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Earthworm invasion causes declines across soil fauna size classes and biodiversity facets in northern North American forests

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Anthropogenic pressures alter the biodiversity, structure and organization of biological communities with severe consequences for ecosystem processes. Species invasion is such a human-induced ecosystem change with pronounced impacts on recipient ecosystems. Around the globe, earthworms invade habitats and impact abiotic soil conditions and a wide range of above- and belowground organisms. In northern North America, where earthworms have been largely absent since the last glaciation period and most earthworm species present today have only been (re-)introduced a few hundred years ago, invasion impacts have been intensively studied. However, despite several studies assessing impacts of invasive earthworms on soil fauna, studies have rarely investigated the simultaneous responses of different soil-fauna size groups and biodiversity facets which might respond differently to earthworm invasion and independently affect ecosystem processes. Our study goes beyond previously-established knowledge on earthworm-invasion effects by simultaneously assessing differences in four biodiversity facets, namely the abundance, biomass, richness and Shannon index of soil invertebrate macro-, meso- and microfauna communities between high- and low-invasion status plots (n = 80) and in relation to invasion intensity measured as earthworm biomass across four northern North American forests sampled between 2016 and 2017. Across forests and soil-fauna groups, we found reduced abundance (−33 to −45%) and richness (−18 to −25%) in high compared to low-invasion status areas. Additionally, meso- (−14%) and microfauna biomass (−38%) and macro- (−7%) and microfauna Shannon index (−8%) were reduced. Higher invasion intensity (earthworm biomass) was additionally related to reduced soil-fauna biodiversity. While the studied biodiversity facet was important for the soil fauna response, soil-fauna size group was comparably unimportant. Given the global ubiquity of earthworm invasion and the importance of soil fauna for key ecosystem processes, our observational results help to assess future impacts of this invasion and the consequences for anthropogenically-altered ecosystem functioning.

Keywords: belowground, biodiversity, community ecology, ecosystem engineer, invasion

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Introduction

Human society depends on nature for a wide range of ecosystem services (Reid et al. 2005, Díaz et al. 2018, IPBES 2019). However, anthropogenic transformation of many ecosystems has put natural ecosystems and biodiversity at risk (Reid et al. 2005, Díaz et al. 2018). Despite the high number of important global-change drivers (Sala et al. 2000, Reid et al. 2005, Maxwell et al. 2016, IPBES 2019) and their variable impact on different ecosystem properties, compartments such as above- and below-ground systems, and organisms of differing body size, most studies focus on the effects of specific drivers on single or few facets of biodiversity for specific organism groups or ecosystem properties (Rillig et al. 2019). This may be problematic as we are rarely only interested in how a single facet of biodiversity is changed, but how changes in driving variables affect the functioning of ecosystems, which often depends on multiple facets of biodiversity (Stevens and Tello 2014, Craven et al. 2018) as well as changes in body-size structure (Barnes et al. 2018). Consequently, when studying global-change impacts, it is crucial to cover a variety of responses in different biodiversity facets to meaningfully assess the consequences of biodiversity and ecosystem change for sustained ecosystem functioning.

Species invasion is among the most important drivers of abiotic and biotic ecosystem and biodiversity change (Reid et al. 2005, Wardle et al. 2011, Simberloff et al. 2013, Maxwell et al. 2016, IPBES 2019). Invasion events can lead to competitive exclusion (Bøhn et al. 2008), trigger cascading effects across trophic levels (Estes et al. 2011) and enable or facilitate further invasions (O'Loughlin and Green 2017) via invasional meltdown (Simberloff and Von Holle 1999) with consequences for recipient communities and ecosystems. As such, invasion events are often associated with both species gains and losses and therefore are crucial drivers of biodiversity change across scales (Wardle et al. 2011). Research has shown that the success probability and impact of biological invasions partly depends on the functional similarity of the invader and the existing community with higher dissimilarity increasing the success rate and impact (Wardle et al. 2011). Additionally, the invasion of certain taxa with distinct functionality has been found to have disproportionate effects on ecosystems. This certainly is the case for so-called ecosystem engineers in soil ecosystems (Jones et al. 1996, Jouquet et al. 2006) which have dramatic consequences for belowground biodiversity and ecosystem functioning (Wardle et al. 2011, Bardgett and van der Putten 2014).

Compared to aboveground biodiversity, soil biodiversity patterns and their responses to global-change drivers are relatively understudied (Veresoglou et al. 2015, Phillips et al. 2017, Cameron et al. 2018, Guerra et al. 2020), despite their crucial role for ecosystem functioning (Bardgett and van der Putten 2014). The same is true for belowground invasions – they are relatively understudied but expected to heavily impact ecosystem functioning in invaded systems (Ehrenfeld and Scott 2001, Eisenhauer et al. 2019). Consequently, belowground invasions might trigger strong changes in ecosystem functioning (Bardgett and van der Putten 2014), but might go unnoticed if not specifically addressed, as aboveground biodiversity patterns have been found to regularly differ from those below the ground (Cameron et al. 2019). As such, given the importance of soil biodiversity for ecosystem functioning and the relatively understudied consequences of belowground invasions, studies investigating the impact of such invasions are urgently needed.

