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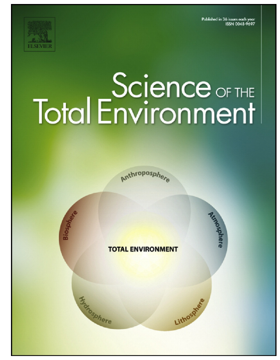

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PII: S0048-9697(20)33890-0

DOI: https://doi.org/10.1016/j.scitotenv.2020.140368

Reference: STOTEN 140368

To appear in: *Science of the Total Environment*

Received date: 10 April 2020

Revised date: 13 June 2020

Accepted date: 17 June 2020

Please cite this article as: C. Malheiro, D.N. Cardoso, S. Loureiro, et al., Effects of climate conditions on the avoidance behavior of Folsomia candida and Enchytraeus crypticus towards metal(loid)-contaminated soils, *Science of the Total Environment* (2018), https://doi.org/10.1016/j.scitotenv.2020.140368

Effects of climate conditions on the avoidance behavior of *Folsomia candida* and *Enchytraeus crypticus* towards metal(loid)-contaminated soils

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Abstract

Global climate changes are predicted for the 21st century. Alterations in soil contaminants' availability and soil invertebrates' behavior are expected, which may interfere with the avoidance capacity that invertebrates may have towards contaminated soils and, therefore, compromise their role in soil functioning. This study aimed to assess the individual effects of air temperature, ultraviolet (UV) radiation and atmospheric CO_2 concentration on the avoidance behavior of the arthropod *Folsomia candida* and the soft-bodied oligochaete *Enchytraeus crypticus* towards metal(loid)-contaminated soils. Avoidance behavior was evaluated under distinct climate treatments (simulating increases in air temperature, UV radiation exposure or atmospheric CO_2 concentration) and compared to the response obtained at the standard conditions recommended by ISO guidelines. Both soil invertebrate species behave differently under standard conditions, with *F. candida* not avoiding the contaminated soils while *E. crypticus* did. Increases in air temperature and exposure to UV radiation did not change *F. candida* behaviour towards contaminated soils. However, high atmospheric CO_2 concentration modified this pattern and induced avoidance towards contaminated soils. As for *E. crypticus*, contaminated soils were also avoided under the different climate treatments simulated. Thus, our study shows that, depending on the species and the climate factor, changes in climate conditions may alter soil invertebrates' behavioral patterns towards meta(loid)-contaminated soils.

Keywords: Anthropogenic activities; Multiple stressors; Soil invertebrates; Air temperature; UV radiation; Atmospheric CO_2

1. Introduction

Soil invertebrates have the ability to avoid soil contamination. They possess sensory organs (chemical, mechanical) that allow them to detect and escape from unfavorable and/or harmful soil conditions (Slifer and Sekhon, 1978; Moment and Johnson, 1979; Edwards and Bohlen, 1992; Curry and Schmidt, 2007). Avoidance behavior varies among invertebrate species and may depend on multiple factors: i) organism development stage (e.g., juveniles or adults); ii) organism mode of life traits (e.g., physiological, morphological and behavioural traits); iii) soil characteristics (e.g., texture, pH, salinity, organic matter, nutrients availability); iv) contaminants presence in the system and their availability; v) climate conditions (e.g., different temperature, moisture level) (Boyd et al., 2002; Loureiro et al., 2005; Lukkari and Haimi, 2005; Natal-da-Luz et al., 2008; Chelinho et al., 2014; Gainer et al., 2019; González-Alcaraz et al., 2019). The vast majority of studies have been focused on the characteristics of invertebrates and/or the soil environment. Still, there is a lack of literature on the influence of climate conditions.

Climate conditions may modify the availability of soil contaminants, due to changes in key soil parameters (e.g., pH, salinity, organic matter), but also the performance of soil invertebrates and/or their susceptibility to contaminants (Peijnenburg and Jager, 2003; Maraldo et al., 2009; Duval et al., 2011; Karmakar et al., 2016). This may have significant implications for the risk assessment of contaminants and contaminated soils in the context of climate change, especially considering the IPCC predictions for the end of the century (IPCC, 2013, 2014a). In fact, some authors have already pointed out that soil ecotoxicity risks may be altered when changing the climate conditions (e.g., Holmstrup et al., 2010; González-Alcaraz and van Gestel, 2015; Lima et al., 2015; Jegede et al., 2017). These studies have mostly looked at traditional endpoints (i.e., invertebrates' survival and reproduction) and found synergistic interactions in most case studies, while behavioral endpoints have received less attention.

To our knowledge, so far, only two studies have evaluated the possible effects of climate conditions on the avoidance behavior of soil invertebrates (Lobe et al., 2018; González-Alcaraz et al., 2019). Lobe et al. (2018) found that heat stress may influence the avoidance behavior of *Enchytraeus albidus* towards silver nitrate and silver nanoparticles. As for González-Alcaraz et al. (2019), the authors showed that the capacity of *Folsomia candida* and *Enchytraeus crypticus* to avoid metal(loid)-contaminated soils might be compromised under water stress situations (high and low soil water availability due to intense rainfalls/floods and dry spells, respectively) and that this behavioral shift may be related to changes in the composition of the soil pore water. Both studies were developed modifying the climate conditions (air temperature and soil moisture content, respectively) recommended by the existing standardized ISO guidelines to evaluate invertebrate avoidance behavior (ISO, 2008, 2011). Despite this first step, more research is needed on the effects of climate factors, including others different to temperature and moisture, on soil invertebrate avoidance behavior.

Considering all this, the present study aimed to assess the individual effects of three climate factors (air temperature, ultraviolet – UV – radiation and atmospheric CO_2 concentration) on the avoidance behavior of different soil invertebrate species towards metal(loid)-contaminated soils. The model invertebrate species *F. candida* (phylum Uniramia, class Collembola, family Isotomidae) and *E. crypticus* (phylum Annelida, class Oligochaeta, family Enchytraeidae) were selected due to their key role in soil functioning processes (e.g., organic matter/nutrient cycling, soil bioturbation, soil structure improvement) and their use as bioindicators of stress conditions (Didden and Römbke, 2001; Fountain and Hopkin, 2005). Avoidance behavior tests were performed under different climate treatments, simulated by individually changing specific climate factors, and comparing to the response under the standardized climate conditions. We initally hypothesized that changes in air temperature, UV radiation and atmospheric CO_2 concentration would affect the avoidance behavior of *F. candida* and *E. crypticus* towards metal(loid)-contaminated soils.

2. Material and methods

2.1. Culture conditions of soil invertebrate species

Soil invertebrates were cultured under controlled laboratory conditions. *Folsomia candida* was cultured in plastic boxes containing moist plaster of Paris mixed with activated charcoal (9:1 w:w) and kept at 20 °C with a 16:8 h light:dark photoperiod (ISO, 1999). Once or twice a week, granulated dry baker's yeast (*Sacchromyces cerevisae*) was added as food source and distilled water was replenished to maintain the medium moist, as needed. Age-synchronized collembolans (10-12 d old) were used for the performance of the avoidance behavior tests (ISO, 2011). *Enchytraeus crypticus* was cultured in plastic boxes containing agar medium dissolved in aqueous soil extracts and kept at 20 °C under complete darkness. The culture was fed once or twice a week with a mixture of oatmeal, dry yeast, yolk powder and fish oil, as needed. Enchytraeids with clearly visible clitellum and similar size (~1 cm length) were used for the performance of the avoidance behavior tests. In both cases, only organisms showing no visible problems were used.

