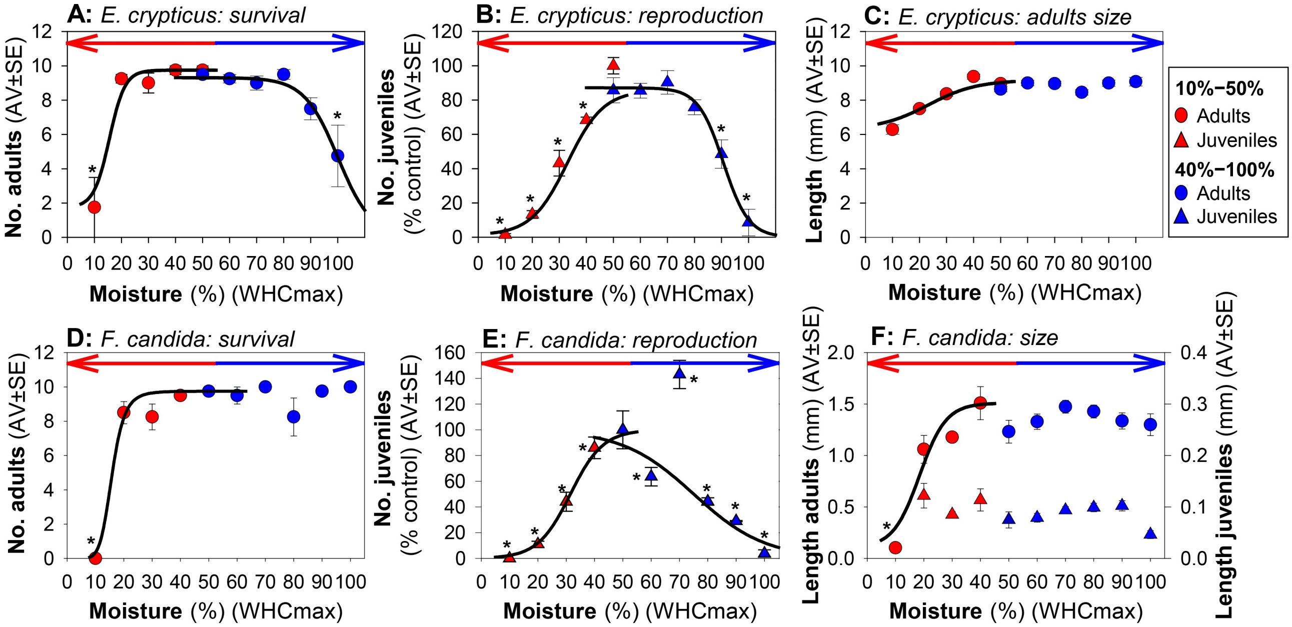

Fig. 1 Results of the standard Enchytraeid Reproduction Test (ERT) (21 days) for *Enchytraeus crypticus* survival (A), reproduction (B) and size (length) (C); and of the collembolan standard test (28 days) for *Folsomia candida* survival (D), reproduction (E) and size (length) (F). Exposure in LUFA 2.2 soil moist to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the soil WHC. All values are expressed as average ± standard error (AV ± SE). For reproduction values are expressed as percentage of the control (50% moisture standard). The solid lines represent the model fit to data. [For *F. candida* 70% moisture (outlier) was not included in the model].

Table 1 Summary of the Effect Concentrations (ECx), for *Enchytraeus crypticus* and *Folsomia candida* when exposed to moisture range in LUFA 2.2 soil.