As ecosystem engineers, earthworms are key players in many terrestrial ecosystems (Edwards 2004) influencing above- and belowground biodiversity (Brown 1995, Eisenhauer 2010) and ecosystem processes (Van Groenigen et al. 2014, Frelich et al. 2019). They burrow, cast and mix litter and soil and thereby vertically and horizontally re-distribute nutrients throughout their belowground habitat. These activities influence various biotic and abiotic characteristics of soil systems and their food webs, consequently affecting plant communities and globally-important nutrient cycles (carbon sequestration and greenhouse gas flux) (Eisenhauer et al. 2019). What is commonly perceived as a service to ecosystems and humankind where earthworms are naturally present (Blouin et al. 2013), can be a threat to ecosystems and human wellbeing where they are introduced and become invasive which is the case in many ecosystems around the globe (Bohlen et al. 2004, Hendrix et al. 2008, Frelich et al. 2019). While the impact of invasive earthworms is not equally negative everywhere and depends on the ecosystem type – some of their effects may be advantageous, for example in agricultural settings or urban gardens – their effects on native forests are usually negative (Hendrix and Bohlen 2002). Recent meta-analyses have revealed earthworm invasion to affect soil-abiotic conditions (Ferlian et al. 2020), soil microbial and soil-fauna communities (Ferlian et al. 2018), as well as plant communities (Craven et al. 2017). For instance, earthworm invasion was shown to reduce mycorrhizal fungi and the diversity and density of several soil-fauna groups (Ferlian et al. 2018). However, there was also a lot of variation between studies and a strong focus on soil mesofauna, with limited knowledge on soil macro- and microfauna (Ferlian et al. 2018; but see Burtis et al. 2014 and McCay and Scull 2019 for macrofauna responses). Additionally, information regarding earthworm invasion effects on soil-fauna biomass or different measures of biodiversity, is rare. Moreover, while most of our knowledge on ecosystem responses to earthworm invasion is based on comparing high-invasion to low-invasion areas, less is known about the effects of invasion intensity. Despite increasing attention to the effects of invasive earthworm density (Suárez et al. 2006), biomass (Mahon et al. 2020) or community composition (Crumsey et al. 2013, Chang et al. 2016), most of these studies focus on responses in leaf litter decomposition or nutrient dynamics while knowledge on soil-fauna responses to invasion intensity remains limited.

Soil organisms span a large gradient in body size (Veresoglou et al. 2015, Thakur et al. 2020). The sensitivity of different size groups to global-change drivers can differ, for example because of physiological differences and trophic level

(Voigt et al. 2007, Hines et al. 2015). Furthermore, different biodiversity facets have been shown to independently affect ecosystem function and stability (Craven et al. 2018). Given the importance of such different biodiversity facets for matter and energy flux through ecological communities, which is tightly linked to various ecosystem functions and services (Barnes et al. 2014, 2016), these facets need to be investigated to improve our understanding of how earthworm invasion affects the functioning of ecosystems (Barnes et al. 2018). Finally, only the simultaneous assessment of several biodiversity facets across different soil fauna size groups will allow for a comprehensive assessment of earthworm invasion impacts on belowground systems as a whole.

Earthworm invasion has been heavily studied in northern North American forest ecosystems (Hendrix and Bohlen 2002, Bohlen et al. 2004, Hendrix et al. 2008). Although there are a few native earthworm species with significant northern ranges, also inside the previously-glaciated area (Hendrix and Bohlen 2002, Csuzdi et al. 2017, McCay et al. 2017, Ikeda et al. 2020), most earthworm species present in northern North America today have been naturally absent since the last glaciation (James and Hendrix 2004) and have only been re-introduced by European settlers (Bohlen et al. 2004) a few hundred years ago. Northern North American forests provide a perfect example of how an organism group that is functionally different from native fauna groups present across large parts of the continent can easily invade and have dramatic impacts on natural ecosystems (Ferlian et al. 2018, Eisenhauer et al. 2019).

To overcome previous limitations of studying single biodiversity facets of only a subset of the soil-fauna community in regionally-restricted frameworks and with potentially variable sampling effort or methodology, we simultaneously studied responses of soil-dwelling macro-, meso- and microfauna to earthworm invasion across four northern North American forests within the EcoWorm project (described in Eisenhauer et al. 2019). Aiming to cover different biodiversity facets with their differential capacity to respond to and drive ecosystem change, we assessed the abundance, biomass, species/taxon richness and Shannon's diversity index (hereafter: Shannon index) for each of these different size groups. We expected higher earthworm invasion status to be related to reduced soil-fauna abundance, biomass, richness and Shannon index (H1) and we expected the magnitude of these effects to differ between the three size groups and the four biodiversity facets (H2). For example, we expected larger size groups to be more strongly affected because of longer regeneration times and we expected soil fauna abundance to be more strongly affected than species richness because it is likely that populations will first suffer before they locally disappear. However, these are just example responses and alternative responses are equally possible as we are far from understanding the full complexity of earthworm-invasion impacts. In addition to the effect of invasion status, we assessed responses of biodiversity facets and size groups to increasing invasive earthworm biomass to gain an understanding of invasion-intensity effects on soil-fauna communities. Here, we expected soil-fauna biodiversity to decline with increasing invasive earthworm biomass

(H3). By taking a multitrophic approach to study the effect of invasion status and intensity on multiple biodiversity facets of macro-, meso- and microfauna with an observational approach across four different forests, we aim to contribute to an improved understanding of how earthworm invasion affects the invaded communities.