2.2. Metal(loid)-contaminated test soils

Two soils contaminated with metal(loid)s from different types of anthropogenic sources were selected in central-northern Portugal: a soil from the abandoned mining district of Braçal (Sever do Vouga, Northeast of Aveiro; hereafter mining soil) (Anjos et al., 2012; Vidal et al., 2012), and a soil from a former agricultural area near the Estarreja chemical complex (Estarreja, Northeast of Aveiro; hereafter agricultural soil) (Inácio et al., 2008; Rodrigues et al., 2012). Both test soils showed loamy sand texture (~ 77-86% sand, ~ 8-16% silt, and ~ 6-7% clay; data not shown), acidic pH (~ 4.7-5.9 in 0.01M $CaCl_2$), low salinity (electrical conductivity – EC – ≤ 0.3 dS m^{-1}) and low values of cation exchange capacity (CEC ≤ 10 $cmol_c$ kg^{-1}) (Table 1). Both test soils showed different contents of total organic carbon (TOC ~ 46 mg kg^{-1} in the mining soil and ~ 26 mg kg^{-1} in the agricultural soil), but similar contents of total nitrogen (TN ~ 1.7-1.8 mg kg^{-1}) (Table 1). Despite this, the agricultural soil showed greater concentrations of dissolved organic carbon (DOC ~ 42 mg kg^{-1} in the mining soil and ~ 125 mg kg^{-1} in the agricultural soil) and total dissolved nitrogen (TDN ~ 13 mg kg^{-1} in the mining soil and ~ 22 mg kg^{-1} in the agricultural soil) (Table 1). The concentrations of total and exchangeable (extracted with 0.01M $CaCl_2$) metal(loid)s (Al, As, Cd, Cu, Mn, Pb and Zn) were high in both test soils (Table S1, Supplementary material). A complete description of the characterization of both test soils is provided by González-Alcaraz et al. (2019).

Soil samples were taken from the top 20 cm, air-dried, sieved (2 mm mesh), thoroughly homogenized and stored at 4 °C prior to the performance of the avoidance behavior tests.

2.3. Experimental set-up

2.3.1. Avoidance behavior evaluation

Invertebrates' avoidance behavior was evaluated following the instructions of the standardized ISO guidelines 17512 (ISO, 2008, 2011), by giving *F. candida* and *E. crypticus* the option to choose between a clean soil (hereafter control soil) and one of the metal(loid)-contaminated test soils. The natural standard soil Lufa 2.2 (Speyer, Germany) was used as control soil (sandy loam texture; pH in 0.01M $CaCl_2$ ~ 5.3; EC ~ 0.1 dS m^{-1}; WHC ~ 45.3%). Before starting the avoidance behavior tests, both the control soil and the metal(loid)-contaminated test soils were moistened at 50% of their maximum WHC (i.e., soil moisture content recommended by the ISO guidelines).

Avoidance behavior tests were performed in two-section containers (cylindrical plastic containers of ~ 8 cm diameter and ~ 6 cm height), where one section contained the control soil and the other the metal(loid)-contaminated soil to be tested (n = 5). Dual control tests (both sections filled with the control soil) were also performed to prove the validity of the avoidance behavior tests according to the ISO guidelines (n = 5). Organisms were placed in the soil midline between both sections (20 for *F. candida* and 10 for *E. crypticus*). Test containers were covered with a perforated plastic lid and kept for 48 h in acclimatized chambers/rooms with a 16:8 h light:dark photoperiod and under the different climate treatments simulated (see Section 2.3.2). After 48 h, a removable plastic split (~ 1 mm thickness) was placed in the soil midline between both sections and the number of surviving organisms in each test container section was recorded: *F. candida* (simultaneous addition of water to both container sections, gentle soil agitation and count of organisms by eye); *E. crypticus* (soil transfer to 250 μm sieves, water addition to removing soil particles and count of organisms by eye). For more details on the procedure see González-Alcaraz et al. (2019).

The avoidance behavior of *F. candida* and *E. crypticus* towards each metal(loid)-contaminated test soil was calculated by applying Equation 1 (in %): C (number of organisms in control soil); T (number of organisms in metal(loid)-contaminated test soil); N (total number of surviving organisms). Positive values indicated that soil invertebrates avoided the metal(loid)-contaminated test soil (i.e., avoidance response) and negative values that invertebrates preferred the metal(loid)-contaminated test soil (i.e., preference response or non-avoidance response). When avoidance behavior equals zero it means that invertebrates were equally distributed between the control soil and the metal(loid)-contaminated test soil (i.e., neutral response).

$$Avoidance = \frac{(\mathrm{C} - \mathrm{T})}{\mathrm{N}} \times 100 \qquad \text{(Equation 1)}$$

Additionally, during the performance of the avoidance behavior tests, extra containers without soil invertebrates were prepared for each of the metal(loid)-contaminated test soils and the control soil to check for the loss of soil moisture content under the different climate treatments simulated (n = 3).

2.3.2. Climate treatments simulated

The standardized ISO guidelines 17512 (ISO, 2008, 2011) recommend evaluating the avoidance behavior of soil invertebrates in acclimatized chambers/rooms with a constant air temperature of ~ 20 °C, without controlling both the UV radiation exposure and the atmospheric CO_2 concentration (hereafter standard conditions) (Table S2, Supplementary material). To evaluate the individual effects of air temperature, UV radiation and atmospheric CO_2 concentration, a set of climate treatments were established based on the IPCC projections for southern Europe for the year 2100 (IPCC, 2013, 2014a, 2014b) and compared to the response under the standard conditions (Figure 1; Table S2, Supplementary material). The different climate treatments established were simulated by varying one individual climate factor (air temperature, UV radiation or atmospheric CO_2 concentration) while keeping the remaining factors at the levels recommended by the standardized ISO guidelines (Table S2, Supplementary material).

For the climate factor air temperature, three treatments were established considering the air temperature rise predictions (Figure 1a; Table S2, Supplementary material). They consisted of repetitive air temperature regimes of 24 h duration and thermal amplitude of 10 °C mimicking increase and decrease in daily temperature (minimum – maximum air temperature reached): i) 15 – 25 °C (characteristic air temperature regime for central-northern Portugal during the summer period); ii) 20 – 30 °C (intermediate exposure scenario for air temperature); iii) 25 – 35 °C (worst-case scenario for air temperature). Each regime started from the minimum air temperature (15, 20 and 25 °C, respectively) at 8:00 and gradually increased until 12:00 when the maximum air temperature was reached (25, 30 and 35 °C, respectively) and maintained until 16:00. From this time on the air temperature gradually decreased until 4:00 when the minimum air temperature was reached again and continued until 8:00 to start a new air temperature regime cycle. Avoidance behavior tests with air temperature treatments were performed in a KBWF 720 Binder (Germany) acclimatized chamber.

