Received: 28 May 2019 | Revised: 28 October 2019 | Accepted: 1 November 2019

DOI: 10.1111/mec.15299

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Climatic vulnerabilities and ecological preferences of soil invertebrates across biomes

Felipe Bastida[1] | **David J. Eldridge**[2] | **Sebastián Abades**[3] | **Fernando D. Alfaro**[3,4,5] | **Antonio Gallardo**[6] | **Laura García-Velázquez**[6] | **Carlos García**[1] | **Stephen C. Hart**[7] | **Cecilia A. Pérez**[4] | **Fernanda Santos**[7] | **Pankaj Trivedi**[8] | **Mark A. Williams**[9] | **Manuel Delgado-Baquerizo**[10]

[1]Soil and Water Conservation, CEBAS-CSIC, Murcia, Spain

[2]University of New South Wales, Sydney, NSW, Australia

[3]GEMA Center for Genomics, Ecology & Environment, Universidad Mayor, Santiago, Chile

[4]Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile

[5]Centro de Biodiversidad y Genética (CBG), Universidad Mayor de San Simón, Cochabamba, Bolivia

[6]Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Sevilla, Spain

[7]Department of Life and Environmental Sciences, Sierra Nevada Research Institute, University of California, Merced, CA, USA

[8]Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

[9]Agricultural and Life Sciences, Virginia Polytechnic and State University, Blacksburg, VA, USA

[10]Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Madrid, Spain

Correspondence
Felipe Bastida, Soil and Water Conservation, CEBAS-CSIC, Murcia, Spain.
Email: fbastida@cebas.csic.es

Funding information
Spanish Ministry of Science, Innovation and Universities and FEDER funds, Grant/Award Number: AGL2017-85755-R and CGL2017-88124-R; Millennium Scientific Initiative, Grant/Award Number: P05-002 ; CONICYT, Grant/Award Number: PFB-23 ; IAI-CRN, Grant/Award Number: 3005; Spanish Research Council (CSIC), Grant/Award Number: 201740I008 and LINKA20069; FONDECYT, Grant/Award Number: 1170995; European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie, Grant/Award Number: 702057; Fundación Séneca from Murcia province, Grant/Award Number: 19896/GERM/15

Abstract

Unlike plants and vertebrates, the ecological preferences, and potential vulnerabilities of soil invertebrates to environmental change, remain poorly understood in terrestrial ecosystems globally. We conducted a cross-biome survey including 83 locations across six continents to advance our understanding of the ecological preferences and vulnerabilities of the diversity of dominant and functionally important soil invertebrate taxa, including nematodes, arachnids and rotifers. The diversity of invertebrates was analyzed through amplicon sequencing. Vegetation and climate drove the diversity and dominant taxa of soil invertebrates. Our results suggest that declines in forest cover and plant diversity, and reductions in plant production associated with increases in aridity, can result in reductions of the diversity of soil invertebrates in a drier and more managed world. We further developed global atlases of the diversity of these important soil invertebrates, which were cross-validated using an independent database. Our study advances the current knowledge of the ecological preferences and vulnerabilities of the diversity and presence of functionally important soil invertebrates in soils from across the globe. This information is fundamental for improving and prioritizing conservation efforts of soil genetic resources and management policies.

KEYWORDS
aridity, biogeography, climate change, diversity, plant diversity, soil invertebrates

1 | INTRODUCTION

Biodiversity loss resulting from global change is predicted to have negative consequences for human wellbeing and ecosystem sustainability (Chapin et al., 2000; Eisenhauer, Bonn, & Guerra, 2019; Gossner et al., 2016; Hooper et al., 2012; Sala et al., 2000; Wagg, Bender, Widmer, & van der Heijden, 2014; Wall, Nielsen, & Six, 2015). In the case of plants and vertebrates, the identification of ecological preferences, diversity hotspots and vulnerabilities has enabled conservation agencies to prioritise the protection of these biological resources (Malcolm, Liu, Neilson, Hansen, & Hannah, 2006; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). Unlike plants, vertebrates and soil microbial communities such as bacteria and fungi (Delgado-Baquerizo et al., 2018; Liang et al., 2016; Reich, Walters, & Ellsworth, 1997; Tedersoo et al., 2014; Willig, Kaufman, & Stevens, 2003), information on invertebrate diversity is still lacking for the most ubiquitous and functionally important taxa such as nematodes, arachnids and rotifers (Eisenhauer et al., 2019). The major ecological predictors of the diversity of soil invertebrates remain widely unexplored and poorly understood at large spatial scales. Recent studies suggest a strong societal and research bias against soil invertebrates in favour of more “charismatic” organisms such as plants and vertebrates (e.g., birds; Troudet, Grandcolas, Blin, Vignes-Lebbe, & Legendre, 2017). Soil invertebrates are critically important for human well-being, contributing to soil function by regulating key ecosystem services such as litter decomposition (García-Palacios, Shaw, Wall, & Hättenschwiler, 2016), nutrient cycling, plant nutrient uptake and climate regulation such as CO_2 fluxes (Frouz, 2018). Therefore, improving our understanding of the potential global vulnerabilities and ecological preferences of soil invertebrates is fundamental to manage the predicted changes in ecosystem services under global change scenarios.

Recently, there has been increasing call for a greater focus on the role of invertebrates in ecosystem functioning at global scales, and the number of studies warning about the potential vulnerabilities of these organisms to global change is rising (Eisenhauer et al., 2019). Traditionally, logistic limitations have precluded large scale investigations on the diversity of soil invertebrates. However, sequencing of 18S rRNA genes and barcoding have been suggested as appropriate methods to characterize the diversity of soil invertebrates at large spatial scales, opening the door to advance our understanding of the ecological preferences and vulnerabilities of soil invertebrates (Drummond et al., 2015; Oliverio, Gan, Wickings, & Fierer, 2018; Ramirez et al., 2014; Wu, Ayres, Bardgett, Wall, & Garey, 2011). Pioneer studies have shown that many invertebrates are globally ubiquitous in soil (Ramirez et al., 2014; Wu et al., 2011). However, much less is known about their ecological preferences and potential vulnerabilities of the diversity (i.e., richness) and dominant taxa of globally widespread soil invertebrates (phylotypes, syn. species) that would be expected to play important roles in terrestrial ecosystems (i.e., nutrient cycling, organic matter breakdown, etc.). Global environmental changes such as those resulting from climate change and land use change (e.g., deforestation) are expected to affect the diversity of soil organisms. However, the linkage between important climatic factors such as aridity (rainfall and temperature) and vegetation types (e.g., forest and grasslands), and the diversity of the main invertebrate groups remains largely unexplored. Increases in aridity by the end of this century (Huang, Yu, Guan, Wang, & Guo, 2015) are predicted to reduce perennial plant diversity (Maestre et al., 2012) and affect soil microbial communities (Maestre et al., 2015). Increases in aridity might also affect the diversity of soil invertebrates globally, but this has never been tested. Identifying the major ecological predictors of soil invertebrate diversity will allow us to better predict how soil invertebrates might change under different global change scenarios and how this might flow on to important ecosystem functions mediated by invertebrates (Wall et al., 2008).