Material and methods

Study sites

We sampled soil macro-, meso- and microfauna in four different northern North American forests in Canada and the USA between 2016 and 2017. The three Canadian forests (Barrier Lake North – 51°02'6''N, 115°03'54''W, hereafter Barrier North; Barrier Lake South – 51°00'54''N, 115°04'23''W, hereafter Barrier South and Bull Creek Hills – 50°24'0''N, 114°33'14''W, hereafter Bull Creek) are situated in the Canadian Rocky Mountains, Kananaskis Valley, southwest Alberta, along Highway 40 and 541. Situated between 1400 and 1500 m a.s.l., these forests are dominated by trembling aspen *Populus tremuloides* and interspersed with balsam poplar *Populus balsamifera*, with a dense understorey and a soil classified as orthic grey luvisol. They are characterized by a combination of short, dry summers and cold winters with the soil typically remaining frozen from November through March, a mean annual precipitation and soil temperature of 625 mm and 3.8°C, respectively (Eisenhauer et al. 2019). The Barrier North and South forests last burned in 1909, Bull Creek in 1858. In this part of western Canada, only very few native earthworm species are present (Hendrix and Bohlen 2002), one example being *Bimastos* (formerly *Dendrodrilus*) *rubidus* (Savigny 1826) which has been reported in Alberta (Scheu and McLean 1993). Despite such native earthworm species generally being present in Alberta, previous assessments of earthworms in glacial refugia in the area reported no native taxa (Hilton and Reynolds 1983). The US forest is situated near St. John's Abbey (45°34'23''N, 94°23'53''W, ~360 m a.s.l., hereafter St John's), northern Minnesota, with a mean annual temperature of 7.2°C, and precipitation of 776 mm, respectively. Soils are loamy sand Udipsamments. The forest is approximately 100–120 years old with sugar maple *Acer saccharum*, American basswood *Tilia americana*, northern red oak *Quercus rubra* and white oak *Quercus alba* dominating. Note that, despite being located within the previously-glaciated part of the continent, previous studies have reported native earthworms of e.g. the genus *Sparganophilus* in the vicinity (Ikeda et al. 2020. The forests at Barrier Lake and St John's were sampled in July and September 2016, respectively, whereas Bull Creek forest was sampled in early August 2017.

Verification of invasion status, plot setup and earthworm community assessment

For each of the four forests, before setting up the sampling plots, we determined the invasion status of different areas

using a combination of digging for earthworms and mustard extraction along a lose transect following the previously-determined or assumed direction of invasion (Eisenhauer et al. 2007, Straube et al. 2009). Wherever possible, high-invasion plots were set up in areas with all three ecological groups of earthworms (epigeics – living in and feeding on leaf litter, endogeics – inhabiting the mineral soil layer, creating non-permanent, horizontal burrows and anecics – living in permanent, deep, vertical, mineral-soil burrows, but feeding on surface leaf litter; Hendrix and Bohlen 2002, Eisenhauer and Eisenhauer 2020) present, whereas low-invasion plots contained no earthworms or only epigeic earthworms (Eisenhauer et al. 2019). Subsequently, in each forest, we set up 20 quadratic plots of 1 × 1 m each, ten in the low-invasion and ten in the high-invasion forest area. For each of these plots, we assessed earthworm, as well as invertebrate macrofauna, mesofauna and microfauna communities.

Earthworm sampling

After plot-establishment, we assessed earthworm communities on an area of 50 × 50 cm within each plot using a combination of digging and hand-sorting (upper 10 cm of the soil) and earthworm extraction using mustard solution (Gunn 1992). Mustard solution was prepared by thoroughly mixing 100 g of dry mustard powder, 20 ml of vinegar and 10 l of water in a jerry can and letting the solution incubate overnight as a standard procedure. After removing the upper soil layers down to 10 cm depth, half of this suspension (5 l) was applied to the 0.25 m^2 hole, which was then watched for 15 min, and all earthworms emerging were caught by hand. Afterwards, this procedure was repeated with the second half of the suspension for another 15 min. The dug-up soil was sorted for earthworms by hand. Retrieved earthworms were stored in 70% ethanol, taken to the lab, identified to species (Supporting information), and their individual fresh body mass weighed. Based on individual-level data on these 1625 earthworms, we calculated earthworm area-specific abundance (ind. m^{-2}), fresh biomass (g m^{-2}), earthworm species richness and ecological group richness for each plot (Supporting information). Note that we found nine individuals of the native North American earthworm species *Bimastos rubidus* in overall five plots of the two forests at Barrier Lake. Due to the very low representation of this native species in the total earthworm abundance (0.5%) and biomass (0.1%) collected in our study, and due to a previous report showing that this native earthworm species does not exert detrimental effects on native fauna that are comparable to those of invasive earthworm species (McCay and Scull 2019), the impact of these individuals on our results is expected to be negligible. However, as we could not be sure if these earthworms would affect other soil fauna in a way similar to that of invasive earthworms or what their history in our specific research plots was, we took a conservative approach here and did not remove these individuals from our analyses and just treated them as all other earthworms in our study.

Sampling procedure for soil-fauna communities

Mobile macrofauna in the upper soil and litter layer was sampled with a combination of sieving and hand-sorting on an area of 50 × 100 cm (0.5 m^2) within each plot. First, all litter and organic soil from a 50 × 50 cm quadrant was collected from the top soil layer by hand, sieved into a box through a coarse, 2 cm-mesh sieve, and then hand-sorted for animals visible to the naked eye. Simultaneously, the bare plot area was surveyed for 10 min, and emerging macrofauna was caught with forceps. Afterwards, the same procedure was applied to a second quadrant of 50 × 50 cm. All animals were stored in 70% ethanol, identified to species or morphospecies level based on several taxonomic keys, and body lengths were measured for up to five individuals per plot and morphospecies (Supporting information). Mites and earthworms were removed from the macrofauna data. Based on the obtained body lengths, average individual fresh body mass per taxon and plot was calculated using literature-derived length-mass regressions for specific taxa (Wardhaugh 2013, Sohlström et al. 2018) (Supporting information).

Soil mesofauna was sampled by taking one 5-cm diameter soil core to a depth of 10 cm per plot. Soil cores were split into the upper and lower 5 cm soil layer and further processed in the lab. For the analyses of this paper, data was pooled across the two layers. Mesofauna was then extracted following a heat-extraction protocol (Macfadyen 1961). Extracted animals were transferred into 70% ethanol, identified to species or genus level (Collembola and Oribatida) or higher-order taxon (other Acari) level using two main keys (Christiansen and Bellinger 1998, Weigmann 2006) (Supporting information), and body lengths were measured for up to five individuals per plot and lowest-resolution taxon to calculate fresh body masses. Based on the obtained body lengths, average individual fresh body mass per taxon and plot was calculated using literature-derived length-mass regressions for specific taxa (Mercer et al. 2001) (Supporting information).