For the climate factor UV radiation, one treatment was established (Figure 1b; Table S2, Supplementary material). It consisted of a repetitive UV radiation regime of 24 h duration (6 h with UV radiation emission and 18 h without UV radiation). For creating the regime we considered the UV radiation data existing for Lisbon (Portugal) since it is the closest city to the area where the metal(loid)-contaminated test soils were collected with a detailed UV radiation record (Tropospheric Emission Monitoring Internet Service, TEMIS; http://www.temis.nl/index.php). The regime was based on the UV index data of the summer period (higher UV exposure) between 2002 and 2018, selecting the average minimum (~ 7) and maximum (~ 10) UV indexes. The regime started without UV radiation emission from 8:00 to 10:00. At this time, UV radiation corresponding to a UV index of ~ 7 started emitting until 12:00 when it was intensified up to ~ 10. The UV index of ~ 10 was maintained until 14:00 when it decreased again to ~ 7 until 16:00. From this time on, the UV radiation emission ceased until 8:00 to start a new cycle of the UV radiation regime. This regime resulted in a daily UV dose of ~ 4400 J m^{-2} (regular daily UV dose in Lisbon during the summer period) (Table S3, Supplementary material). Avoidance behavior tests with UV radiation emission were performed in an acclimatized room. UV radiation was provided by two UV lamps (Spectroline XX15F/B, Spectronics Corporation, USA) with peak emissions at 365 nm for UVA (~ 320-400 nm) and 312 nm for UVB (~ 280-320 nm). The UV lamps were covered by cellulose acetate sheets, previously burned under the lamps for 12 h (Figure S1, Supplementary material). This allowed the stabilization of the UV radiation intensity passing through and prevented the exposure of the avoidance behavior test containers to UVC, which is not typically found in nature due to its strong adsorption to the stratosphere ozone layer. The different UV indexes selected were obtained by keeping the UV lamps in permanent, static position respect to fixed platforms where the test containers were located (Figure S1, Supplementary material). One lamp was set at ~ 75 cm distance from the test containers to reach the UV index of ~ 7 and the other lamp at ~ 65 cm distance to reach the UV index of ~ 10. The test containers were moved between platforms to be exposed to the corresponding UV indexes and the perforated plastic lids were removed during the periods of UV radiation emission. To ensure that the selected UV indexes were reached, a spectroradiometer connected to a monochromator (Bentham Instruments, UK) was used to measure the UV irradiance reaching the test containers (Figure S1, Supplementary material); the spectroradiometer was placed in between the avoidance behavior test containers. The UV irradiance measurements were corrected according to a weighing factor concerning the erythema reference action spectrum established by the International Commission on Illumination (CIE) to obtain the biologically effective UV irradiance. Then, the daily UV dose (total amount of biologically effective UV irradiance reaching the avoidance behavior test containers per day) was calculated by applying Equation 2 (in J m^{-2}): I_i (biologically effective UV irradiance in mW m^{-2}); t_i (exposure time in s); i (UV regime phase: 1 and 3 refers to the UV radiation exposure period to the UV index of ~ 7, and 2 to the UV exposure period to the UV index of ~ 10).

$$Daily\ UV\ dose = \left(\frac{I_1 \times t_1}{1000}\right) + \left(\frac{I_2 \times t_2}{1000}\right) + \left(\frac{I_3 \times t_3}{1000}\right) \quad \text{(Equation 2)}$$

For the climate factor atmospheric CO_2 concentration, three treatments were established considering the atmospheric CO_2 rise predictions (Figure 1c; Table S2, Supplementary material). The treatments consisted of setting the atmospheric CO_2 concentration at three different constant levels, with a difference of 200 ppm of CO_2: i) 600 ppm (low exposure scenario for atmospheric CO_2 concentration); ii) 800 ppm (intermediate exposure scenario for atmospheric CO_2 concentration); iii) 1000 ppm (worst-case scenario for atmospheric CO_2 concentration). The atmospheric CO_2 concentration was controlled by a IRGAs system coupled to the acclimatized chamber, where the avoidance behavior tests were performed (FitoclimaD1200 Aralab, Portugal).

Avoidance behavior tests under standard conditions were performed separately for each of the climate factors evaluated.

2.4. Statistical analyses

Statistical analyses were performed with GraphPad Prism 6.0 and IBM SPSS Statistics 22 softwares; differences were considered significant at $p < 0.05$. Fisher's exact test was used to check for differences in the avoidance behavior of soil invertebrates towards each metal(loid)-contaminated soil when varying individual climate factors, by comparing the observed distribution of organisms between both test container sections with an expected distribution where no avoidance response occurred (Natal-da-Luz et al., 2008): i) one-tailed tests were applied to analyze the results from the avoidance behavior tests (control soil vs. metal(loid)-contaminated test soil); ii) two-tailed tests were applied to analyze the results from the dual controls (control soil vs. control soil). To deepen into the effects of the climate factors evaluated, the total number of organisms in the control section of the test containers was compared by a two-way ANOVA followed by Tukey's post-hoc test (Natal-da-Luz et al., 2008). The factors included in the analysis were the soil type (mining and agricultural) and the climate conditions (climate treatments simulated within each climate factor). Data were log-transformed when they failed to pass the Leven's test for the homogeneity of variance.

Student's t-test was used to check for differences in the survival of organisms and the loss of soil moisture content under the different climate treatments simulated, by comparing each climate treatment with the standard conditions.

3. Results

3.1. Validity of avoidance behavior tests with soil invertebrates

Avoidance behavior tests need to fulfill two assumptions to be considered valid (ISO 2008, 2011): i) the number of surviving organisms > 80% for *F. candida* and > 90% for *E. crypticus* per combination tested, and ii) homogeneous distribution of organisms in dual control tests (40% to 60% in each test container section). Taking this into account, the validity criteria were met for both soil invertebrate species in all the cases (standard conditions and climate treatments simulated) except at the highest air temperature regime tested (25 – 35 ºC) (Tables 2 and 3; Tables S4 and S5, Supplementary material). For *F. candida*, survival decreased significantly (t-test, $p < 0.05$) at 25 – 35 ºC in the dual control tests and in the test performed with the agricultural soil, compared to the standard conditions (~ 71% and 74% of surviving organisms, respectively), and no homogeneous distribution of organisms was observed in the dual control tests (77% to 23%; significant differences, Fisher's test, $p < 0.05$) (Table 2; Table S4, Supplementary material). For *E. crypticus*, survival decreased at 25 – 35 ºC compared to the standard conditions in the test performed with the mining soil (~ 84% of surviving organisms) and organisms were not homogeneously distributed in the dual control tests (74% to 27%; significant differences, Fisher's tests, $p < 0.05$) (Table 3; Table S5, Supplementary material).

3.2. Avoidance behavior of soil invertebrates under standard conditions

Both soil invertebrate species showed a different behavior towards the metal(loid)-contaminated test soils under the standard conditions and their response was consistent across the different tests performed (Figure 2). The collembolan *F. candida* did not avoid the metal(loid)-contaminated test soils (Figure 2). Organisms were equally distributed between the control soil and the metal(loid)-contaminated soil tested or showed a slight preference for the contaminated section of the containers, especially in the presence of the agricultural soil (~ 14-16% of preference). Contrariwise, *E. crypticus* in most cases avoided significantly (Fisher's test, $p < 0.05$) both metal(loid)-contaminated test soils when exposed to the standard conditions (~ 40-48% and ~ 77-90% of avoidance towards the mining and the agricultural soils, respectively) (Figure 2).

3.3. Effects of climate factors on the avoidance behavior of soil invertebrates

3.3.1. Air temperature

In general, when exposed to the different air temperature treatments, *F. candida* did not avoid both metal(loid)-contaminated test soils and a higher number of collembolans were found in the contaminated section of the containers (Figure 2a). This was significant (Fisher's test, $p < 0.05$) for the mining soil at 25 – 35 ºC (~ 30% of preference) and the agricultural soil both at 15 – 25 ºC and 25 – 35 ºC (~ 82% and ~ 66% of preference, respectively). The two-way ANOVA showed that *F. candida* behavior was significantly ($p < 0.05$) affected by the soil type, the climate conditions and their interaction (Table S6, Supplementary material) due to the greater preference response for the agricultural soil with increasing air temperature. In the case of *E. crypticus*, avoidance response was observed towards both metal(loid)-contaminated test soils when exposed to most of the air temperature treatments simulated (Figure 2a). Enchytraeids avoided significantly (Fisher's test, $p < 0.05$) the mining soil at 20 – 30 ºC (~ 64% of avoidance) and the agricultural soil, under all air temperature treatments (~ 31-76% of avoidance). The two-way ANOVA showed that *E. crypticus* behavior was significantly ($p < 0.05$) affected only by the factor soil type (Table S6, Supplementary material), due to the higher avoidance response towards the agricultural soil.