Moist scenario	Endpoint	EC_{10}	EC_{50}	EC_{90}	Model&parameters
Enchytraeus crypticus					
Flooding	Survival	86 75<CI<97	99 94<CI<104	113 98<CI<127	log 2 par S: 0.4; Y0: 10
	Reproduction	76 72<CI<80	85 83<CI<87	95 91<CI<99	log 2 par S: 0.5; Y0: 945
Drought	Survival	22 17<CI<27	16 12<CI<19	9 3<CI<15	log 2 par S: 0.9; Y0: 8.2
	Reproduction	48 44<CI<52	33 31<CI<35	18 15<CI<22	log 2 par S: 0.4; Y0: 331
	Size (adults)	43 38<CI<48	23 21<CI<26	4 0<CI<10	log 2 par S: 0.3; Y0: 3
Folsomia candida					
Flooding	Reproduction	72 66<CI<78	78 76<CI<80	84 80<CI<88	log 2 par S: 0.2; Y0:865
Drought	Survival	22 19<CI<25	16 13<CI<19	12 8<CI<17	log 2 par S: 4; Y0: 9.7
	Reproduction	44 39<CI<49	31 29<CI<34	19 14<CI<24	log 2 par S: 0.4; Y0: 865
	Size (adults)	29 22<CI<36	18 15<CI<22	8 0<CI<17	log 2 par S: 0.5; Y0: 1.4

Results show EC estimates and 95% confidence intervals. S: slope; Y0: intercept.

4 Discussion

For *Enchytraeus crypticus,* although survival was only affected at the extremes of drought (10% moisture) and flooding (100% moisture), reproduction showed a narrower range for average performance, between 50%−70% moisture. The recommended soil moisture in the standard guideline (OECD, 2016a) is between 40% to 60% of the WHC, hence this is within the adequate conditions also observed in the present study.

For *Folsomia candida,* survival was affected by extreme drought (10% moisture) but not by flooding. This could be related to the fact that these animals have a water repellent exoskeleton cuticle and are actually able to stand on the water surface, walking on the soil particles, rather than within and in close contact like enchytraeids. Reproduction was viable between 40%−70% moisture, becoming impacted at ⩽30% and ⩾80% moisture. It is perhaps of interest to note that reproduction was well above average at 70% moisture, indicating that the increase in soil moisture seems preferred compared to decrease for *F. candida* in LUFA 2.2 soil. This is slightly higher than the recommended in the standard guideline (40% to 60%) (OECD, 2016b), but still aligned and is well-known to depend on the species and soil type, among others. Sandy soils will allow much higher drainage than clay, in which the soils will remain soaked for much longer periods. For instance, a study using OECD standard soil with *F. candida* exposed to 30%, 50% and 70% moisture (Bandow et al., 2014) showed a similar pattern, with reproduction decreased at 30% and increased at 70%. On the other hand, a similar exposure using LUFA 2.2 soil with *F. candida* exposed to 30%, 50% and 75% (Szabó et al., 2022) showed reproduction similar to 50% moisture. Another study, using LUFA 2.2 soil with *F. candida* exposed to 25%, 50% and 75% moisture conditions (Silva et al., 2022), showed differences in reproduction compared to 50% for drought conditions, but not for flooding. Hence, it seems that there is a chance for higher variability in this moisture interval. Overall, previous results offer agreement regarding the preference for increased moisture, with significant increase at 70%, although by 80% already a decrease occurs (EC_{50} =78%, EC_{90}=84%).

Despite the large impact of extreme events, both species showed a remarkable tolerance for drought and flooding levels. *E. crypticus* and *F. candida* showed a survival threshold to drought up to 10% to 20% moisture, respectively. For flooding, survival threshold was much higher for *F. candida*. It is noted that drought caused a strong impact on size of *F. candida* adults, but not flooding, and this can be linked to these differences: larger animals have higher survival than smaller.