To assess soil microfauna, nematodes were sampled by taking three 5-cm-diameter soil cores to a depth of 10 cm per plot. As for the mesofauna, the soil cores were split into an upper and lower 5 cm subsample, but data were subsequently pooled across depths. The three samples from each depth layer were mixed and then sieved through a 2-mm sieve. The soil was kept cool and taken to the laboratory, where nematodes were extracted from a soil subsample of fresh soil following a modified Baermann technique with an extraction of 48 h (Cesarz et al. 2019). All extracted nematodes were transferred to formalin (4%) and counted to obtain the total abundance of nematodes per g soil dry weight. Subsequently, ~100 individuals, or all individuals in case of densities lower than 100 per sample, were randomly selected and identified to genus (adults and most of the juveniles) or family level (juveniles) (Bongers 1994) (Supporting information). For the nematodes, genus- and family-specific body masses were retrieved from the nemaplex online resource (<http://nemaplex.ucdavis.edu/>) on 19 August 2019. Data was available for all but one nematode taxon (Rhabditidae larvae, which was subsequently removed

from biomass calculations). If available, genus-specific body mass values were used; otherwise, family-specific values were used. As nemaplex nematode body masses are only available for females, all adult nematodes were assigned the female weights. As our data contained juveniles, those individuals were assumed to – on average – weigh half the female weight.

Soil bulk density measurement

As nematodes were counted for soil subsamples of known soil fresh and dry weight, we additionally assessed soil bulk density in high-invasion and low-invasion areas of the four study forests. Using the individual counts and biomass of known-soil-mass subsamples and soil-bulk density, we scaled total nematode abundance and biomass to an area of 1 m^2 (at a depth between 0 and 10 cm). Between October and November 2019, five soil cores (ten for St John's forest) of 10 cm depth and 5 cm diameter were taken per forest area (high versus low-invasion areas) in each of the forests. Samples were taken to the laboratory, dried at 105°C for 24 h, and then weighed. Soil bulk density (dry weight per volume) was then averaged over the five (ten for St John's forest) samples per invasion status area for each forest (Supporting information for bulk density results).

Assessment of additional soil-abiotic properties

In addition to soil bulk density, we assessed soil carbon (C), nitrogen (N) and water content, as well as humus layer thickness in each observational plot across the four forests. These data were not used in our main analysis, but illustrate how soil abiotic properties differ between low earthworm invasion and high invasion areas (see Supporting information for methods and results).

Calculation of biodiversity facets

From the community data on macro-, meso- and microfauna, we calculated four size-group and plot-specific biodiversity facets. 1) Individual density per square meter (hereafter: abundance) was calculated by scaling up from the sampled area/volume to one square meter. 2) Fresh biomass (g m^{-2}) was calculated by multiplying the taxon-specific average body masses by the taxon-specific abundance per sample and then by scaling up from the sampled area to one square meter. Note that for the meso- and microfauna, abundance and biomass are for the uppermost 10 cm of soil depth below one square meter. 3) Species/taxon richness was calculated by counting the identified taxa (morphospecies, genera, higher taxa, for the different size groups, respectively) per sample. This was not scaled up to a specific area. Finally, 4) Shannon index, was calculated using the function 'diversity' in R package vegan (Oksanen et al. 2016). Although there have been substantial advances in the field of partitioning biodiversity that advocate the use of Shannon diversity rather than Shannon index because of the former's easier interpretation as an effective number of species (Jost 2006, Ellison 2010),

we deliberately used Shannon index as a commonly-used biodiversity facet that is structurally different (entropy measure rather than species number) from species richness. Please note that we present abundance and biomass scaled to SI units to provide comparability within our study (size groups) and across studies. For the specific area of original sampling for each size group, please refer to the description of sampling methodology above. Additionally, please note that different taxa could only be identified to a certain taxonomic resolution. Within size classes, all samples (across all forests) were identified by the same taxonomists to minimize potential issues resulting from this taxonomic resolution.

Statistical analysis

As all four assessed earthworm biodiversity facets were well represented by the invasion status of the forest areas (low and high invasion; Supporting information), we used this as a categorical predictor variable to assess the effects of earthworm invasion on invaded soil-fauna communities. All analyses were performed in R ver. 3.6.2 (<www.r-project.org>). To test our first hypothesis (H1) expecting lower abundance, biomass, richness and Shannon index in high-invasion status plots, we fitted separate linear mixed effects models with biodiversity facets as response variables, invasion status as the predictor (fixed effect), and forest identity (n = 4) as a random-intercept blocking factor. Models were fitted with function 'lme' from the package 'nlme' (Pinheiro et al. 2014) in R. After visual inspection of diagnostic plots, response variables were log_{10}-transformed to meet model assumptions, where necessary (Table 1 for details).