3.3.2. UV radiation

When exposed to UV radiation, *F. candida* showed a neutral response in the case of the mining soil (collembolans were equally distributed between both container sections), while it significantly (Fisher's test, $p < 0.05$) preferred the agricultural soil (~ 36% of preference) (Figure 2b). On the contrary, *E. crypticus* avoided significantly (Fisher's test, $p < 0.05$) both metal(loid)-contaminated test soils with UV radiation exposure (~ 40% and ~ 52% of avoidance towards the mining and the agricultural soils, respectively) (Figure 2b). For both soil invertebrate species, the two-way ANOVA did not show effects for any of the factors analyzed (soil type and climate conditions) or their interaction (Table S7, Supplementary material).

3.3.3. Atmospheric CO_2 concentration

When exposed to rising atmospheric CO_2 concentration, *F. candida* tended to avoid both metal(loid)-contaminated test soils (Figure 2c). This was more evident as atmospheric CO_2 concentration increased, with collembolans avoiding significantly (Fisher's test, $p < 0.05$) the mining soil at 800 and 1000 ppm of atmospheric CO_2 (~ 29-48% of avoidance) and the agricultural soil at 1000 ppm of atmospheric CO_2 (~ 56% of avoidance). The two-way ANOVA showed a significant ($p < 0.05$) effect of the factor climate conditions (Table S8, Supplementary material), with the highest avoidance behavior towards metal(loid)-contaminated soils occurring at 1000 ppm of atmospheric CO_2. As for *E. crypticus*, enchytraeids avoided significantly (Fisher's test, $p < 0.05$) both metal(loid)-contaminated test soils regardless of the atmospheric CO_2 concentration tested (~ 38-100% and ~ 34-72% of avoidance towards the mining and agricultural soils, respectively) (Figure 2c). The two-way ANOVA showed that *E. crypticus* behavior was significantly ($p < 0.05$) affected by the interaction soil type x climate conditions (Figure S8, Supplementary material).

3.4. Soil moisture content loss during the performance of avoidance behavior tests

There was a general decrease in soil moisture content after 48 h of exposure to the different climate treatments simulated, including on the standard conditions (Figure S2, Supplementary material). Exposure to the standard conditions led to decreases in the soil moisture content of ~ 15-17% in all the soils (control, mining and agricultural soils). The simulation of different air temperature treatments led to significant (t-test, $p < 0.05$) losses of soil moisture content, especially at the highest temperatures (Figure S2a, Supplementary material): control soil (~ 30-44% decrease; only significant at 15 – 25 ºC); mining soil (~ 27% decrease at 15 – 25 °C and ~ 51-52% decrease at 20 – 30 °C and 25 – 35 °C); agricultural soil (~ 34% decrease at 15 – 25 °C and ~ 56-61% decrease at 20 – 30 °C and 25 – 35 °C). Exposure to UV radiation led to significant (t-test, $p < 0.05$) decreases of soil moisture content in all the soils (~ 39-44%) (Figure S2b, Supplementary material). Exposure to atmospheric CO_2 led to losses of soil moisture content in all the soils (control soil ~ 23-31%, mining soil ~ 36-42% and agricultural soil ~ 25-47%) (Figure S2c, Supplementary material). These decreases were significant (t-test, $p < 0.05$) only for the agricultural soil at 600 and 1000 ppm of atmospheric CO_2.

4. Discussion

4.1. Effects of climate factors on the performance of soil invertebrates without metal(loid) soil contamination

The experimental set-up with dual control tests helped relate the performance of soil invertebrates directly to the climate conditions to which they were exposed for 48 h, without the influence of metal(loid) contamination. This set-up, essential to understanding the main effects of climate factors, also facilitated the evaluation of invertebrates response when confronted with the metal(loid)-contaminated test soils.

Air temperature was the climate factor inducing greater effects in the absence of metal(loid)s. In the case of *F. candida,* its survival decreased at 25 – 35 ºC (~ 28% lower compared to the standard conditions) (Table 2). This negative effect could have been related to the exposure of *F. candida* to temperatures above its thermal optimum

(~ 10-26 ºC) (Snider and Butcher, 1973; Fountain and Hopkin, 2005; Jänsch et al., 2005a), but also to the high loss of soil moisture content at these elevated temperatures (~ 44% decrease) (Figure S2a, Supplementary material). Without any possible shelter besides soil particles, the only option would have been to burrow into the soil to reduce heat stress. However, collembolans were most of the time on the first centimeters of the topsoil (authors' visual observation). This agrees with the findings of Boiteau and MacKinley (2013) that showed no relocation of *F. candida* to deeper soil layers to avoid the stress conditions induced by temperature changes.

Unlike *F. candida*, the survival of *E. crypticus* was not affected by any of the air temperature regimes tested (Table 3). This difference could have been related to the greater resistance of *E. crypticus* to high temperatures (thermal tolerance limit ~ 15-30 ºC) (Jänsch et al., 2005a). Moreover, the burying behavior of enchytraeid species could have guaranteed some desiccation protection (Didden, 1993; Jänsch et al., 2005b) and, thus, diminished the effects of these elevated temperatures. Besides invertebrates' survival, at 25 – 35 ºC, both *F. candida* and *E. crypticus* tended to stay in one of the container sections of the dual control tests instead of showing a homogeneous distribution as in the other temperature treatments (Tables 2 and 3). This could have been related to the fact that the soil does not dry evenly when exposed to high temperatures, and invertebrates could have remained in specific spots with higher soil moisture content and/or grouped to minimize their body dehydration. This possible seek for areas with greater soil moisture content was already reported by González-Alcaraz et al. (2019) who found a clear avoidance response of both invertebrate species towards soil dryness when having the option to choose between different soil moisture levels.

Different from air temperature, the exposure to both UV radiation and atmospheric CO_2 concentration did not induce any effect on invertebrates' performance in duals control tests (Tables 2 and 3). In the case of *F. candida*, Cardoso et al. (2014) also showed no effects of UV radiation on collembolan survival (UV doses from 1121 to 3795 J m^{-2}). As for the effects of UV radiation on *E.* crypticus or atmospheric CO_2 on both soil invertebrate species, the absence of previous studies to our knowledge does not allow results comparison and emphasizes the need for considering climate factors.

4.2. Effects of climate factors on the avoidance behavior of soil invertebrates towards metal(loid) soil contamination

Under standard conditions, both soil invertebrate species showed an opposite response towards metal(loid) soil contamination (Figure 2). The collembolan *F. candida* was homogeneously distributed between the test container sections or showed a slight preference for the contaminated section, especially for the agricultural soil (~ 14-16% of preference). The latter could have been favored by the high availability of DOC and TDN of the agricultural soil (Table 1) (Natal-da-Luz et al., 2008). On the contrary, *E. crypticus* avoided both metal(loid)-contaminated test soils (≥ 40% of avoidance). González-Alcaraz et al. (2019) also found the same behavior in both invertebrate species under varying soil moisture conditions. The authors pointed out that this behavioral response could be related to the different sensitivity/resistance of both invertebrate species to metal(loid) soil contamination. Collembolans are generally more tolerant than enchytraeids to metal(loid)s due to the different main routes of exposure (Fountain and Hopkin, 2005; Römbke, 2003). In addition, *F. candida* is able to minimize the internal concentration of metal(loid)s through the exfoliation of the midgut epithelium as part of the detoxification system (Fountain and Hopkin, 2001, 2005).