Herein, we conducted a cross-biome survey across 83 locations in six continents, and including multiple climates (tropical, temperate, polar and arid) and ecosystem types (e.g., forest, grasslands and shrublands; Figure S1) to identify the ecological preferences and potential vulnerabilities of the diversity of dominant soil invertebrate taxa across the globe. Using this information, we predicted the global distribution of the diversity of soil invertebrates and generated global atlases which were cross-validated using an independent global data set.

2 | MATERIALS AND METHODS

2.1 | Global survey

Soil and vegetation data were collected between 2016 and 2017 from 83 locations in six continents (Figure S1). The field survey was designed to include globally distributed locations spanning a wide range of climate (tropical, temperate, polar and arid), vegetation (including grasslands, shrublands, forests and forblands) types and soil properties. Field surveys were conducted according to a standardized sampling protocol (Maestre et al., 2012). In each location, we surveyed a 50 m × 50 m plot. Three parallel transects of the same length, spaced 25 m apart were added. Perennial plant richness (number of species) was estimated at the plot level. We estimated the mean annual plant productivity for the 2008–2017 period using the Normalized Difference Vegetation Index (NDVI) from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA's Terra satellites at 250 m resolution (Delgado-Baquerizo et al., 2018). This measure of plant production aims to summarize the production of plants over multiple years, rather than at one point in time.

2.2 | Soil sampling

Soil sampling was designed to cover the entire spatial heterogeneity within each plot. Five composite topsoil (of five 0–10 cm soil cores) samples were collected under the dominant vegetation within each location. Thus, a total of 25 soil cores were collected at each location, and merged in five soil samples. Following field sampling, soils were sieved (2 mm) and separated into two portions. One portion was air-dried and used for soil physical and chemical analyses. The other portion of soil was immediately frozen at –20°C for molecular analyses. This storage approach is commonly used in global surveys (Maestre et al., 2012, 2015; Tedersoo et al., 2014). Ten grams of frozen soil sample, from composite soil samples corresponding to ~5 kg of soil, as explained above, were ground using a mortar and liquid N, aiming to homogenize soils and obtain a representative sample for sequencing.

2.3 | Soil properties

For all soil samples, we measured electrical conductivity, pH, texture (% of clay plus silt content), soil organic carbon (SOC) concentration and total soil phosphorus (P) concentration. Soil properties were determined using standardized protocols (Maestre et al., 2012). Soil pH was measured in all the soil samples with a pH metre, in a 1:2.5 mass: volume soil and deionized water suspension. Soil texture (% of fine fractions: clay + silt) was determined as described elsewhere (Kettler, Doran, & Gilbert, 2001). Mean annual temperature ranged from –1.8 to 21.6°C, mean annual precipitation ranged from 104 to 2,833 mm; soil organic C (SOC) concentration ranged between 0.1% and 35%; total soil P between 9 and 2,558 mg P/kg soil; pH between 3.8 and 9.1 and the % of clay + silt varied between 0.3% and 71%.

2.4 | Soil invertebrate diversity

The diversity of soil invertebrates was assessed by amplicon sequencing using the Illumina MiSeq platform. Soil DNA was extracted using the Powersoil DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's instructions. A portion of the eukaryotic 18S rRNA genes were sequenced after PCR using the Euk1391f (5′-GTACACCGCCCGTC-3′)/EukBr (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) primer sets according to (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009; Ramirez et al., 2014). This is the primer set most commonly used by global surveys such as the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/18s/) or the Australian Microbiome Initiative (Bissett et al., 2016). The PCR protocol utilized here was exactly the one described by the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/18s/). Duplicate PCR reactions were completed for each of the 415 extracted samples. The water utilized in the last step of DNA extraction was sequenced as a negative control to check for potential contamination. We normalized sample concentrations with the SequalPrep Normalization Plate Kit (Invitrogen) and then sequenced samples with the 2 × 300 bp paired-end chemistry on the Illumina MiSeq platform.

Bioinformatic processing was performed using a combination of QIIME (Caporaso et al., 2010), USEARCH (Edgar, 2010) and UNOISE3 (Edgar, 2017). Raw reads were demultiplexed and then merged and quality filtered with UPARSE (Edgar, 2013). Primers were removed from our amplicons before zOTU clustering. We then identified exact sequence variants with UNOISE3 (Edgar, 2017). Sequences were quality filtered to a maximum expected error threshold of 1.0 and then exact sequence variants (ESVs) were identified, as per UNOISE3 defaults. We used exact sequence variants (100% similarity) rather than clustering sequences based on an arbitrary sequence similarity threshold (e.g., 97%). Thus, zOTUs corresponded to phylotypes identified at the 100% identity level. Even so, we would like to clarify that the phylotypes identified here do not equate to species-level diversity (e.g., comparable to species of plants or animals).

The Protist Ribosomal Reference database (PR2) database was used for sequence identification (Guillou et al., 2013) using the RDP Classifier algorithm. We filtered taxa assigned to Embryophyta, Fungi and Protists for downstream analyses. A total of 531,836 reads were obtained. The original OTU abundance tables (sample-level) were rarefied at 300 sequences/sample, respectively, to ensure even sampling depth within each belowground group of organisms. Before conducting further analyses, we ensured that our choice of rarefaction level, taken to maximize the number of samples in our study, was not obscuring our results. Thus, using the samples with the highest sequence/sample yield, we tested for the impact of different levels of rarefaction on invertebrate richness. We found highly statistically significant correlations between the diversity of invertebrates (rarefied at 300 vs. 1,800 sequences/sample; Pearson's $r = .96$; $p < .001$), providing evidence that our choice of rarefaction level did not affect our results or conclusions. Moreover, we found strong positive and significant correlations for the richness of main invertebrate groups calculated from a single (300 sequences/samples) or independent (75 sequences/samples) rarefied OTU tables (Nematoda–Chromodorea: Pearson's $r = .86$, $p < .001$; Nematoda–Enoplea: Pearson's $r = .87$, $p < .001$; rotifers: Pearson's $r = .78$, $p < .001$; Arachnida: Pearson's $r = .85$, $p < .001$). Given these results, we provided evidence that the selected rarefaction level did not influence the obtained conclusions in this study. In addition, we repeated our analyses using a widely used normalization method in the literature (Trimmed mean of M [TMM]: Robinson & Oshlack, 2010) to further cross-validate our rarefaction approach. We found that the richness of main invertebrate groups calculated from rarefied OTU tables (300 sequences/samples) was highly positively and significantly correlated with the same indexes calculated from a normalized OTU table using the TMM normalization approach (Nematoda–Chromodorea: Pearson's $r = .91$, $p < .001$; Nematoda–Enoplea: Pearson's $r = .94$, $p < .001$; rotifers: Pearson's $r = .86$, $p < .001$; Arachnida: Pearson's $r = .91$, $p < .001$).