To assess effect sizes of soil fauna community change from low to high invasion status sites for each of the size groups, we calculated log-response ratios as $LNRR = ln(x_h/x_l)$ for high/low intensity invasion data, where x_h is the response-variable mean of the high-invasion group, and x_l is the mean of the low-invasion group. The variance of the log-response ratio was calculated using

$$V = S^2_{pooled} \left(\frac{1}{n_h \left(x_h \right)^2} + \frac{1}{n_l \left(x_l \right)^2} \right)$$

where S^2_{pooled} is the pooled standard deviation, n_h is the sample size of the high-invasion group and n_l is the sample size of the low-invasion group. Effect sizes and variances were calculated using R-package 'metafor' (Viechtbauer 2010) with random-effects models (restricted maximum likelihood estimators), treating 'forest' as a random blocking factor. Confidence intervals (CI, 95%) for each effect-size measure were calculated using bias-corrected bootstrapping with functions 'boot' and 'boot.ci' in R-package 'boot' (Canty and Ripley 2020) (R = 1000 replicates, method = 'basic'). Confidence intervals were bootstrapped because of the small sample size and the conservative nature of the method employed here. The effect was treated as significantly different from zero if 95% confidence intervals did not overlap zero. Because of the

Table 1. Results of linear mixed effects models relating soil fauna biodiversity facets to earthworm invasion status. For each model, the table shows the response variable, sample size (n), whether response variables were transformed to meet model assumptions, slope estimates, approximated lower and upper 95% confidence intervals (using function 'intervals' from R package 'nlme' (Pinheiro et al. 2014)), F- and p-value and marginal (only fixed effects) and conditional (fixed and random effects) pseudo R^2 values calculated using function 'rsquared' from R package 'piecewiseSEM' (Lefcheck 2016). Predictor variables are invasion status (fixed effect) and forest identity (random intercept effect) for all models. p-values significant to an alpha level of 0.05 are set in bold. Values are rounded to two decimals.

Resp. variable	n	Resp. transf.	Slope est.	Lower int.	Upper int.	F-value	p-value	Pseudo R^2 mar.	Pseudo R^2 cond.
macro_abundance	80	log10	−0.20	−0.29	−0.12	23.98	**0.00**	0.19	0.36
macro_biomass	80	log10	−0.09	−0.22	0.04	1.88	0.17	0.02	0.36
macro_richness	80	None	−7.65	−10.39	−4.91	31.00	**0.00**	0.27	0.32
macro_shannon	80	None	−0.22	−0.41	−0.03	5.06	**0.03**	0.06	0.07
meso_abundance	80	log10	−0.34	−0.47	−0.21	28.68	**0.00**	0.19	0.49
meso_biomass	80	log10	−0.22	−0.37	−0.06	7.94	**0.01**	0.09	0.10
meso_richness	80	None	−2.33	−3.52	−1.13	14.96	**0.00**	0.16	0.16
meso_shannon	80	None	0.04	−0.10	0.18	0.25	0.62	0.00	0.12
micro_abundance	78	log10	−0.28	−0.36	−0.20	46.57	**0.00**	0.19	0.68
micro_biomass	78	log10	−0.33	−0.50	−0.17	16.06	**0.00**	0.14	0.34
micro_richness	79	log10	−0.10	−0.13	−0.06	30.20	**0.00**	0.26	0.32
micro_shannon	79	None	−0.23	−0.36	−0.11	14.26	**0.00**	0.14	0.22

differences in methodology between the linear mixed effects models and the log-response ratio effect-size calculation, slight differences between the results are expected. In addition to the log-response ratios, we also calculated percentage change in response variables from areas of low to high earthworm invasion status (Supporting information).

In a second step, we used a model selection approach to test if soil fauna community responses to earthworm invasion were dependent on the size group or biodiversity facet in focus (H2). First, we scaled all plot-specific response variables (abundance, biomass, richness, Shannon index) to zero mean and unit variance within each size group and across all four forests. Then, we combined these scaled response variables into a single variable 'scaled response' and created additional categorical predictor variables 'sizegroup' (n = 3; macro-, meso- and microfauna) and biodiversity 'facet' (n = 4; abundance, biomass, richness, Shannon index). Subsequently, we set up a global linear mixed effect model as follows: scaled response ~ worms × sizegroup × facet with forest identity as a random blocking factor. Finally, we used the 'dredge' function in the R package 'MuMIn' (Barton 2015) to compute models for all possible combinations of predictor variable terms (direct effects, two-way and three-way interactions). The resulting 19 candidate models were then ranked by AICc, their Akaike weights calculated and the summed Akaike weights of all variable-containing models were calculated for each of the three predictor terms (earthworm invasion status, fauna size group, biodiversity facet). These results allowed us to assess both the relative performance of each candidate model and the importance of the three predictor variables. This analysis enabled us to decide if the choice of focal size group and biodiversity-facet identity in a given study would affect conclusions on how soil-fauna communities respond to earthworm invasion. For this exercise, we did not use a strict delta AICc threshold approach to decide on a best model but simply used model delta AICc as a way of quantitatively comparing model performance.

Finally, we tested our third hypothesis (H3) expecting reduced soil fauna biodiversity facets across size groups with increasing earthworm biomass. To do so, we fitted an additional set of linear mixed effects models with each of the four biodiversity facets as response variables, $\log_{10}$-transformed earthworm biomass as the predictor (fixed effect), and forest identity (n = 4) as a random-intercept blocking factor. We used the same R-package, tested model assumptions and log-transformed response variables analogous to the models fit for testing the effects of earthworm invasion status (H1) (Table 2 for details).

We acknowledge that testing hypotheses H1 and H3 involved testing 24 statistical models (Fig. 1, 3, Table 1, 2). However, we refrained from applying a p-value adjustment due to multiple statistical tests following the mathematical, logical and practical argumentation by Moran (2003). Specifically, we would like to argue that our presentation of results is robust for three reasons: 1) Our statistical analysis for H1 and H3 resulted in very clear declines across biodiversity facets and size groups, with very few exceptions. 2) The p-values of those effects found to be significant (Table 1, 2) are generally very low and we provide effect sizes measured with two different approaches (Fig. 2 and Supporting information) to allow further insights into the strength of these effects. 3) Finally, adjusting p-values for these models using the 'Holm' method (Holm 1979) in R function 'p.adjust', separated into the two sets of 12 models (Fig. 1, 3, Table 1, 2), led to only one formerly-significant effect turning insignificant (invasion-status effect on macrofauna Shannon index), so adjusting p-values would not have any major effect on our conclusions.