When changing the climate factors air temperature and UV radiation *F. candida* did not modify its response towards the metal(loid)-contaminated test soils (Figures 2a and 2b). Similar to the standard conditions, collembolans showed a homogeneous distribution in most of the climate treatments simulated or, in the case of the agricultural soil, a clear preference for the contaminated section of the test containers (≥ 36% of preference). No major differences were observed among soils in terms of loss of moisture content after 48 h exposure to the different air temperature and UV radiation treatments simulated (Figures S2a and S2b, Supplementary material). Hence, once again, the high DOC and TDN concentrations of the agricultural soil (Table 1) could have been the major factor that led *F. candida* to remain in this soil, even taking into consideration the stress related to air temperature and UV radiation treatments (Figures 2a and 2b).

Unlike air temperature and UV radiation, when avoidance behavior tests were performed with atmospheric CO_2, *F. candida* completely changed its behavioral pattern towards the metal(loid)-contaminated test soils (Figure 2c). As atmospheric CO_2 concentration increased, collembolans started avoiding the contaminated section of the test containers and tended to stay in the control soil, especially when exposed to 1000 ppm of atmospheric CO_2 (≥ 48% of avoidance). Soil invertebrates are assumed to be relatively tolerant to elevated CO_2 pressure since they live in a medium with higher CO_2 concentration than in the atmosphere (Glinski and Stepniewski, 1985; Zinkler and Platthaus, 1996). Despite this, exposure to high atmospheric CO_2 concentration could have interfered with the ability of *F. candida* to compensate for high metal(loid) concentrations through internal regulation. If so, with increasing atmospheric CO_2, collembolans may have felt threatened by the metal(loid)-contaminated test soils and, to avoid this pressure, they may have needed to escape to a more favorable place. Rising atmospheric CO_2 concentration could have also led to some modifications in key soil parameters, indirectly affecting the response of *F. candida*. Although we did not evaluate soil physico-chemical characteristics after avoidance behavior tests, we hypothesize that greater CO_2 diffusion to the soil could have lowered the pH due to the formation of carbonic acid and the dissociation of H^+ ions. This could have been favored by the low buffer capacity of the metal(loid)-contaminated test soils (Table 1). Caramanna et al. (2013) reported a decrease in soil pH after one hour of CO_2 injection into soil columns. He et al. (2016) also found soil acidification after short periods of CO_2 injection into plant germination boxes. Soil acidification could have promoted greater availability of some of the metals present in the contaminated test soils and turned the medium less favorable for *F. candida*. Unfortunately, the lack of studies on the effects of atmospheric CO_2 concentration on the avoidance behavior of soil invertebrates towards soil contamination does not allow comparing the results obtained. Moreover, the information gathered highlights how climate change might compromise and impact *F. candida* in anthropogenic-contaminated areas. The migration (avoidance or escape) of collembolans may lead to changes in the structure of soil communities and/or loss of the beneficial functions they perform, if no other organisms can assume their role, impairing ecosystems functionality and sustainability.

Differently to *F. candida*, the avoidance behavior that *E. crypticus* showed towards metal(loid) soil contamination under standard conditions was not modified by the different climate factors evaluated (Figure 2). Enchytraeids avoided both metal(loid)-contaminated test soils when exposed to the distinct treatments of air temperature, UV radiation and atmospheric CO_2 concentration simulated, which highlights how unfavorable the test soils were for these organisms. The only difference observed was the high data variability obtained at the highest air temperature regime tested (25 – 35 ºC), especially for the mining soil in which enchytraeids were equally distributed between the test container sections (Figure 2a). The latter could have been related to the exposure of *E. crypticus* to temperatures above its upper thermal tolerance limit (> 30 ºC), which could result in a higher vulnerability of enchytraeids to metal(loid)s. In fact, González-Alcaraz and van Gestel (2015) and González-Alcaraz et al. (2015) found greater toxicity of soils affected by metal(loid) mine wastes to *E. crypticus* with increasing air temperature. In addition, the high vulnerability of enchytraeid species to desiccation could have led *E. crypticus* to lose, to some extent, their capacity to avoid metal(loid) soil contamination at these elevated temperatures (Lindberg et al., 2002; Maraldo et al., 2008, 2009). Indeed, González-Alcaraz et al. (2019) demonstrated that *E. crypticus* might lose its capacity to avoid metal(loid)-contaminated soils under dry soil conditions. The consistent avoidance response of *E. crypticus* towards the metal(loid)-contaminated soils tested, regardless of the climate factors evaluated, highlights the importance of considering these organisms for the risk assessment of natural contaminated sites. If metal(loid) contamination jeopardizes soil invertebrates, namely enchytraeids in this case, all soil functions and services provided by them may be impaired.

5. Conclusions

The soil invertebrate species *F. candida* and *E. crypticus* presented different behavioral strategies towards metal(loid) soil contamination. *Folsomia candida* did not avoid the metal(loid)-contaminated soils tested, neither when exposed to the standard conditions recommended by the ISO guidelines nor when increasing air temperature or when exposed to UV radiation. However, under rising atmospheric CO_2 concentration, *F. candida* changed its behavioral pattern and avoided the metal(loid)-contaminated soils. On the contrary, *E. crypticus* always avoided the metal(loid)-contaminated soils tested, regardless of the exposure climate conditions. Therefore, the present study shows that changes in climate conditions may alter the behavioral patterns of soil invertebrates towards meta(loid)-contaminated soils. However, this depends on the invertebrate species tested and the climate factor modulated. Moreover, the information gathered highlights how climate change might compromise and impact soil-dwelling organisms, preventing them from performing vital processes that may potentially impair terrestrial ecosystems functionality and sustainability.

Acknowledgments

The present study was supported by the project METOXCLIM (PTDC/CTA-AMB/29557/2017), funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES; by the project GLOBALTOX, funded by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 704332. Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds. C. Malheiro holds a PhD grant (PD/BD/135577/2018) from the Doctoral Program in Biology and Ecology of Global Changes of the University of Aveiro funded by FCT.

Conflict of interest

There is no conflict of interest.

References

Anjos, C., Magalhães, M.C., & Abreu, M.M., 2012. Metal (Al, Mn, Pb and Zn) soils extractable reagents for available fraction assessment: Comparison using plants, and dry and moist soils from the Braçal abandoned lead mine area, Portugal. Journal of Geochemical Exploration, 113, 45-55.

Boiteau, G., MacKinley, P., 2013. Role of avoidance behavior in the response of *Folsomia candida* to above-freezing cooling temperatures. Entomologia Experimentalis et Applicata 147, 50-60.

Boyd, W.A., Brewer, S.K., Williams, P.L., 2002. Altered behaviour of invertebrates living in polluted environments, in: Dell'Omo (Ed.), Behavioural Ecotoxicology. John Wiley & Sons, LTD, pp. 293-376.

Caramanna, G., Wei, Y., Maroto-Valer, M.M., Nathanail, P., Steven, M., 2013. Laboratory experiments and field study for the detection and monitoring of potential seepage from CO_2 storage sites. Applied Geochemistry 30, 105-113.

Cardoso, D.F.N., Bastos, A.C., Soares, A.M.V.M., Loureiro, S., 2014. Short-term exposure to carbaryl and UV radiation increases the reproduction output of the collembolan *Folsomia candida*. Journal of Soils and Sediments 14, 1559-1567.

Chapman, H.D., 1965. Cation-exchange capacity, in: Black, C.A. (Ed.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 891-900.

Chelinho, S., Domene, X., Campana, P., Andrés, P., Römbke, J., Sousa, J.P., 2014. Toxicity of phenmedipham and carbendazim to *Enchytraeus crypticus* and *Eisenia andrei* (Oligochaeta) in Mediterranean soils. Journal of Soils and Sediments 14, 584-599.

Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms - a review. Pedobiologia, 50, 463-477.

Didden, W.A.M., 1993. Ecology of terrestrial enchytraeidae. Pedobiologia 37, 2-29.

Didden, W.A.M., Römbke, J., 2001. Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. Ecotoxicology and Environmental Safety, 50, 25-43.