Based on our sample-level OTU tables, we merged information for all replicates to obtain a plot-level estimation of invertebrate diversity, and thus account for the spatial heterogeneity within each plot. In particular, we merged (averaging the rarefied reads/zOTU) the information derived from the zOTU abundance tables coming from the five replicates/plot to obtain site-level estimates. Information within the plot-level OTU table was then transformed to % of relative abundance of each OTU (phylotype hereafter). Even so, hereafter, we always used presence-absence OTU tables for calculating richness or presence of specific taxa. Using the merged OTU table, we calculated the richness of soil invertebrates present in our samples. Thus we obtained information on the diversity of soil invertebrates at the plot-level, rather than at the sample level, which could be misleading given the capacity of soil invertebrates to move in soils. We used the richness of soil invertebrates as our measure of invertebrate diversity. Although our study included 415 soil samples (five replicates × 83 locations), not all samples passed our rarefaction cutoff. We obtained information for 81 of the 83 plots.

2.5 | Statistical analyses

We first quantified the % of phylotypes of different groups of soil invertebrates in our study sites. We then focused on the four most dominant groups of invertebrates found in our survey (two types of Nematoda, Arachnida and rotifers; Figure 1a) for downstream analyses to ensure enough resolution to address our research questions. Then, Spearman correlations were used to evaluate the associations between diversity of predominant groups of organisms and environmental factors. Environmental factors included distance from equator, climate (aridity, mean annual temperature [MAT], precipitation seasonality [PSEA], mean diurnal temperature range [MDR]), variables related to soil chemistry (soil organic C and total P contents, C:N ratio and pH), soil physics (texture), plant community features (plant richness and mean annual net primary productivity [NPP]), and dominant vegetation types (forests, shrublands and grasslands). Aridity was calculated as 1 − Aridity Index. By using Spearman correlations, we aimed to identify the most important correlations among environmental data and richness of invertebrates. Spearman rank correlations measure the strength and direction of association between two ranked variables. Moreover, Spearman rank correlations do not require normality of data, and more importantly, linearity is not strictly an assumption of these correlations.

2.5.1 | Structural equation modelling

We used structural equation modelling (SEM; a priori model in Figure S2) to provide a comprehensive and integrated view of the major ecological predictors of invertebrate diversity across a broad range of climates, vegetation types and soil properties (Figure S1; see Section 2). Our model included the important environmental factors considered above. The association between aridity with soil C and NPP has been previously reported to show a curvilinear shape (Delgado-Baquerizo et al., 2013). Because of this, these relationships were introduced in our a priori SEM as a second-order polynomial. To introduce polynomial relationships into our model, we calculated the square of aridity and introduced it into our model using a composite variable approach (Grace, 2006). The use of composite variables does not alter the underlying SEM model, but collapses the effects of multiple conceptually-related variables into a single composite effect, aiding interpretation of model results. We evaluated the fitting of our model using the model chi-square test and root mean square error of approximation (RMSEA). Because, the residuals of some data were not normally distributed, we confirmed fit using the Bollen-Stine bootstrap test. Also, we aimed to be conservative with our predictions and only reported highly significant associations ($p < .01$). All the SEM analyses were conducted using AMOS 20.0 (AMOS IBM).

2.5.2 | Global mapping

We used the prediction-oriented regression model Cubist (Quinlan, 1993) to predict the diversity of the dominant groups of invertebrates. The Cubist algorithm uses a regression tree analysis to generate a set of hierarchical rules using information on environmental covariates, which are later used for spatial prediction (Kuhn, Weston, Keefer, & Coulter, 2016). Covariates in our models include 10 environmental predictors for which global information is widely available: soil properties (soil C, pH and texture), climate (MAT, MDR, PSEA, Aridity), and vegetation attributes (annual NPP from 2008 to 2017, and major vegetation types: forests and grasslands. The inclusion of these variables in our models was limited to the existence of high resolution global maps. Information for other environmental predictors was not available at the global scale or was not comparable with our data. Global predictions were made on a 25 km resolution grid. Global information on soil properties for this grid was obtained using the ISRIC (global gridded soil information) Soil Grids (https://soilgrids.org/#!/?layer=geonode:taxnwrb_250m). Similarly, global information on the major vegetation types in this study (grasslands and forests) was obtained using the Globcover2009 map from the European Space Agency (http://due.esrin.esa.int/page_globcover.php; Hengl et al., 2017). Global information on climate and NPP (2008–2017) were obtained from the WorldClim database (www.worldclim.org; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and NASA satellites (https://neo.sci.gsfc.nasa.gov), as explained above. We used the package Cubist in R to conduct these analyses (Kuhn et al., 2016). Finally, we cross-validated our maps using two different approaches. First, we evaluated the correlation between observed and predicted (maps) data in our global data set. Second, we used the database from an independent global database (Ramirez et al., 2014) to further test the reliability of our maps. We evaluated the correlation between predicted soil diversity of invertebrates in our global maps with the diversity of the same invertebrates obtained for 48 soil samples in Ramirez et al. (2014).

2.5.3 | Ecological clusters of ubiquitous phylotypes of soil invertebrates

We then identified the potential ecological preferences of relatively ubiquitous phylotypes of soil invertebrates (those present

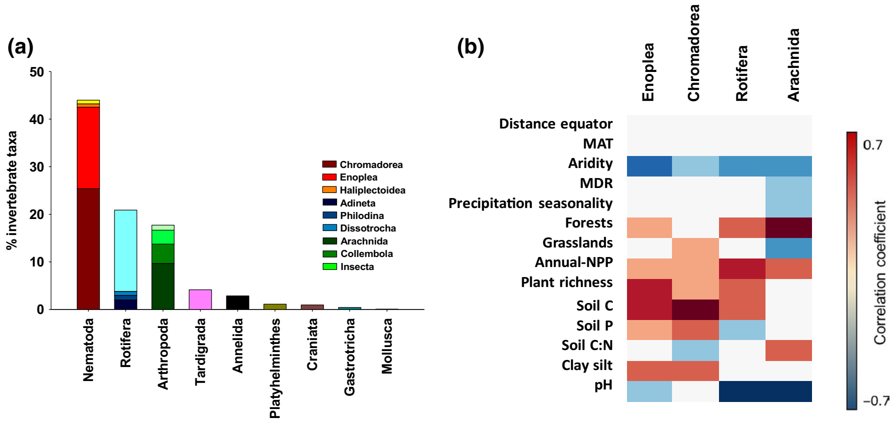

FIGURE 1 (a) Percentage (%) of phylotypes of soil invertebrates across different groups and (b) correlations between invertebrate richness and environmental predictors. Only significant correlations ($p < .05$) are displayed

in at least 20% of locations). We clustered these soil phylotypes into different ecological groups. We used Spearman correlations to identify potential associations between environmental predictors and the presence/absence of ubiquitous invertebrate phylotypes. Information on correlations (significant $p < .05$ correlation coefficients) was then used to cluster our dominant phylotypes in different ecological clusters with hierarchical cluster analysis (as implemented in the hclust function in the R package stats; see Delgado-Baquerizo et al., 2018 for a similar approach). See a rationale on the utility of this type of correlations above. We then computed the relative abundance of each cluster per sample by averaging the presence/absence information of the phylotypes that belong to each ecological cluster. Thus, the relative presence of each cluster ranged between 0 and 1.