Results

Our analysis of soil-fauna biodiversity facets in low and high-invasion plots across northern North American forest systems revealed a general reduction of biodiversity from low-invasion to high-invasion status plots (H1, Fig. 1). Across size

Table 2. Results of linear mixed effects models relating soil fauna biodiversity facets to earthworm biomass. For each model, the table shows the response variable, sample size (n), whether response variables were transformed to meet model assumptions, slope estimates, approximated lower and upper 95% confidence intervals (using function 'intervals' from R package 'nlme' (Pinheiro et al. 2014), F- and p-value and marginal (only fixed effects) and conditional (fixed and random effects) pseudo R^2 values calculated using function 'rsquared' from R package 'piecewiseSEM' (Lefcheck 2016). Predictor variables are $\log_{10}$-transformed earthworm biomass (fixed effect) and forest identity (random intercept effect) for all models. p-values significant to an alpha level of 0.05 are set in bold. Values are rounded to two decimals.

Resp. variable	n	Resp. transf.	Slope est.	Lower int.	Upper int.	F-value	p-value	Pseudo R^2 mar.	Pseudo R^2 cond.
macro_abundance	80	log10	−0.16	−0.22	−0.09	25.10	**0.00**	0.22	0.38
macro_biomass	80	log10	−0.04	−0.14	0.06	0.72	0.40	0.01	0.35
macro_richness	80	log10	−0.13	−0.18	−0.09	37.55	**0.00**	0.32	0.38
macro_shannon	80	None	−0.19	−0.33	−0.05	7.42	**0.01**	0.09	0.16
meso_abundance	80	log10	−0.31	−0.40	−0.23	51.10	**0.00**	0.25	0.67
meso_biomass	80	log10	−0.21	−0.32	−0.10	15.00	**0.00**	0.16	0.22
meso_richness	80	None	−1.72	−2.59	−0.85	15.48	**0.00**	0.17	0.19
meso_shannon	80	None	0.01	−0.10	0.11	0.02	0.90	0.00	0.12
micro_abundance	78	log10	−0.17	−0.24	−0.10	23.39	**0.00**	0.11	0.67
micro_biomass	78	log10	−0.28	−0.41	−0.16	20.60	**0.00**	0.21	0.31
micro_richness	79	None	−4.08	−5.54	−2.62	30.95	**0.00**	0.29	0.32
micro_shannon	79	None	−0.22	−0.31	−0.14	26.77	**0.00**	0.26	0.26

groups and biodiversity facets, we found significantly lower (percentage reduction of 6.7 ± 9.9 to 45.3 ± 6.8, mean ± standard error (SE); Supporting information, Table 1) soil-fauna biodiversity in forest areas with high compared to low-invasion status. Only two of the 12 tested response variables were not significantly affected by earthworm invasion status, namely macrofauna biomass and mesofauna Shannon index.

For the soil macrofauna, which was based on the assessment of 3496 individuals, abundance, richness and Shannon index were significantly lower in high versus low invasion status areas (p < 0.001, p < 0.001 and p < 0.05, respectively; Fig. 1, Table 1) with effect sizes of LNRR = −0.47 (CI: −0.87 to −0.07), −0.37 (CI: −0.73 to −0.01) and −0.08 (CI: −0.25 to 0.09), respectively (Fig. 2). For the soil mesofauna, based on the assessment of 9394 individuals, abundance, biomass and richness were significantly lower in high versus low invasion status areas (p < 0.001, p < 0.01 and p < 0.001, respectively; Fig. 1, Table 1) with effect sizes of LNRR = −0.83 (CI: −1.84 to −0.13), −0.55 (−1.45 to 0.04) and −0.24 (−0.43 to −0.001), respectively (Fig. 2). For the soil microfauna, based on 50 157 counted and 11 627 identified nematodes (and soil bulk density measurements, Supporting information), abundance, biomass, richness and Shannon index were found to be significantly lower in high versus low invasion status areas (all p < 0.001; Fig. 1, Table 1) with effect sizes of LNRR = −0.62 (CI: −0.88 to −0.46), −0.65 (CI: −0.94 to −0.23), −0.22 (CI: −0.28 to −0.15) and −0.09 (CI: −0.18 to −0.01), respectively (Fig. 2). Note that the difference between effects showing 95% confidence intervals overlapping the zero line in Fig. 2 (LNRR) but found to be significant reductions in Fig. 1 (mixed effects models), specifically macrofauna Shannon index and mesofauna biomass, might be caused by the extremely conservative method to calculate LNRR and their bootstrapped confidence intervals. In summary, across soil fauna size groups, abundance tended to show the

strongest negative effect sizes, with richness and Shannon index showing weaker reductions, and biomass responding at variable strength (second-strongest and strongest for meso- and microfauna, respectively; non-significant for macrofauna).

The fixed effect 'invasion status' in our significant linear mixed effects models explained a variable amount of variation, as indicated by marginal R^2 values ranging from as low as 0.06 (macrofauna Shannon index) to 0.27 for macrofauna richness (Table 1 for more detail and for conditional R^2 values). There were also some differences among the four forests (Supporting information). For example, for the macrofauna, effects in Barrier South forest differed from the three other forests (reverse trends) and, for the mesofauna, Bull Creek forest showed different results (reversed trends). For the microfauna, such differences were not as pronounced.