Oligochaete species are in general very sensitive to drought stress, due to their permeable skin surface, however this sensitivity is species dependent (Maraldo et al., 2009). For example, soil water potential (SWP) of –9.8 bar was lethal for *Cognettia sphagnetorum* (Abrahamsen, 1971; Maraldo et al., 2009). *Fridericia galba* survived to SWP < –9.8 bar in soil (equivalent moist to 20% of WHC) for more than 49 days (Dözsa-Farkas, 1977; Maraldo et al., 2009). A study with *E. albidus* and *E. crypticus* (chronic exposure, 6 and 4 weeks, respectively) to drought levels showed impacts on survival and reproduction at mild levels of drought: 32% to 30% moisture (Maraldo et al., 2009). The *E. albidus* and *E. crypticus* 50% lethality drought level (LD_{50}) were from –2 to –2.5 bar (SWP), corresponding to 32% to 30% moisture (Maraldo et al., 2009). The soil used in this study consisted of peat (25%) and agricultural loamy sand (75%, consisting of: 35% coarse sand, 45% fine sand, 9.4% silt, 8.9% clay and 1.7% organic matter) (Maraldo et al., 2009). The SWP for a given soil is influenced by properties like soil texture, organic matter, among others (Ma et al., 2022). The differences for the results observed in our study, with previous studies, can be related with the different soil properties. For example, same moisture levels in soil LUFA 2.2 (our study) could correspond to higher SWP levels, i.e., 30% moisture correspond to > –2.5 bar. In a field experiment for 2 months, exposure to extreme drought conditions (average of 50 mm of irrigation), showed that enchytraeid populations (most abundant species: *C. sphagnetorum*) recovered within 3 months (Maraldo and Holmstrup, 2009). Enchytraeids can survive drought conditions via migration to microhabitats with higher moister levels (Uhía and Briones, 2002). Results from our laboratory study cannot confirm this as exposure is not fit for purpose [in our laboratory study the soil vials (test vessels) are homogenously moistened (mixed), i.e. no micro-habitat], but migration to more moist habitats (e.g., deeper soil layers or micro-patches) could reveal a very important mitigation behaviour in the real environment. Another important coping strategy for enchytraeids is that their production of cocoons, which are more tolerant to drought than juveniles, could facilitate recovery (Lagerlöf and Strandh, 1997). This was in agreement with the observations by the mentioned study of (Maraldo et al., 2009) where *E. crypticus* juveniles were less tolerant to drought conditions, showing a 50% reduction of reproduction (ED_{50}) of –0.07 bar, much higher than for survival (LD_{50}: –2.5 bar). In this study, both species showed a higher tolerance to drought conditions after acute exposure (2 days) (*E. albidus* LD_{50} = –13.9 bar; *E. crypticus* LD_{50} = –12.3 bar) (Maraldo et al., 2009). For *E. albidus* the osmotic pressure of body fluids was higher, which partly explained the higher tolerance (Maraldo et al., 2009). In our study, it is a possibility that *E. crypticus* activated physiological mechanisms to increase osmotic pressure, but we have not recorded this. In stressful environments, also as the experienced via drought soil regimes, earthworms coil into a tight ball and excrete a protective mucus to reduce water loss (McDaniel et al., 2013). This behaviour response has also been observed in enchytraeids under stress conditions, e.g., contaminated soils, it is usually observed through the glass test vessel during exposure. It is possible that enchytraeids have behaved with such response, but we have not noticed it.

For *F. candida*, a study on drought (Silva et al., 2022) showed no effect on survival up to 25% soil moisture, but reproduction was reduced, in agreement with our current results. For collembolan species the physiological adaptations to drought have been described by (Holmstrup et al., 2001), showing their higher tolerance to drought regulated via e.g. water vapour absorption, high osmotic pressure, accumulation of sugars and polyols and also free amino acids (Holmstrup et al., 2015). Moreover, morphological adaptations have also been described for euedaphic collembolan species (*F. candida*), like small body size and high cuticular permeability (Kærsgaard et al., 2004). In our study *F. candida* adults were smaller at lower soil moisture (EC_{50} = 18% moisture), hence this could be a strategy or consequence.

Less studies have investigated the impacts of flooding scenarios in soil invertebrates. Flooding events can create hypoxia or even anoxia conditions in soil (Coyle et al., 2017). Our results showed that *E. crypticus* was less tolerant than *F. candida* in terms of survival, although reproduction was similarly impacted (*E. crypticus*: EC_{50}=85%; *F. candida*: EC_{50}=78% moisture). For oligochaetes, oxygen retrieval occurs from direct diffusion between water and epidermis from the surrounding (pore) water. Although recent evidence show that enchytraeids can be either epigeic, epi-endogeic or endogeic (Korobushkin et al., 2024), the trophic niche for *E. crypticus* in natural habitats is still not known. In our experiments, during exposure in flooded soil (100% moisture), animals were observed within the water layer in the test vessels. Hence, death due to hypoxic conditions would likely be occurring. This has been suggested as a major impact of flooding events in endogeic animals because they are unable to avoid or rapidly escape flooding conditions (Coyle et al., 2017). A field study with enchytraeid populations (most abundant species: *Cognettia glandulosa*, *Henlea ventriculosa* and *Fridericia bulboides*) showed that these were severely reduced after long periods (up to 5 months) of flooding (5 mm h^{-1}), although these populations show a rapid recovery within 2 to 3 months (Plum and Filser, 2005). The oligochaetes of the genus *Enchytraeidae* are known to be amongst the most abundant in flooded rice fields (Schmidt et al., 2015). Clearly, enchytraeids tolerance to flooded conditions is species dependent.