3 | RESULTS

A total of 1,266 soil invertebrate phylotypes were retrieved in our database across 83 globally distributed locations. Most identified soil phylotypes belonged to Nematoda (43% of the total phylotypes), including Chromadorea and Enoplea as dominant classes (Figure 1a). Following Nematoda, Rotifera was the most diverse group of soil invertebrates (18% of the identified phylotypes), including Adineta, Phylodina and Dissotrocha, and followed by Arthropoda (16%), including Arachnida (mostly mites), Collembola and Insecta as dominant taxa. Other less diverse soil taxa included members from Tardigrada, Annelida, Platyhelminthes, Craniata, Gastrotricha and Mollusca. Considering the high diversity of Nematoda (Chromadorea and Enoplea), Rotifera and Arachnida, in comparison with the other invertebrate groups, we focused on these main groups to ensure enough resolution and worked with them in downstream analyses.

We then conducted correlation analyses to gain more knowledge of the potential ecological factors associated with the diversity of the most dominant soil invertebrates in our survey: Chromadorea, Enoplea, Rotifera and Arachnida (Figure 1b). We found that aridity and abiotic factors associated with the presence of forest, plant richness and mean annual NPP were the most important factors associated with the diversity of nematodes (Chromadorea and Enoplea), rotifers and arachnids. Aridity was consistently negatively related to the diversity of all these organisms. Conversely, forest and mean annual NPP was always positively correlated with the diversity of these soil organisms. Plant richness was positively associated with the diversity of nematodes and rotifers, but not to Arachnida (Figure 1b).

We evaluated the direct and indirect associations of multiple environmental factors and the diversity of soil invertebrates. Our SEMs explained between 48% and 69% of the variation of the diversity of soil invertebrates (Figure 2). We found multiple organism-dependent associations between forests, plant diversity, mean annual plant productivity and aridity with the diversity nematodes, arachnids and rotifers (Figure 2). Plant richness and mean annual NPP had positive associations and aridity negative associations with the diversity of nematodes. Aridity also had an indirect negative effect on the diversity of nematodes via declines in annual NPP. Plant richness showed the only significant positive effect on the diversity of rotifers. Forest and pH showed positive and negative associations with the diversity of Arachnida, respectively. Soil properties and other environmental factors also showed some organism-dependent associations on the diversity of soil invertebrates (Figure 2).

Furthermore, global atlases of the distribution of soil invertebrates were developed. This was possible for three main reasons. First, we found that environmental factors can predict an important portion of the variation in the distribution of the diversity of soil invertebrates in our study (Figures 1b and 2). Second, our database included wide gradients of factors covering most ranges of environmental conditions and soil properties found on Earth. Finally, high resolution maps including information on reported important environmental predictors of soil diversity are available at the global scale. Following the approach explained in the Methods section, we generated a novel global atlas for the diversity of soil invertebrates (Figure 3).

We conducted two independent cross-validations of our global atlas of soil invertebrates. First, we found that predicted and observed values of the diversity of individual groups of soil taxa were positively and significantly correlated in our database: Chromadorea ($r = .59$; $p < .001$; $n = 81$); Enoplea ($r = .58$; $p < .001$; $n = 81$); Rotifera ($r = .47$; $p < .001$; $n = 81$); Arachnida ($r = .36$; $p < .001$; $n = 81$). Second,