Our model selection approach to test if soil fauna community differences related to earthworm invasion were dependent on the focal size group or biodiversity facet provided some support for both aspects to be of some importance. The best-performing model (AICc of 2595.43, Akaike weight of 0.80; Supporting information) included invasion status (worms) and biodiversity-facet identity (facet) and the interaction of the two, but not the soil fauna size group (sizegroup). However, the two next-best performing models (delta AICc of 4.10 and 4.36 and Akaike weights of 0.10 and 0.09, respectively) both included soil fauna size group. The third-best performing model additionally included the interaction between size group and earthworm invasion status. All other models had delta AICc values of 10.94 and higher and Akaike weights of below 0.01. The summed Akaike weights of predictor-variable containing candidate models showed the highest importance of predictor variables invasion status, biodiversity-facet identity and their interaction (all Akaike weight of 1), compared to size group and its interaction with invasion status (Supporting
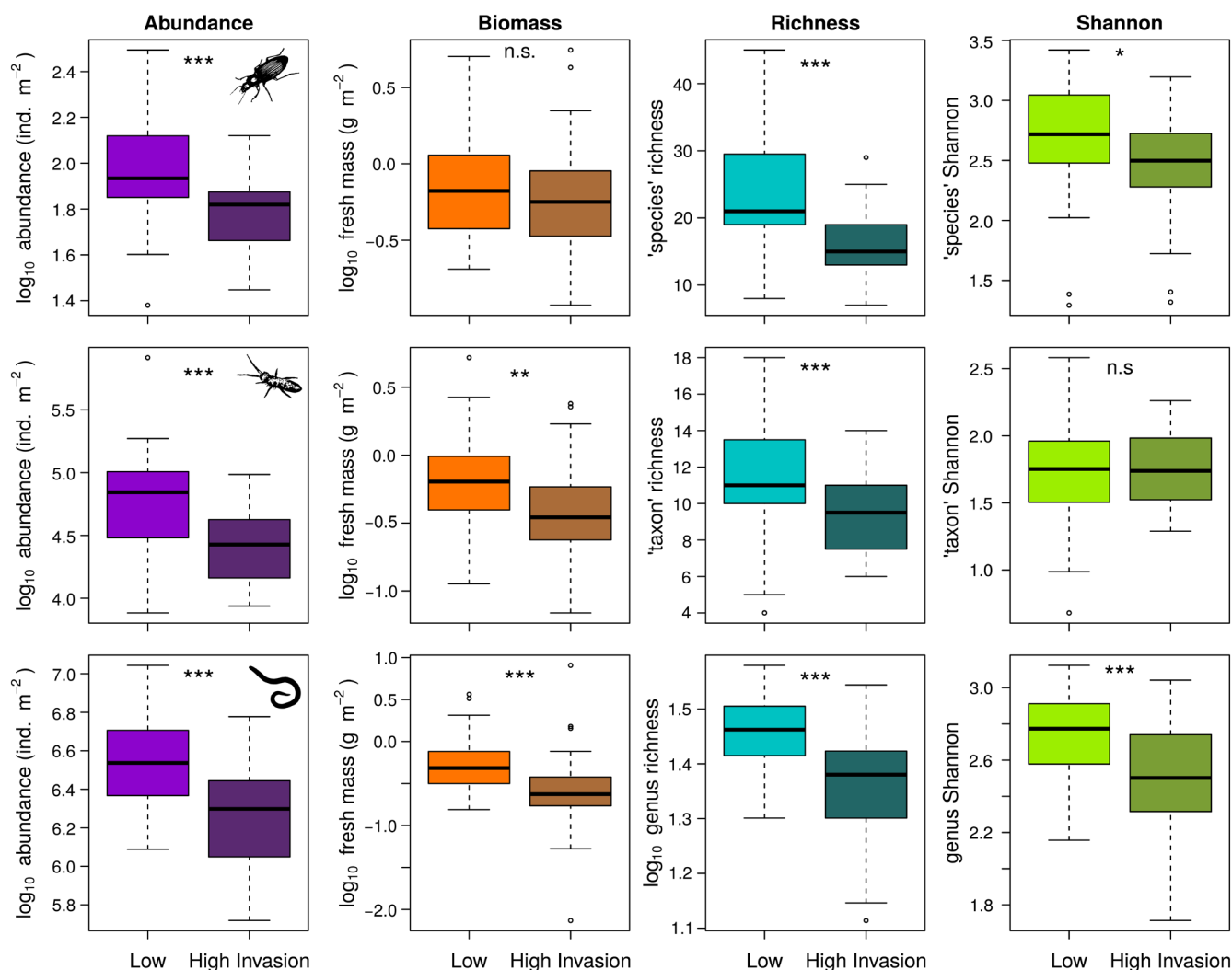
Figure 1. Boxplots showing differences in abundance, biomass, richness (species, taxon and genus richness) and Shannon index (left to right columns) of soil fauna size groups (macrofauna, mesofauna, microfauna, top to bottom rows) in low (left, bright color shade) and high invasion (right, dark color shade) sites across the four northern North American forests (n=78–80, Table 1). Asterisks and 'n.s.' show significance levels ('n.s.' not significant, $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$) of associated linear mixed effects models with 'forest' as random effect. Table 1 for detailed results of linear mixed effects models. Note that due to transformation of response variables, not all y-axes are on a comparable scale. Also, variables have been scaled to SI units where possible; for information on the original area of the different sampling techniques, please refer to the methods. Animal silhouettes are from PhyloPic.org (nematode) and Florian Schneider (collembola and beetle).

information). Both the interaction of biodiversity facet with size group and the three-way interaction showed the lowest predictor variable importance values (both below 0.01). We conclude that biodiversity facet identity provides valuable information when assessing soil-fauna relationships with earthworm invasion status, while animal size group was comparably unimportant. In other words, when assessing relationships with earthworm invasion status, we found pronounced differences among biodiversity facets but not among soil fauna size groups.