As to collembolans, *F. candida* obtains oxygen from air, via their cuticle (Fountain and Hopkin, 2005). *F. candida* can survive in pockets of gas, between soil particles unsaturated with water, during flooding, where levels of oxygen can be below the optimal (Fountain and Hopkin, 2005). A study showed that during hypoxia, *F. candida* increases heart contraction frequency, hence animals can maintain partial pressure differences between external medium and tissues (Paul et al., 1997; Fountain and Hopkin, 2005). Also, *F. candida* showed metabolic changes (increased lactate levels) under anaerobic conditions (Marx et al., 2009; Marx et al., 2012). Further, it has also been described that *F. candida* can survive at the surface of water tables (Peterson et al., 2006). During our study, animals were observed always standing above the surface of the water layer that was formed in the test vessels. On the other hand, in terms of reproduction, the eggs of *F. candida* are known to be very sensitive to high levels of moisture (Fountain and Hopkin, 2005), which explains the observed impact in reproduction, while not on survival.

The global predictions are not only for more regions in the planet to suffer from extreme scenarios events but also an increase on their frequency (Tabari, 2020; Spinoni et al., 2021). Hence, it is of vital importance to understand the potential impacts and if predictions surpass species threshold limits. As previously shown, drought conditions caused a reduction in earthworms` functional diversity and species richness, with consequent slowdown of litter decomposition processes (da Silva et al., 2020). Hence, a redistribution of soil invertebrate species functional groups can be expected to occur, this under the present and future climate change scenarios, with new and more tolerant species to prevail in different habitats. This will have direct consequences on ecosystem services and goods, like the agricultural sector.

Beyond survival thresholds, other mechanisms may play an intermediate role, e.g., as measured by size, overall drought impacted negatively both *F. candida* and *E. crypticus*, A survival strategy can impose costs on other traits, e.g., size and reproduction. This has been also observed for *Enchytraeus albidus* under temperature stress (Holmstrup et al., 2022). Regardless of which specific physiological and or morphological mechanisms might be involved, our study shows evidence of adaptative phenotypic plasticity for both species to differences in moisture. Namely, within drought conditions, changes in the phenotypes were observed while surviving, eventually shifting to a new optimum that guarantees the individual survival. Such type of phenotypic plasticity, where shifts in life-history and morphological traits occur, have been shown for several taxa, from protists to vertebrates (Miner et al., 2005; Pigliucci, 2005). Long-term studies with extreme scenarios followed by a recovery phase would be extremely relevant to 1) further understand possible consequences at the population level and, 2) understand if *E. crypticus* and *F. candida* phenotypic plasticity reflects adaptation responses to drought conditions, like an increased tolerance to drought that can be maintained or passed throughout generations. For example, for another enchytraeid species (*Cognettia sphagnetorum*) this was not shown, after several years of intermediate exposure to drought conditions, populations still maintained the same sensitivity levels to low moisture conditions (Maraldo et al., 2008). Nevertheless, long-term studies with several drought levels followed by a recovery period, also covering several life stages, are necessary to fully understand *E. crypticus* responses and the mechanisms behind drought tolerance.