Duval, B.D. Dijkstra, P., Natali, S.M., Megonigal, J.P., Ketterer, M.E., Drake, B.G., Lerdau, M.T., Gordon, G., Anbar, A.D., Hungate, B.A., 2011. Plant-soil distribution of potentially toxic elements in response to elevated atmospheric CO_2. Environmental Science and Technology 45, 2570-2574.

Edwards, C.A., Bohlen, P.J., 1992. The effects of toxic chemicals to earthworms. Reviews of Environmental Contamination and Toxicology 125, 23-99.

Fountain, M.T., Hopkin, S.P., 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. Ecotoxicology and Environmental Safety 48, 275-286.

Fountain, M.T., Hopkin, S.P., 2005. *Folsomia candida* (Collembola): a ‘‘standard’’ soil arthropod. Annual Review of Entomology 50, 201-222.

Gainer, A., Akre, R., Owojori, O.J., Sicilianol, S.D., 2019. Protecting vulnerable individuals in a population: is the avoidance response of juvenile soil invertebrates more sensitive than the adults response? Chemosphere 220, 658-667.

Gee, G.W., Bauder, J.W., 1986. Particle Size Analysis, in: Methods of Soil Analysis, Part A. Klute (ed.). 2 Ed., Vol. 9 nd. Am. Soc. Agron., Madison, WI, pp: 383-411.

Glinski, J., Stepniewski, W., 1985. Soil aeration and its role for plants. CRC Press, Boca Raton, Florida, USA. pp. 237.

González-Alcaraz, M.N., Malheiro, C., Cardoso, D.N., Loureiro, S., 2019. Soil moisture influences the avoidance behavior of invertebrate species in anthropogenic metal(loid)-contaminated soils. Environmental Pollution 248, 546-554.

González-Alcaraz, M.N., Tsitsiou, E., Wieldraaijer, R., Verweij, R.A., van Gestel, C.A.M., 2015. Effects of climate change on the toxicity of soils polluted by metal mine wastes to *Enchytraeus crypticus*. Environmental Toxicology and Chemistry 34, 346-354.

González-Alcaraz, M.N., van Gestel, C.A.M., 2015. Climate change effects on enchytraeid performance in metal-polluted soils explained from changes in metal bioavailability and bioaccumulation. Environmental Research 142, 177-184.

He, W., Moonis, M., Chung, H., Yoo, G., 2016. Effects of high soil CO_2 concentrations on seed germination and soil microbial activities. International Journal of Greenhouse Gas Control 53, 117-126.

Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C., Spurgeon, D.J., 2010.

Interactions between effects of environmental chemicals and natural stressors: A review. Science of the Total Environment 408, 3746-3762.

Inácio, M., Pereira, V., Pinto, M., 2008. The Soil Geochemical Atlas of Portugal: Overview and applications. Journal of Geochemical Exploration 98, 22-33.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

IPCC, 2014a. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp.

IPCC, 2014b. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R., C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 688.

ISO, 1998. ISO 11274: Soil Quality - Determination of the water-retention characteristic. ISO (International Organization for Standardization), Geneva, Switzerland.

ISO, 1999. ISO 11267: Soil Quality – Inhibition of reproduction of collembolan (*Folsomia candida*) by soil pollutants. ISO (International Organization for Standardization), Geneva, Switzerland.

ISO, 2008. ISO 17512: Soil Quality - Avoidance test for determining the quality of soils and effects of chemicals on behavior - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). Geneva, Switzerland.

ISO, 2011. ISO 17512: Soil Quality - Avoidance test for determining the quality of soils and effects of chemicals on behavior - Part 2: Test with collembolans (*Folsomia candida*). Geneva, Switzerland.

Jänsch, S., Amorim, M.J., Römbke, J., 2005a. Identification of the ecological requirement of important terrestrial ecotoxicological test species. Environmental Reviews 13, 51-83.

Jänsch, S., Römbke, J., Didden, W., 2005b. The use of enchytraeids in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62, 266-277.

Jegede, O.O., Owojori, O.J., Römbke, J., 2017. Temperature influences the toxicity of deltamethrin, chlorpyrifos and dimethoate to the predatory mite *Hypoaspis aculeifer* (Acari) and the springtail (Collembola). Ecotoxicological and Environmental Safety 140, 214-221.

Karmakar, R., Das, I., Dutta, D., Rakshit, A., 2016. Potential Effects of Climate Change on Soil Properties: A Review. Science International , 51-73.

Lima, M.P.R., Cardoso, D.N., Soares, A.M.V.M., Loureiro, S., 2005. Carbaryl toxicity prediction to soil organisms under high and low temperature regimes. Ecotoxicology and Environmental Safety 114, 263-272.

Lindberg, N., Bengtsson, J., Persson, T., 2002. Effects of experimental irrigation and drought on the composition and diversity of soil fauna in a coniferous stand. Journal of Applied Ecology 39, 924-936.

Lobe, P.D.D., Filsera, J., Otomo, P.V., 2018. Avoidance behaviour of *Enchytraeus albidus* (Oligochaeta) after exposure to AgNPs and $AgNO_3$ at constant and fluctuating temperature. European Journal of Soil Biology 87, 40-45.

Loureiro, S., Soares, A.M.V.M., Nogueira, A.J.A., 2005. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution 138, 121-131.

Lukkari, T., Haimi, J., 2005. Avoidance of Cu- and Zn-contaminated soil by three ecologically different earthworm species. Ecotoxicology and Environmental Safety 62, 35-41.

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.

Moment, G.B., Johnson Jr., J.E., 1979. The structure and distribution of external sense organs in newly hatched and mature earthworms. Journal of Morphology 159, 1-15.

Natal-da-Luz, T., Römbke, J., Sousa, J.P., 2008. Avoidance tests in site-specific risk assessment – influence of soil properties on the avoidance response of collembola and earthworms. Environmental Toxicology and Chemistry 27, 1112-1117.

NEIKER, 2005. Determinación del contenido de amonio en aguas, lixiviados y extractos (PEC/EN/A-059).

Peijnenburg, W.J.G.M., Jager, T., 2003. Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. Ecotoxicology and Environmental Safety 56, 63-77.

Rodrigues, S.M., Pereira, E., Duarte, A.C., Römkens, P.F.A.M., 2012. Derivation of soil to plant transfer functions for metals and metalloids: impact of contaminant's availability. Plant and Soil 361, 329-341.

Römbke, J., 2003. Ecotoxicological laboratory tests with enchytraeids: A review. Pedobiologia 47, 607-616.

Slifer, E.H., Sekhon, S.S., 1978. Sense organs on the antennae of two species of collembola (insecta). Journal of Morphology 157, 1-19.

Snider, R.M., Burcher, J.W., 1973. The life history of *Folsomia candida* (Willen) (Collembola: Isotomidae) relative to temperature. The Great Lakes Entomologist 6, 97-106.

van Gestel, C.A.M., 2012. Soil ecotoxicology: state of the art and future directions. ZooKeys 176, 275-296.

Vidal, T., Pereira, J.L., Abrantes, N., Soares, A.M.V.M., Gonçalves, F., 2012. Ecotoxicological assessment of contaminated river sites as a proxy for the water framework directive: an acid mine drainage case study. Water, Air and Soil Pollution 223, 6009-6023.

Zinkler, D., Platthaus, J., 1996. Tolerance of soil-dwelling Collembola to high carbon dioxide concentrations. European Journal of Entomology 93, 443-450.