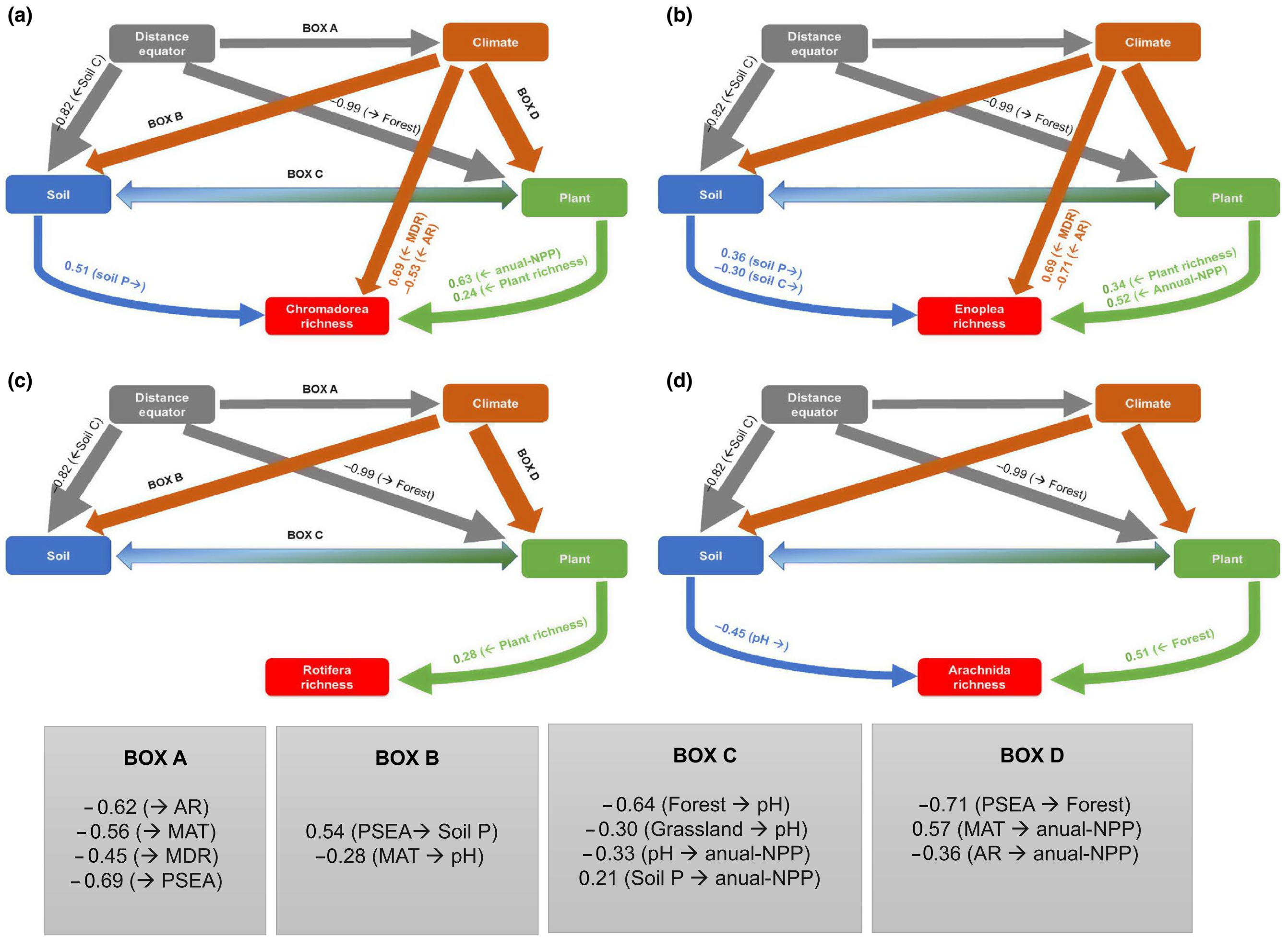

FIGURE 2 Structural equation model (SEM) describing the major ecological predictors of the soil invertebrate biodiversity: (a) Chromadorea (R^2 = .61); (b) Enoplea (R^2 = .69); (c) Rotifera (R^2 = .48); (d) Arachnida (R^2 = .58)

despite the differences in sampling and molecular protocols, we found that our predicted and observed values for the diversity of individual taxa were, in general, positively and significantly correlated with the same measures using independent data from Ramirez et al. (2014): Chromadorea (*r* = .36; *p* = .01; *n* = 47); Enoplea (*r* = .58; *p* < .001; *n* = 47); Rotifera (*r* = .46; *p* = .001; *n* = 81); and Arachnida (*r* = .27; *p* = .06; *n* = 47).

Our maps predicted the existence of potential global hotspots of the most diverse taxa (Figure 3). For example, both Chromadorea and Enoplea nematodes were expected to be highly diverse in the West and East coasts of North-America, Central America, sub-Saharan fringe, South and northwest China, South East Asia, South-Siberia, East Australia and temperate regions of Europe. Enoplea was predicted to be more diverse in South America than Chromadorea. Further, hotspots of rotifers diversity were predicted in South-America, East-Northern America, sub-Saharan fringe and South East Asia. The diversity of Arachnida was expected to be high in north latitudes of the globe, some regions in South America and the sub-Saharan fringe.

Finally, we identified the potential ecological preferences of relatively ubiquitous soil invertebrates (present at >20% of locations). We found 79 relatively ubiquitous soil invertebrate phylotypes across contrasting locations. The most ubiquitous taxa included soil phylotypes associated with Arthropoda (mostly Arachnida), Nematoda and Rotifera. Our results suggest that the ubiquitous soil invertebrate phylotypes can be classified into major ecological clusters that share similar habitat preferences. These phylotypes group into six reasonably well-defined ecological clusters sharing environmental preferences for: (a) high mean annual-NPP, (b) low aridity, (c) high plant richness, (d) forest environments, (e) high content of clay plus silt, and (f) high MAT (Figure 4). A complete list of these soil phylotypes and their environmental preferences is shown in Table S1. These ecological clusters always included taxa within Nematoda, Arthropoda and Rotifera, suggesting that phylotypes within these soil groups might have very different environmental preferences. Other clusters were dominated by single soil groups. For example, the low aridity and high clay plus silt clusters were dominated by Nematoda (eight and 18 phylotypes, respectively; Table S1). Further, up to eight phylotypes of Rotifera, four of Nematoda and four of Arhtropoda were classified into the high annual-NPP cluster.

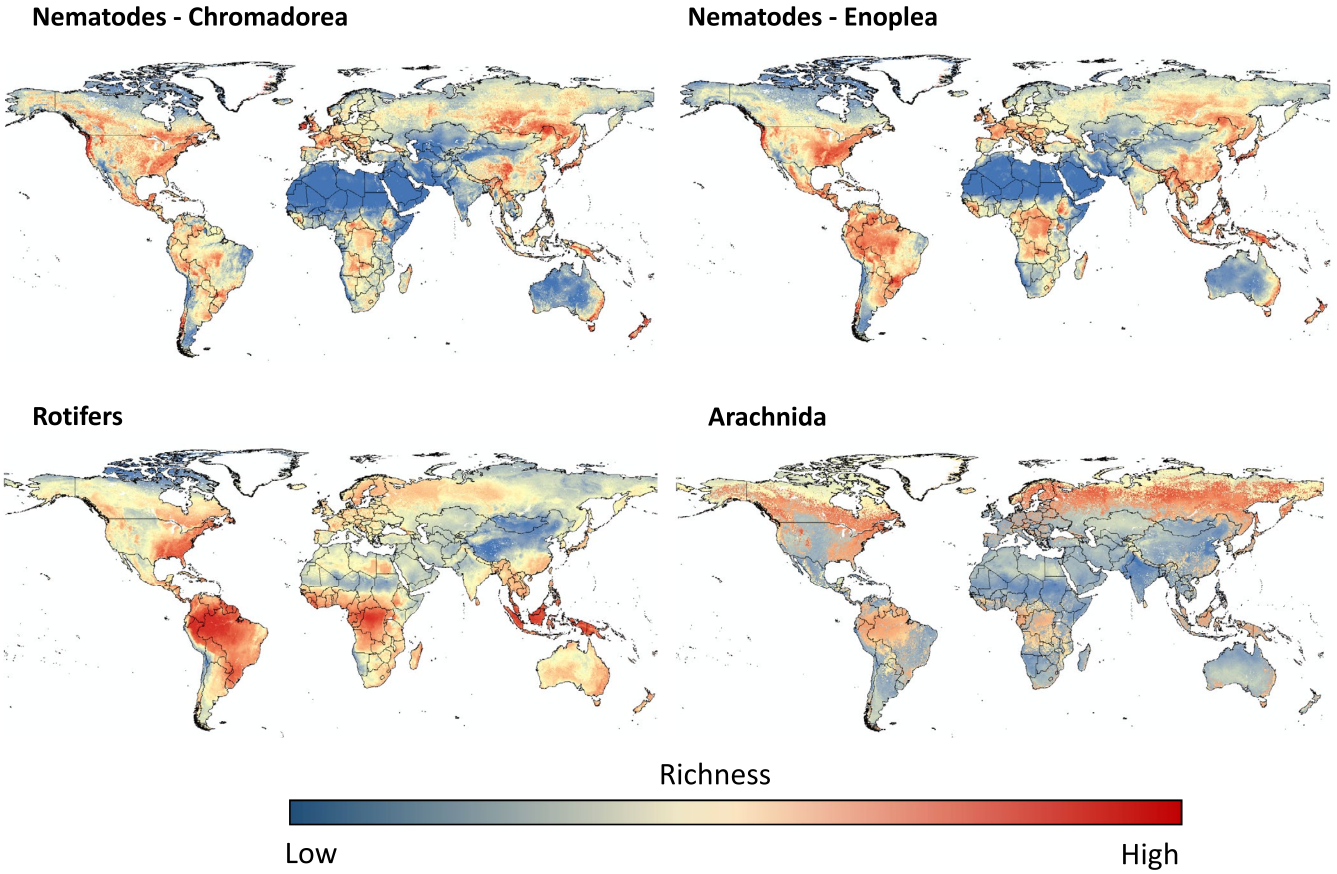

FIGURE 3 Predicted global richness of different invertebrate groups: Chromadorea (r = .59); Enoplea (r = .58); Rotifera (r = .47); and Arachnida (r = .36). All correlations had a p-value <.001