5 Conclusions

E. crypticus and *F. candida* showed high tolerance for drought and flooding scenarios, with survival threshold being between 10% and 90% for *E. crypticus* and 10% and 100% moisture for *F. candida*, whereas reproduction was more affected for both species, decreasing roughly outside ⩽30% and >70% moisture. Mainly for drought conditions, size was impacted (decrease in body size). These morphological adaptations support evidence of adaptative phenotypic plasticity for both species, but highest for *F. candida*. As learned from current limits of these soil invertebrates, a redistribution of soil invertebrate species can be expected to occur, this under the present and future climate change scenarios, with new and more tolerant species to prevail in different habitats. This will have direct consequences on ecosystem services and goods, like the agricultural sector and a profound impact on human health. Extreme drought or flooding are expected to occur in more regions of our planet and at higher frequence due to climate change. These extreme events can change the abundance and diversity of soil invertebrate communities. Animals have the capacity to respond to environmental changes, namely recover, but at this level and frequency it will force a structural change.

Declaration of competing interest

The authors declare no competing interests.

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References

Abrahamsen, G., 1971. The influence of temperature and soil moisture on the population density of *Cognettia sphagnetorum* [Oligochaeta: Enchytraeidae] in cultures with homogenized raw humus. Pedobiologia 11, 417–424.

Albaladejo, J., Díaz-Pereira, E., de Vente, J., 2021. Eco-holistic soil conservation to support land degradation neutrality and the sustainable development goals. CATENA 196, 104823.

Amorim, M.J.B., Gansemans, Y., Gomes, S.I.L., Van Nieuwerburgh, F., Scott-Fordsmand, J.J., 2021. Annelid genomes: *Enchytraeus crypticus*, a soil model for the innate (and primed) immune system. Lab Animal 50, 285–294.

Amundson, R., Berhe, A.A., Hopmans, J.W., Olson, C., Sztein, A.E., Sparks, D.L., 2015. Soil and human security in the 21st century. Science 348, 6235.

Bandow, C., Karau, N., Römbke, J., 2014. Interactive effects of pyrimethanil, soil moisture and temperature on *Folsomia candida* and *Sinella curviseta* (Collembola). Applied Soil Ecology 81, 22–29.

Barrett, J.E., Virginia, R.A., Wall, D.H., Adams, B.J., 2008. Decline in a dominant invertebrate species contributes to altered carbon cycling in a low-diversity soil ecosystem. Global Change Biology 14, 1734–1744.

Bicho, R.C., Faustino, A.M.R., Rêma, A., Scott-Fordsmand, J.J., Amorim, M.J.B., 2021. Confirmatory assays for transient changes of omics in soil invertebrates – Copper materials in a multigenerational exposure. Journal of Hazardous Materials 402, 123500.

Bicho, R.C., Roelofs, D. Mariën, J., Scott-Fordsmand, J.J., Amorim, M.J.B., 2020a. Epigenetic effects of (nano)materials in environmental species – Cu case study in *Enchytraeus crypticus*. Environment International 136, 105447.

Bicho, R.C., Scott-Fordsmand, J.J., Amorim, M.J.B., 2020b. Developing an epigenetics model species - From blastula to mature adult, life cycle methylation profile of *Enchytraeus crypticus* (Oligochaete). Science of the Total Environment 732, 139079.

Briones, M.J.I., Ineson, P., Piearce, T.G., 1997. Effects of climate change on soil fauna; responses of enchytraeids, Diptera larvae and tardigrades in a transplant experiment. Applied Soil Ecology 6, 117–134.

Ciobanu, M., Eisenhauer, N., Stoica, I.A., Cesarz, S., 2019. Natura 2000 priority and non-priority habitats do not differ in soil nematode diversity. Applied Soil Ecology 135, 166–173.

Coyle, D.R., Nagendra, U.J., Taylor, M.K., Campbell, J.H., Cunard, C.E., Joslin, A.H., Mundepi, A., Phillips, C.A., Callaham., M.A.Jr., 2017. Soil fauna responses to natural disturbances, invasive species, and global climate change: current state of the science and a call to action. Soil Biology and Biochemistry 110, 116–133.

Cragg, R.G., Bardgett, R.D., 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology and Biochemistry 33, 2073–2081.

da Silva, P.M., Nascimento, E., Reis, F., Briones, M.J.I., Brussaard, L., Sousa, J.P., 2020. More frequent droughts slow down litter decomposition across European agroecosystems and increase the importance of earthworm functional diversity. Applied Soil Ecology 153, 103628.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences of the United States of America 105, 6668–6672.