Figure captions

Figure 1. Climate treatments simulated by modulating individual climate factors during the performance of the avoidance behavior tests (48 h) with the soil invertebrate species: a) air temperature (15 – 25, 20 – 30 and 25 – 35 ºC); b) ultraviolet (UV) radiation (without and with UV radiation emission); c) atmospheric CO_2 concentration (600, 800 and 1000 ppm). In the UV radiation treatment "on" indicates that the UV lamps were emitting UV radiation (in total 6 h per day) and "off" that there was no UV radiation emission (in total 18 h per day). For each climate factor modulated the remaining factors were kept at the levels recommended by the standardized ISO guidelines (20 °C and no control of UV radiation and atmospheric CO_2 concentration). Soil moisture content was established at 50% of the soil maximum water holding capacity for all the climate treatments simulated.

Figure 2. Avoidance behavior of *Folsomia candida* and *Enchytraeus crypticus* in tests performed for 48 h under the standard conditions (climate conditions recommended by the standardized ISO guidelines) and the climate treatments simulated by individually modulating the climate factors (a) air temperature, (b) ultraviolet (UV) radiation and (c) atmospheric CO_2 concentration. Lufa 2.2 soil was used as control soil. Data are average ± SD (n = 5). Positive values indicate metal(loid)-contaminated test soil avoidance, negative values metal(loid)-contaminated test soil preference, and zeros neutral response. Asterisks (*) indicate significant differences in control and metal(loid)-contaminated test soils (Fisher's test, $p < 0.05$).

Credit Author Statement

Catarina Malheiro: Methodology, Investigation, Data curation, Writing - original draft, and Writing - review & editing.

Diogo N. Cardoso: Investigation, and Writing - Review & Editing.

Susana Loureiro: Conceptualization, Methodology, Writing - Review & Editing, Supervision, and Funding acquisition.

M. Nazaret González-Alcaraz: Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, and Funding acquisition.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1. General characterization of the metal(loid)-contaminated soils from central-northern Portugal (data taken from González-Alcaraz et al., 2019). Values are average ± SD (n=3). EC (electrical conductivity). DOC (dissolved organic carbon). TDN (total dissolved nitrogen). TOC (total organic carbon). TN (total nitrogen). CEC (cation exchange capacity). WHC (water holding capacity).

Soil parameter	Mining soil	Agricultural soil
pH 0.01M $CaCl_2$[a]	5.86 ± 0.01	4.75 ± 0.08
EC (dS m^{-1})[b]	0.34 ± 0.02	0.06 ± 0.001
DOC (mg kg^{-1})[c]	42.4 ± 6.7	125 ± 2
TDN (mg kg^{-1})[c]	13.3 ± 1.5	22.3 ± 2.3
TOC (mg kg^{-1})[d]	45.9 ± 2.2	26.0 ± 1.4
TN (mg kg^{-1})[d]	1.77 ± 0.04	1.65 ± 0.25
CEC ($cmol_c$ kg^{-1})[e]	10.3 ± 1.4	7.8 ± 0.7
WHC (%)[f]	40.0 ± 0.3	36.5 ± 0.9
Texture[g]	Loamy sand	Loamy sand

[a] 1:5 (w:v) soil:0.01M $CaCl_2$ suspensions after 2 h shaking at 200 rpm.

[b] 1:5 (w:v) soil:H_2O suspensions after 2 h shaking at 200 rpm.

[c] DOC and TDN concentrations determined in previous soil:H_2O suspensions with an automatic TOC analyzer (TOC-VCSH Shimadzu).

[d] TOC and TN concentrations determined with an automatic TOC analyzer (TOC-VCSH Shimadzu).

[e] Saturation of soil exchange complex with 1N CH_3COONH_4 pH 7.0 and displacement of adsorbed ammonium with 10% NaCl (Chapman, 1965). Ammonium concentration determination by spectrophotometry at λ = 670 nm (Lambda 25 UV/Vis spectrometer Perkin Helmer) (NEIKER, 2005).

[f] Soil saturation with water for 3 h followed by 2 h of water excess removal (ISO, 1998).

[g] Bouyouco's densimeter method (Gee and Bauder, 1986).

Table 2. Survival and organism distribution between sections of *Folsomia candida* in the dual control tests (both test container sections filled with Lufa 2.2 soil) for the standard conditions (climate conditions recommended by the standardized ISO guidelines) and the climate treatments simulated by individually modulating the climate factors air temperature, ultraviolet (UV) radiation and atmospheric CO_2 concentration. Data are average ± SD (n = 5). For survival asterisks (*) indicate significant differences between climate treatments and standard conditions (Student t-test; $p < 0.05$). For organism distribution between sections asterisks (*) indicate significant differences between observed and expected values (Fisher's test, $p<0.05$).

Climate factor	**Climate treatment**	**Survival (%)**	**Organism distribution between sections (%)**
Air temperature	Standard conditions	98.8 ± 2.5	51.8 ± 6.9 vs. 48.2 ± 6.9
	15 – 25 ºC	92.0 ± 8.4	55.0 ± 11.7 vs. 45.0 ± 11.7
	20 – 30 ºC	98.0 ± 4.5	52.9 ± 6.9 vs. 47.1 ± 6.9
	25 – 35 ºC	71.0 ± 4.5 (*)	77.1 ± 14.7 vs. 22.9 ± 14.7 (*)
UV radiation	Standard conditions	99.0 ± 2.2	49.5 ± 14.6 vs. 50.5 ± 14.6
	With UV	96.7 ± 5.2	55.4 ± 9.9 vs. 44.6 ± 9.9
Atmospheric CO_2	Standard conditions	98.4 ± 2.9	49.4 ± 8.6 vs. 50.6 ± 8.6
	600 ppm	100 ± 0	50.0 ± 6.1 vs. 50.0 ± 6.1
	800 ppm	100 ± 0	45.0 ± 11.7 vs. 55.0 ± 11.7
	1000 ppm	99.0 ± 2.2	54.5 ± 6.2 vs. 45.5 ± 6.2

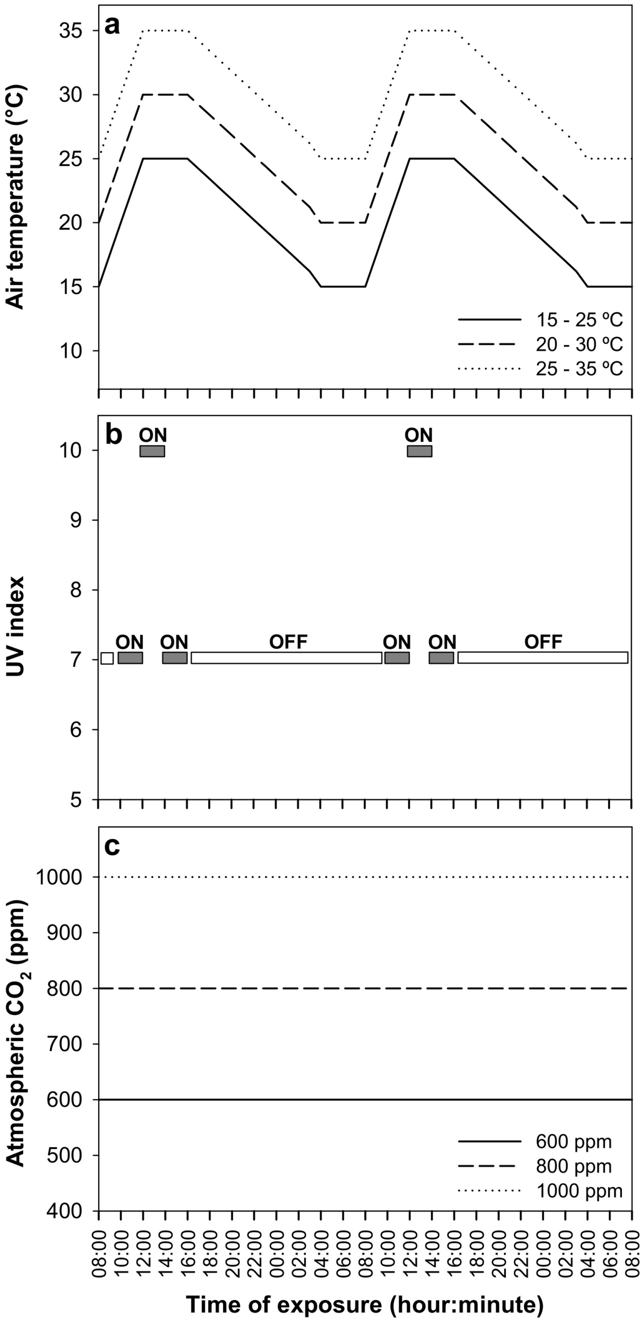
Table 3. Survival and organism distribution between sections of *Enchytraeus crypticus* in the dual control tests (both test container sections filled with Lufa 2.2 soil) for the standard conditions (climate conditions recommended by the standardized ISO guidelines) and the climate treatments simulated by individually modulating the climate factors air temperature, ultraviolet (UV) radiation and atmospheric CO_2 concentration. Data are average ± SD (n = 5). For organism distribution between sections asterisks (*) indicate significant differences between observed and expected values (Fisher's test, $p < 0.05$).