4 | DISCUSSION

Characterisation of invertebrate communities through DNA sequencing of soil samples has increased markedly over the last decade (Oliverio et al., 2018; Treonis et al., 2018; Wu et al., 2009). While some discrepancies between morphological identification of soil invertebrates and amplicon sequencing of DNA extracted from soil may occur at the genus/species level (Treonis et al., 2018; Wu et al., 2009), the primer set used in our study has been shown to be useful for characterising the diversity and community composition of major groups of soil invertebrates in different global and continental surveys (Bissett et al., 2016; Ramirez et al., 2014). Significant correlations have been obtained between similar primer sets and invertebrate data collected from field surveys (Oliverio et al., 2018).

Our study provides novel insights into the cross-biome vulnerabilities, ecological preferences and diversity hotspots of dominant soil invertebrate taxa. Unlike bacterial diversity, which responds most strongly to changes in soils (Bastida et al., 2016; Delgado-Baquerizo, Reich, et al., 2017), we found that climate and plant attributes regulated the diversity of nematodes, arachnids and rotifers, suggesting that these organisms might be more vulnerable directly by changes in climate, or indirectly, by changes in plant communities. For example, our study provides evidence that reductions in forest cover and plant diversity, and declines in plant production associated with increases in aridity might have negative consequences for the diversity and dominant taxa of invertebrates globally. As such, our research draws attention to the potential vulnerabilities of soil invertebrates in a drier and hotter world. Finally, we provide novel global atlases of the potential distribution of soil invertebrates.

Vegetation had a predominant role in predicting the diversity of dominant soil invertebrates. Perennial plant richness was correlated with the diversity of nematodes and rotifers across globally distributed locations, suggesting that the diversity of plants and these soil organisms might share similar cross-biome environmental preferences and vulnerabilities. The positive associations between plant and invertebrate richness can be related to the multiple circular positive loops between the diversity of plants and invertebrates (De Deyn, Raaijmakers, Van Ruijven, Berendse, & Van Der Putten, 2004; Delgado-Baquerizo, Powell, et al., 2017; Hooper et al., 2000). Rotifers are known to feed from litter detritus and many nematodes have important associations with plant roots and litter (García-Palacios et al., 2016; Wu et al., 2011). Indeed, plant richness was particularly important for predicting the presence of a relatively ubiquitous cluster of soil phylotypes belonging to nematodes, including Chromadorea, Enoplea and rotifers (Table S1). Such results suggest that plant richness might promote these organisms (e.g., by increasing habitat heterogeneity),

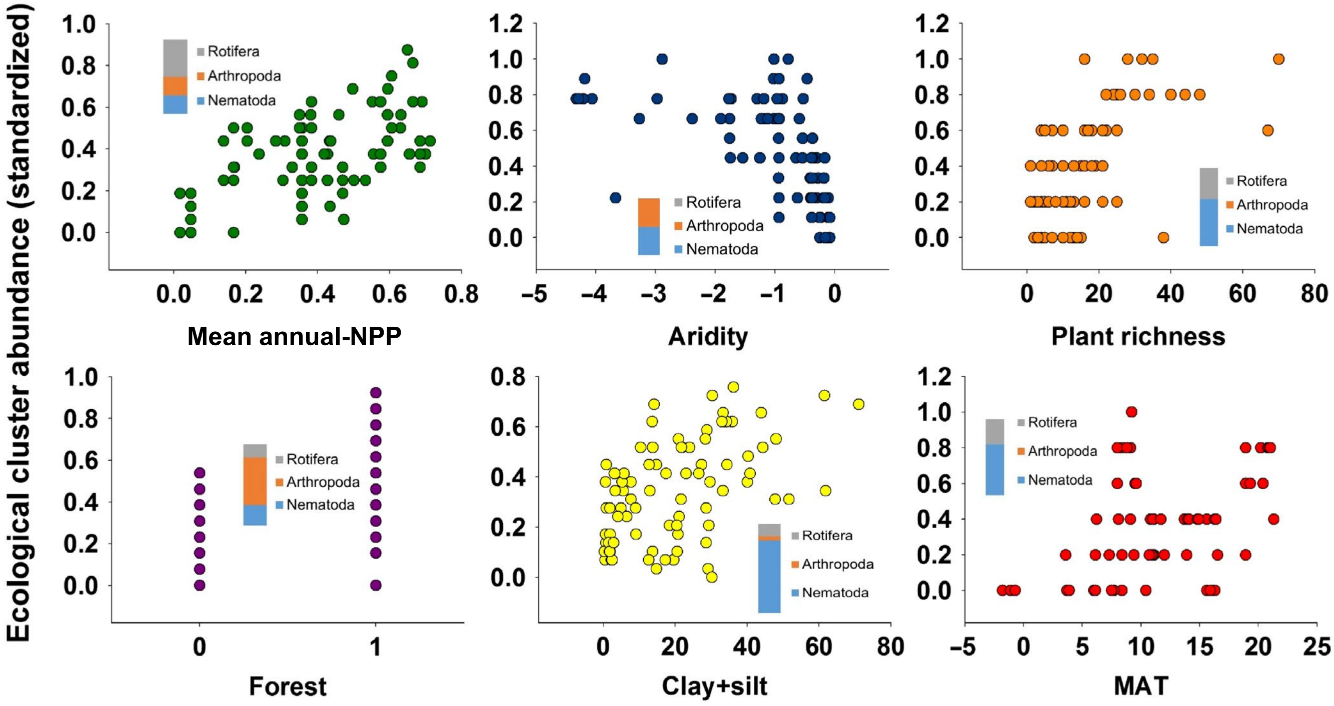

FIGURE 4 Ecological clusters showing the environmental preferences of invertebrates. Bar inset in each figure panel indicates the number of phylotypes of each phylum associated to each specific cluster

as demonstrated for nematodes (De Deyn et al., 2004; Viketoft, Palmborg, Sohlenius, Huss-Danell, & Bengtsson, 2005) and that these dominant taxa of invertebrates could be indirectly preserved by promoting plant diversity. Together, these results suggest that reductions in plant diversity associated with global environmental change can have negative consequences for the diversity of dominant invertebrate taxa, such nematodes and rotifers, which are central to the functioning of ecosystems (Eisenhauer et al., 2019).