Dözsa-Farkas, K., 1977. Beobachtungen über die Trockenheitstoleranz von Fridericia galba (Oligochaeta, Enchytraeidae). Opusc Zool Budapest 14, 77–83.

Duncan, E.J., Leask, M.P., Dearden, P.K., 2020. Genome architecture facilitates phenotypic plasticity in the honeybee (*Apis mellifera*). Molecular Biology and Evolution 37, 1964–1978.

Eekhout, J.P.C., de Vente, J., 2022. Global impact of climate change on soil erosion and potential for adaptation through soil conservation. Earth-Science Reviews 226, 103921.

European Commission, 2019. World Atlas of Desertification [Online]. (accessed May 24, 2019).

Faddeeva-Vakhrusheva, A., Kraaijeveld, K., Derks, M.F.L., Anvar, S.Y., Agamennone, V., Suring, W., Kampfraath, A.A., Ellers, J., Le Ngoc, G., van Gestel, C.A.M., Mariën, Smit, S., van Straalen, N.M., Roelofs, D., 2017. Coping with living in the soil: the genome of the parthenogenetic springtail *Folsomia candida*. BMC Genomics 18, 493.

Fountain, M.T., Hopkin, S.P., 2005. *Folsomia candida* (collembola): a "standard" soil arthropod. Annual Review of Entomology 50, 201–222.

Fusco, G., Minelli, A., 2010. Phenotypic plasticity in development and evolution: facts and concepts. Philosophical Transactions of the Royal Society B: Biological Sciences 365, 547–556.

Guarino, F., Cicatelli, A., Castiglione, S., Agius, D.R., Orhun, G.E., Fragkostefanakis, S., Leclercq, J., Dobránszki, J., Kaiserli, E., Lieberman-Lazarovich, M., Sõmera, M., Sarmiento, C., Vettori, C., Paffetti, D., Poma, A.M.G., Moschou, P.N., Gašparović, M., Yousefi, S., Vergata, C., Berger, M.M.J., Gallusci, P., Miladinović, D., Martinelli, F., 2022. An epigenetic alphabet of crop adaptation to climate change. Frontiers in Genetics 13, 818727.

Guerra, C.A., Bardgett, R.D., Caon, L., Crowther, T.W., Delgado-Baquerizo, M., Montanarella, L., Navarro, L.M., Orgiazzi, A., Singh, B.K., Tedersoo, L., Vargas-Rojas, R., Briones, M.J.I., Buscot, F., Cameron, E.K., Cesarz, S., Chatzinotas, A., Cowan, D.A., Djukic, I., van den Hoogen, J., Lehmann, A., Maestre, F.T., Marín, C., Reitz, T., Rillig, M.C., Smith, L.C., de Vries, F.T., Weigelt, A., Wall, D.H., Eisenhauer, N., 2021. Tracking, targeting, and conserving soil biodiversity. Science 371, 239–241.

Holmstrup, M., Bayley, M., 2013. *Protaphorura tricampata*, a euedaphic and highly permeable springtail that can sustain activity by osmoregulation during extreme drought. Journal of Insect Physiology 59, 1104–1110.

Holmstrup, M., Sjursen, H., Ravn, H., Bayley, M., 2001. Dehydration tolerance and water vapour absorption in two species of soil-dwelling Collembola by accumulation of sugars and polyols. Functional Ecology 15, 647–653.

Holmstrup, M., Slotsbo, S., Rozsypal, J., Henriksen, P.G., Bayley, M., 2015. Accumulation of free amino acids during exposure to drought in three springtail species. Journal of Insect Physiology 82, 114–121.

Holmstrup, M., Sørensen, J.G., Dai, W.C., Krogh, P.H., Schmelz, R.M., Slotsbo, S., 2022. Analysis of heat and cold tolerance of a freeze-tolerant soil invertebrate distributed from temperate to Arctic regions: evidence of selection for extreme cold tolerance. Journal of Comparative Physiology B 192, 435–445.

Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Álvarez Pérez, H.J., Garland, T., 2009. Why tropical forest lizards are vulnerable to climate warming. Proceedings of the Royal Society B: Biological Sciences 276, 1939–1948.