Climate factor	Climate treatment	Survival (%)	Organism distribution between sections (%)
Air temperature	Standard conditions	95.0 ± 5.8	43.9 ± 31.1 vs. 56.1 ± 31.1
	15 – 25 ºC	90.0 ± 12.2	54.6 ± 15.1 vs. 45.4 ± 15.1
	20 – 30 ºC	94.0 ± 8.9	52.3 ± 25.4 vs. 47.7 ± 25.4
	25 – 35 ºC	96.0 ± 8.9	26.5 ± 14.5 vs. 73.5 ± 14.5 (*)
UV radiation	Standard conditions	95.0 ± 5.8	39.2 ± 7.9 vs. 60.8 ± 7.9
	With UV	95.0 ± 5.5	36.9 ± 21.2 vs. 63.1 ± 21.2
Atmospheric CO_2	Standard conditions	91.8 ± 8.1	46.1 ± 23.3 vs. 53.9 ± 23.3
	600 ppm	96.0 ± 8.9	54.0 ± 26.3 vs. 46.0 ± 26.3
	800 ppm	94.0 ± 5.5	57.3 ± 15.9 vs. 42.7 ± 15.9
	1000 ppm	95.0 ± 10.0	35.6 ± 30.4 vs. 64.4 ± 30.4

Graphical abstract

Highlights

- Air temperature, UV radiation and atmospheric CO_2 as climate change drivers
- Non-avoidance behavior of *F. candida* towards metal(loid)-contaminated soils
- *F. candida* changed its behavior to avoidance under high atmospheric CO_2
- Avoidance behavior of *E. crypticus* towards metal(loid)-contaminated soils
- Avoidance behavior of *E. crypticus* not affected when changing climate conditions


a
b
c
Air temperature (°C)
35
30
25
20
15
10
15 - 25 ºC
20 - 30 ºC
25 - 35 ºC
UV index
10
9
8
7
6
5
ON
ON
ON
ON
OFF
ON
ON
OFF
Atmospheric CO_2 (ppm)
1000
900
800
700
600
500
400
600 ppm
800 ppm
1000 ppm
08:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
00:00
02:00
04:00
06:00
08:00
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12:00
14:00
16:00
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20:00
22:00
00:00
02:00
04:00
06:00
08:00
Time of exposure (hour:minute)

Figure 1

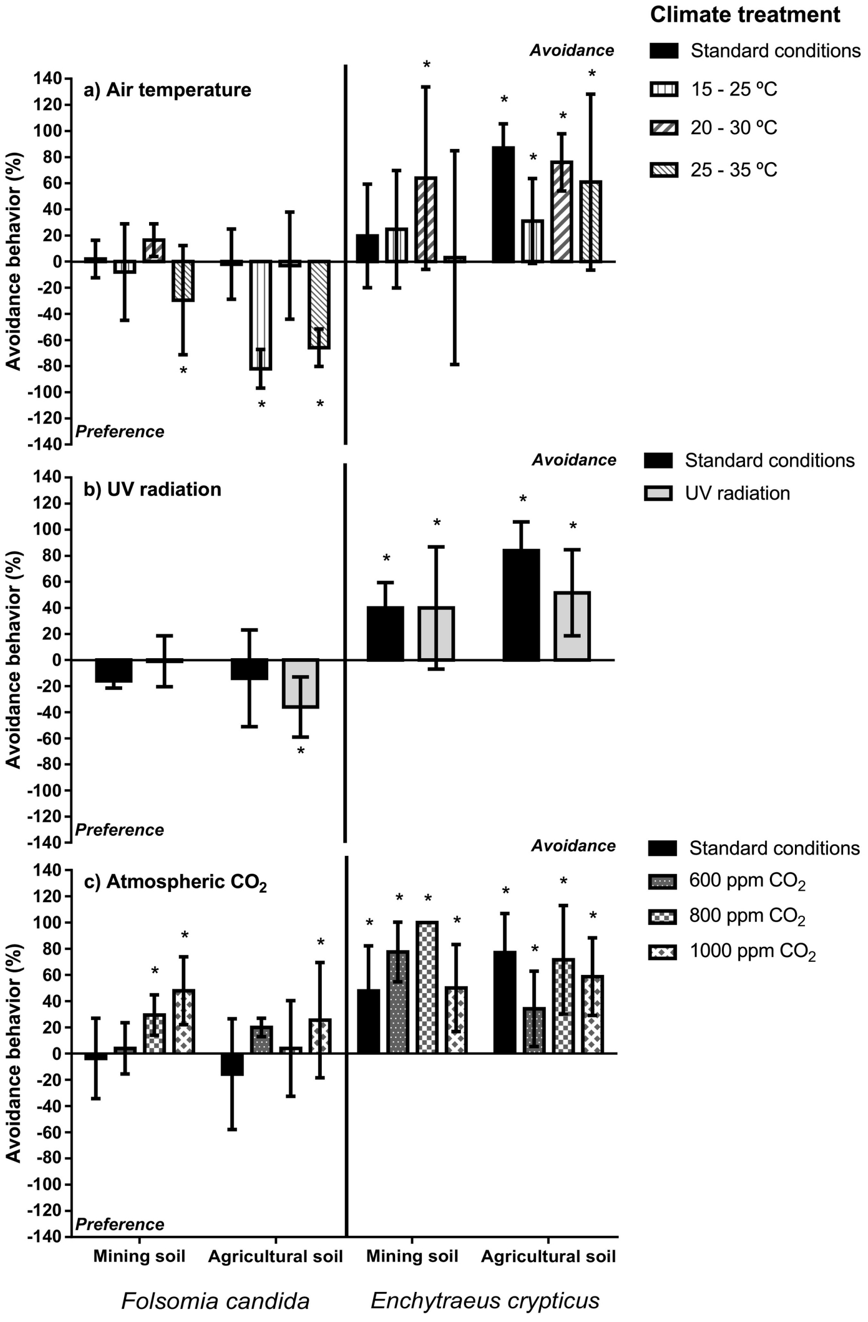
Climate treatment
Standard conditions
15 - 25 ºC
20 - 30 ºC
25 - 35 ºC
a) Air temperature
Avoidance
Preference
Avoidance behavior (%)
b) UV radiation
Standard conditions
UV radiation
c) Atmospheric CO2
Standard conditions
600 ppm CO2
800 ppm CO2
1000 ppm CO2
Mining soil
Agricultural soil
Mining soil
Agricultural soil
Folsomia candida
Enchytraeus crypticus

Figure 2