Our study further identified the potential vulnerabilities for the diversity and dominant taxa of nematodes, rotifers and mites, and suggests that deforestation and increases in aridity might reduce the global diversity of soil invertebrates. For example, forest ecosystems and annual NPP were positively linked to the diversity of nematodes, rotifers and arachnids. Thus, our results suggest that deforestation processes could lead to critical reductions in the diversity of these organisms. Our cross-biome survey provided further evidence that climate exerts a critical direct and indirect control of the diversity and dominant taxa of soil invertebrates. For example, our results indicate that aridity, which is expected to expand in the coming decades (Huang et al., 2015), is negatively associated with the diversity of soil nematodes. We also found indirect negative effects of increases in aridity on the diversity of nematodes by constraining annual NPP in the most arid locations. Our study suggests, therefore, that reductions in plant productivity under a drier world might lead to reductions in the diversity of soil nematodes, with important implications for those ecological functions that they sustain (García-Palacios et al., 2016).

The ability of the studied environmental factors to predict the variation in the distribution of the diversity of soil invertebrates allowed us to create novel global atlases of the diversity of soil nematodes, rotifers and arachnids. These global atlases were successfully cross-validated using a global independent database (Ramirez et al., 2014). Our predictive maps identified potential regions with diversity of nematodes in East and South-East Asia, North and South-America and temperate regions in Europe. Further, the diversity of rotifers was predicted to be extremely high in South America and South-East Asia, and Arachnida in northern latitudes. These global atlases can be used to predict potential variations in soil biodiversity under global change scenarios including climatic changes and land use intensification, and help identifying global hotspots and potential vulnerabilities for soil biodiversity globally. For example, the strong statistical links among temperate forest, mean annual NPP, and soil invertebrate richness suggest that human-induced deforestation of key terrestrial biomes such as temperate forests in North America and Central Europe, may dramatically reduce the diversity of soil invertebrates. Similarly, deforestation of South American forests may have critical consequences for the diversity of rotifers and arachnids.

Taken together, our results provide evidence that vegetation and climate drive the diversity and dominant taxa of soil invertebrates across widely distributed terrestrial ecosystems. Reductions in plant biomass and diversity derived from deforestation and climate change, and increases in aridity associated with climate change will probably lead to reductions in soil invertebrate diversity. We further generated cross-validated global atlases of the diversity of soil invertebrates and identified the potential hotspots for the diversity of dominant soil invertebrates. Our study advances the current knowledge of the ecological preferences and vulnerabilities for the diversity and presence of functionally important soil invertebrates in soils from across the globe. This information is fundamental for improving and prioritizing conservation efforts of soil genetic resources and management policies, as well as for generating hypotheses about the influence of climate change in soil invertebrates which are fundamental for the maintenance of ecosystem services.

ACKNOWLEDGEMENTS

This project received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 702057. F.B. and A.G. thank the Spanish Ministry of Science, Innovation and Universities and FEDER funds for the CICYT project AGL2017-85755-R and CGL2017-88124-R. F.B. and C.G. thanks CSIC for the Intramural grant 201740I008 and the I-Link+2018 (LINKA20069) grant, and Fundación Séneca from Murcia province (19896/GERM/15). M.D.-B. acknowledges support from the Marie Sklodowska-Curie Actions of the Horizon 2020 Framework Programme H2020-MSCA-IF-2016 under REA grant agreement n°702057. S.A. and F.D.A. were supported by FONDECYT 1170995. CAP is grateful to IAI-CRN 3005. C.A.P., and F.D.A. were supported by PFB-23 (from CONICYT) and P05-002 (from Millennium Scientific Initiative) to the Institute of Ecology and Biodiversity, Chile. Noah Fierer, Mathew A. Bowker, Asmeret A. Berhe, Nick A. Cutler, Patrick E. Hayes, Zeng Y. Hseu, M. Kirchmair, Hans Lambers, Sigrid Neuhauser, Victor M. Peña-Ramírez, Sasha C. Reed, Christina Siebe and Benjamin W. Sullivan are acknowledged for their contribution with soil samples.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

F.B., and M.D.-B. designed the research and analyzed data. M.D.-B. designed the field study, coordinated all field and laboratory operations and developed models. The rest of the authors provided soil samples. The paper was written by F.B., and M.D.-B., edited by D.J.E., and all authors approved the final version of the manuscript.

ORCID

Felipe Bastida https://orcid.org/0000-0001-9958-7099
Mark A. Williams https://orcid.org/0000-0002-4766-9399
Manuel Delgado-Baquerizo https://orcid.org/0000-0002-6499-576X

DATA AVAILABILITY STATEMENT

The primary data used in this paper have been deposited in figshare: https://figshare.com/s/4cbe0dc93a79e111956b (https://doi.org/10.6084/m9.figshare.8948927).

REFERENCES

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS ONE*, *4*, e6372.

Bastida, F., Torres, I. F., Moreno, J. L., Baldrian, P., Ondoño, S., Ruiz-Navarro, A., … Jehmlich, N. (2016). The active microbial diversity drives ecosystem multifunctionality and is physiologically related to carbon availability in Mediterranean semi-arid soils. *Molecular Ecology*, *25*, 4660–4673. https://doi.org/10.1111/mec.13783

Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P. M., Reith, F., Dennis, P. G., … Young, A. (2016). Introducing BASE: The Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience*, *5*, 21. https://doi.org/10.1186/s13742-016-0126-5

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336. https://doi.org/10.1038/nmeth.f.303

Chapin, F. S. III, Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., … Díaz, S. (2000). Consequences of changing biodiversity. *Nature*, *405*, 234. https://doi.org/10.1038/35012241

De Deyn, G. B., Raaijmakers, C. E., Van Ruijven, J., Berendse, F., & Van Der Putten, W. H. (2004). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos*, *106*, 576–586. https://doi.org/10.1111/j.0030-1299.2004.13265.x

Delgado-Baquerizo, M., Maestre, F. T., Gallardo, A., Bowker, M. A., Wallenstein, M. D., Quero, J. L., … Zaady, E. (2013). Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature*, *502*, 672. https://doi.org/10.1038/nature12670

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., … Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science*, *359*, 320–325. https://doi.org/10.1126/science.aap9516

Delgado-Baquerizo, M., Powell, J. R., Hamonts, K., Reith, F., Mele, P., Brown, M. V., … Bissett, A. (2017). Circular linkages between soil biodiversity, fertility and plant productivity are limited to topsoil at the continental scale. *New Phytologist*, *215*, 1186–1196. https://doi.org/10.1111/nph.14634

Delgado-Baquerizo, M., Reich, P. B., Khachane, A. N., Campbell, C. D., Thomas, N., Freitag, T. E., … Singh, B. K. (2017). It is elemental: Soil nutrient stoichiometry drives bacterial diversity. *Environmental Microbiology*, *19*, 1176–1188.