Kærsgaard, C.W., Holmstrup, M., Malte, H., Bayley, M., 2004. The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. Journal of Insect Physiology 50, 5–15.

Kaka, H., Opute, P.A., Maboeta, M.S., 2021. Potential impacts of climate change on the toxicity of pesticides towards earthworms. Journal of Toxicology 2021, 8527991.

Korobushkin, D.I., Guseva, P.A., Gongalsky, K.B., Saifutdinov, R.A., Zaitsev, A.S., Degtyarev, M.I., 2024. Are there different trophic niches of enchytraeids? A stable isotopic ($\delta^{13}C$, $\delta^{15}N$) evidence. Soil Biology and Biochemistry 194, 109422.

Lagerlöf, J., Strandh, M., 1997. Hatching of Enchytraeidae (Oligochaeta) from egg cocoons in agricultural soil exposed to different drought regimes - a laboratory study. Pedobiologia 41, 334–341.

Lalejini, A., Ferguson, A.J., Grant, N.A., Ofria, C., 2021. Adaptive phenotypic plasticity stabilizes evolution in fluctuating environments. Frontiers in Ecology and Evolution 9, 715381.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology 42, S3–S15.

Leal Filho, W., Nagy, G.J., Setti, A.F.F., Sharifi, A., Donkor, F.K., Batista, K., Djekic, I., 2023. Handling the impacts of climate change on soil biodiversity. Science of the Total Environment 869, 161671.

Lussenhop, J., 1996. Collembola as mediators of microbial symbiont effects upon soybean. Soil Biology and Biochemistry 28, 363–369.

Ma, Y.Y., Liu, H., Yu, Y., Guo, L., Zhao, W.Z., Yetemen, O., 2022. Revisiting soil water potential: towards a better understanding of soil and plant interactions. Water 14, 3721.

Maraldo, K., Holmstrup, M., 2009. Recovery of enchytraeid populations after severe drought events. Applied Soil Ecology 42, 227–235.

Maraldo, K., Holmstrup, M., 2010. Enchytraeids in a changing climate: a mini-review. Pedobiologia 53, 161–167.

Maraldo, K., Ravn, H.W., Slotsbo, S., Holmstrup, M., 2009. Responses to acute and chronic desiccation stress in *Enchytraeus* (Oligochaeta: Enchytraeidae). Journal of Comparative Physiology B 179, 113–123.

Maraldo, K., Schmidt, I.K., Beier, C., Holmstrup, M., 2008. Can field populations of the enchytraeid, *Cognettia sphagnetorum*, adapt to increased drought stress? Soil Biology and Biochemistry 40, 1765–1771.

Marx, M.T., Guhmann, P., Decker, P., 2012. Adaptations and predispositions of different middle european arthropod taxa (collembola, araneae, chilopoda, diplopoda) to flooding and drought conditions. Animals 2, 564–590.

Marx, M.T., Wild, A.K., Knollmann, U., Kamp, G., Wegener, G., Eisenbeis, G., 2009. Responses and adaptations of collembolan communities (Hexapoda: Collembola) to flooding and hypoxic conditions. Pesquisa Agropecuária Brasileira 44, 1002–1010.

Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K., Relyea, R.A., 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution 20, 685–692.

OECD, 2016a. Test No. 220: Enchytraeid Reproduction Test. OECD (OECD Guidelines for the Testing of Chemicals, Section 2). DOI:10.1787/9789264264472-en.

OECD, 2016b. Test No. 232: Collembolan Reproduction Test in Soil. OECD (OECD Guidelines for the Testing of Chemicals, Section 2). DOI:10.1787/9789264264601-en.

Paul, R.J., Colmorgen, M., Hüller, S., Tyroller, F., Zinkler, D., 1997. Circulation and respiratory control in millimetre-sized animals (*Daphnia magna, Folsomia candida*) studied by optical methods. Journal of Comparative Physiology B 167, 399–408.

Pelosi, C., Römbke, J., 2018. Enchytraeids as bioindicators of land use and management. Applied Soil Ecology 123, 775–779.