Drummond, A. J., Newcomb, R. D., Buckley, T. R., Xie, D., Dopheide, A., Potter, B. C. M., … Nelson, N. (2015). Evaluating a multigene environmental DNA approach for biodiversity assessment. *GigaScience*, *4*(1), 46. https://doi.org/10.1186/s13742-015-0086-1

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*, 996. https://doi.org/10.1038/nmeth.2604

Edgar, R. C. (2017). UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*, 081257. https://doi.org/10.1101/081257

Eisenhauer, N., Bonn, A., & Guerra, C. A. (2019). Recognizing the quiet extinction of invertebrates. *Nature Communications*, *10*, 50. https://doi.org/10.1038/s41467-018-07916-1

Frouz, J. (2018). Effects of soil macro- and mesofauna on litter decomposition and soil organic matter stabilization. *Geoderma*, *332*, 161–172. https://doi.org/10.1016/j.geoderma.2017.08.039

García-Palacios, P., Shaw, E. A., Wall, D. H., & Hättenschwiler, S. (2016). Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecology Letters*, *19*, 554–563. https://doi.org/10.1111/ele.12590

Gossner, M. M., Lewinsohn, T. M., Kahl, T., Grassein, F., Boch, S., Prati, D., … Allan, E. (2016). Land-use intensification causes multitrophic homogenization of grassland communities. *Nature*, *540*, 266. https://doi.org/10.1038/nature20575

Grace, J. B. (2006). *Structural equation modeling and natural systems*. Cambridge, UK: Cambridge University Press.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Reearchs*, *41*, D597–D604.

Hengl, T., Mendes de Jesus, J., Heuvelink, G. B. M., Ruiperez Gonzalez, M., Kilibarda, M., Blagotić, A., … Kempen, B. (2017). SoilGrids250m: Global gridded soil information based on machine learning. *PLoS ONE*, *12*, e0169748. https://doi.org/10.1371/journal.pone.0169748

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978. https://doi.org/10.1002/joc.1276

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., … O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, *486*, 105. https://doi.org/10.1038/nature11118

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Mark dangerfield, J., Wall, D. H., … Wolters, V. (2000). Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *BioScience*, *50*, 1049–1061. https://doi.org/10.1641/0006-3568(2000)050[1049:IBAABB]2.0.CO;2

Huang, J., Yu, H., Guan, X., Wang, G., & Guo, R. (2015). Accelerated dryland expansion under climate change. *Nature Climate Change*, *6*, 166.

Kettler, T. A., Doran, J. W., & Gilbert, T. L. (2001). Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal*, *65*, 849–852. https://doi.org/10.2136/sssaj2001.653849x

Kuhn, M., Weston, S., Keefer, C., & Coulter, N. (2016). *Cubist: Rule- and instance-based regression modeling. R package version 0.0.19.*

Liang, J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., … Reich, P. B. (2016). Positive biodiversity-productivity relationship predominant in global forests. *Science*, *354*, aaf8957. https://doi.org/10.1126/science.aaf8957

Maestre, F. T., Delgado-Baquerizo, M., Jeffries, T. C., Eldridge, D. J., Ochoa, V., Gozalo, B., … Singh, B. K. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 15684–15689. https://doi.org/10.1073/pnas.1516684112

Maestre, F. T., Quero, J. L., Gotelli, N. J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., … Zaady, E. (2012). Plant species richness and ecosystem multifunctionality in global drylands. *Science*, *335*, 214–218. https://doi.org/10.1126/science.1215442

Malcolm, J. R., Liu, C., Neilson, R. P., Hansen, L., & Hannah, L. (2006). Global warming and extinctions of endemic species from biodiversity hotspots. *Conservation Biology*, *20*, 538–548. https://doi.org/10.1111/j.1523-1739.2006.00364.x

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853. https://doi.org/10.1038/35002501

Oliverio, A. M., Gan, H., Wickings, K., & Fierer, N. (2018). A DNA metabarcoding approach to characterize soil arthropod communities. *Soil Biology and Biochemistry*, *125*, 37–43. https://doi.org/10.1016/j.soilbio.2018.06.026

Quinlan, J. R. (1993). Combining instance-based and model-based learning. In *Proceedings of the Tenth International Conference on International Conference on Machine Learning* (pp. 236–243). Amherst, MA: Morgan Kaufmann Publishers Inc.

Ramirez, K., Leff Jonathan, W., Barberán, A., Bates Scott, T., Betley, J., Crowther Thomas, W., … Fierer, N. (2014). Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20141988.

Reich, P. B., Walters, M. B., & Ellsworth, D. S. (1997). From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 13730–13734.

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology*, *11*, R25. https://doi.org/10.1186/gb-2010-11-3-r25

Sala, O. E., Stuart Chapin, F. III, Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., … Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science*, *287*, 1770–1774.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*, 1256688. https://doi.org/10.1126/science.1256688

Treonis, A. M., Unangst, S. K., Kepler, R. M., Buyer, J. S., Cavigelli, M. A., Mirsky, S. B., & Maul, J. E. (2018). Characterization of soil nematode communities in three cropping systems through morphological and DNA metabarcoding approaches. *Scientific Reports*, *8*, 2004. https://doi.org/10.1038/s41598-018-20366-5

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., & Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences. *Scientific Reports*, *7*, 9132. https://doi.org/10.1038/s41598-017-09084-6

Viketoft, M., Palmborg, C., Sohlenius, B., Huss-Danell, K., & Bengtsson, J. (2005). Plant species effects on soil nematode communities in experimental grasslands. *Applied Soil Ecology*, *30*, 90–103. https://doi.org/10.1016/j.apsoil.2005.02.007

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wall, D. H., Bradford, M. A., St. john, M. G., Trofymow, J. A., Behan-pelletier, V., Bignell, D. E., … Zou, X. (2008). Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology*, *14*, 2661–2677. https://doi.org/10.1111/j.1365-2486.2008.01672.x

Wall, D. H., Nielsen, U. N., & Six, J. (2015). Soil biodiversity and human health. *Nature*, *528*, 69. https://doi.org/10.1038/nature15744

Willig, M. R., Kaufman, D. M., & Stevens, R. D. (2003). Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 273–309. https://doi.org/10.1146/annurev.ecolsys.34.012103.144032

Wu, T., Ayres, E., Bardgett, R. D., Wall, D. H., & Garey, J. R. (2011). Molecular study of worldwide distribution and diversity of soil animals. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 17720–17725. https://doi.org/10.1073/pnas.1103824108

Wu, T., Ayres, E., Li, G., Bardgett, R. D., Wall, D. H., & Garey, J. R. (2009). Molecular profiling of soil animal diversity in natural ecosystems: Incongruence of molecular and morphological results. *Soil Biology and Biochemistry*, *41*, 849–857. https://doi.org/10.1016/j.soilbio.2009.02.003

SUPPORTING INFORMATION

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

How to cite this article: Bastida F, Eldridge DJ, Abades S, et al. Climatic vulnerabilities and ecological preferences of soil invertebrates across biomes. *Mol Ecol*. 2019;00:1–10. https://doi.org/10.1111/mec.15299