Pereira, H.M., Navarro, L.M., Martins, I.S., 2012. Global biodiversity change: the bad, the good, and the unknown. Annual Review of Environment and Resources 37, 25–50.

Peterson, J.W., Johnson, E.M., Cencer, J.L., Thomason, C.J., 2006. Physiochemical conditions of *Folsomia candida* occurrence in a shallow coastal Lake Michigan aquifer. Environmental Geology 49, 1125–1138.

Pigliucci, M., 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution 20, 481–486.

Pimentel, D., 2006. Soil erosion: a food and environmental threat. Environment, Development and Sustainability 8, 119–137.

Plum, N.M., Filser, J., 2005. Floods and drought: response of earthworms and potworms (Oligochaeta: Lumbricidae, Enchytraeidae) to hydrological extremes in wet grassland. Pedobiologia 49, 443–453.

Potapov, A., Bellini, B., Chown, S., Deharveng, L., Janssens, F., Kováč, L., Kuznetsova, N., Ponge, J.F., Potapov, M., Querner, P., Russell, D., Sun, X., Zhang, F., Berg, M., 2020. Towards a global synthesis of Collembola knowledge- challenges and potential solutions. Soil Organisms 92, 161–188.

Schilthuizen, M., Kellermann, V., 2014. Contemporary climate change and terrestrial invertebrates: evolutionary versus plastic changes. Evolutionary Applications 7, 56–67.

Schlichting, C.D., Wund, M.A., 2014. Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. Evolution 68, 656–672.

Schmidt, A., John, K., Arida, G., Auge, H., Brandl, R., Horgan, F.G., Hotes, S., Marquez, L., Radermacher, N., Settele, J., Wolters, V., Schädler, M., 2015. Effects of residue management on decomposition in irrigated rice fields are not related to changes in the decomposer community. PLoS One 10, e0134402.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671–675.

SigmaPlot, 1997. Statistical Package for the Social Sciences e SigmaPlot for Windows. Chicago: IL, USA .

Silva, A.R.R., Malheiro, C., Loureiro, S., González-Alcaraz, M.N., 2022. Toxicity of historically metal(loid)-contaminated soils to *Folsomia candida* under the influence of climate change alterations. Environmental Pollution 305, 119256.

Singh, J., Schädler, M., Demetrio, W., Brown, G.G., Eisenhauer, N., 2020. Climate change effects on earthworms - a review. Soil Organisms 91, 114–137.

Sjursen, H., Bayley, M., Holmstrup, M., 2001. Enhanced drought tolerance of a soil-dwelling springtail by pre-acclimation to a mild drought stress. Journal of Insect Physiology 47, 1021–1027.

Spinoni, J., Barbosa, P., Cherlet, M., Forzieri, G., McCormick, N., Naumann, G., Vogt, J.V., Dosio, A., 2021. How will the progressive global increase of arid areas affect population and land-use in the 21st century ? Global and Planetary Change 205, 103597.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proceedings of the Royal Society B: Biological Sciences 278, 1823–1830.

Szabó, B., Bálint, B., Balogh, K., Mézes, M., Seres, A., 2022. Changes in soil moisture and temperature modify the toxicity of sodium selenite and sodium selenate for *Folsomia candida* (Collembola) Willem 1902. Applied Soil Ecology 177, 104543.

Tabari, H., 2020. Climate change impact on flood and extreme precipitation increases with water availability. Scientific Reports 10, 13768.

Uhía, E., Briones, M.J.I., 2002. Population dynamics and vertical distribution of enchytraeids and tardigrades in response to deforestation. Acta Oecologica 23, 349–359.

van Vliet, P.C.J., West, L.T., Hendrix, P.F., Coleman, D.C., 1993. The influence of Enchytraeidae (Oligochaeta) on the soil porosity of small microcosms. In: Brussaard, L., Kooistra, M.J., eds. Soil Structure/Soil Biota Interrelationships. Amsterdam: Elsevier, 287–299.

Yusefi, G.H., Safi, K., Tarroso, P., Brito, J.C., 2021. The impacts of extreme climate change on mammals differ among functional groups at regional scale: the case of Iranian terrestrial mammals. Diversity and Distributions 27, 1634–1647.