The results of our mixed-effects models testing the effect of increasing earthworm biomass on soil fauna biodiversity facets (H3, Fig. 3, Table 2) matched the results of the models using the categorical invasion predictor. Soil fauna

biodiversity facets were found to significantly decline with increasing earthworm biomass across size groups. Macrofauna biomass and mesofauna Shannon index were the only (non-significant) exceptions. Earthworm biomass, as a fixed effect, explained a variable amount of variation, with marginal R^2 values ranging from 0.09 (macrofauna Shannon) to 0.32 (macrofauna richness). Most of the marginal R^2 values were slightly higher than for the invasion status models (except for microfauna abundance, where the invasion-status model R^2 was higher than that of the earthworm-biomass model). Interestingly, for some of the investigated relationships (e.g. macrofauna abundance, macrofauna richness and mesofauna biomass), strong changes in response to earthworm biomass appear to happen at relatively low earthworm densities
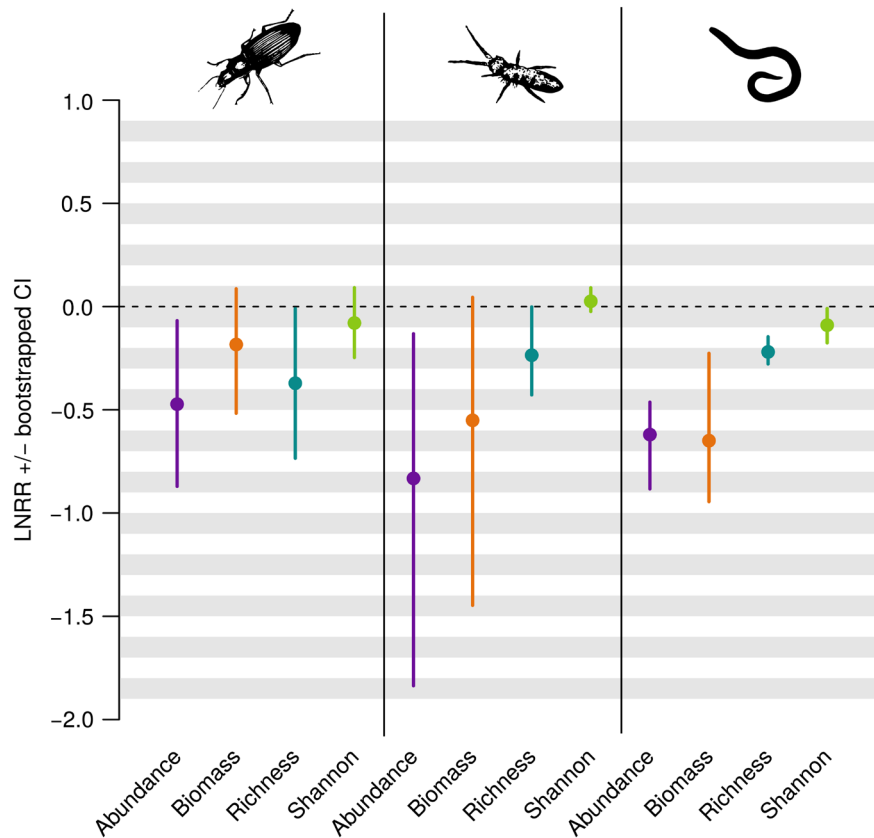
Figure 2. Log response ratio's (LNRR, and bootstrapped 95% confidence intervals, CI) of invasion-induced change in abundance (purple), biomass (orange), richness (turquoise) and Shannon index (green) for soil macro- (left), meso- (center) and micro-fauna (right) from four northern North American forests as shown in Fig. 1. Log-response ratios have been calculated treating 'forest' as a random effect. Methods for further calculation details of log-response ratios and bootstrapped confidence intervals. Animal silhouettes are from PhyloPic.org (nematode) and Florian Schneider (collembola and beetle).

(see the Supporting information for visualization of untransformed data). Note that as our selection of low- and high-invasion plots was based on the presence/absence of the three ecological groups of earthworms and not on earthworm biomass, the two invasion statuses are not entirely separated in figures based on earthworm biomass (Fig. 3).

Discussion

Here, we studied the impacts of earthworm invasion on soil-fauna communities and found a consistent negative association between earthworm invasion and soil-fauna responses (up to 45% decline) across three size groups and four biodiversity facets (with the exception of a weak and non-significant positive effect on mesofauna Shannon index). In addition, we found consistent negative effects of invasion intensity, measured as earthworm biomass, across the four forests. This systematic investigation of invasion impacts across different predictor variables, fauna size groups, biodiversity facets and multiple forest sites closes an important gap in the earthworm-invasion literature by covering understudied taxa (such as macro- and microfauna; Ferlian et al. 2018) and

considering body size traits (Schlaghamersky et al. 2014). The insights and aggregated datasets resulting from this study open up exciting avenues for further research on structural and functional ecosystem change following earthworm invasion in northern North American forest systems.

Earthworm invasion and soil-fauna biodiversity (H1)

Although there is a well-founded expectation of earthworm invasion reducing soil-fauna diversity (Ferlian et al. 2018, Eisenhauer et al. 2019; note that there may be both winners and losers (Bohlen et al. 2004)), this has rarely been systematically studied across body size groups and different facets of biodiversity. Most studies investigating earthworm-invasion impacts on soil fauna (reviewed by Ferlian et al. 2018) have focused on mesofauna (but see Burtis et al. 2014, Shao et al. 2017, McCay and Scull 2019). This reduced attention for micro- and macrofauna responses to earthworm invasion is problematic given the importance of these groups for ecosystem functioning (Bardgett and van der Putten 2014, Handa et al. 2014, van den Hoogen et al. 2019) and their expected sensitivity to habitat changes caused by invading earthworms (Migge-Kleian et al. 2006). We found
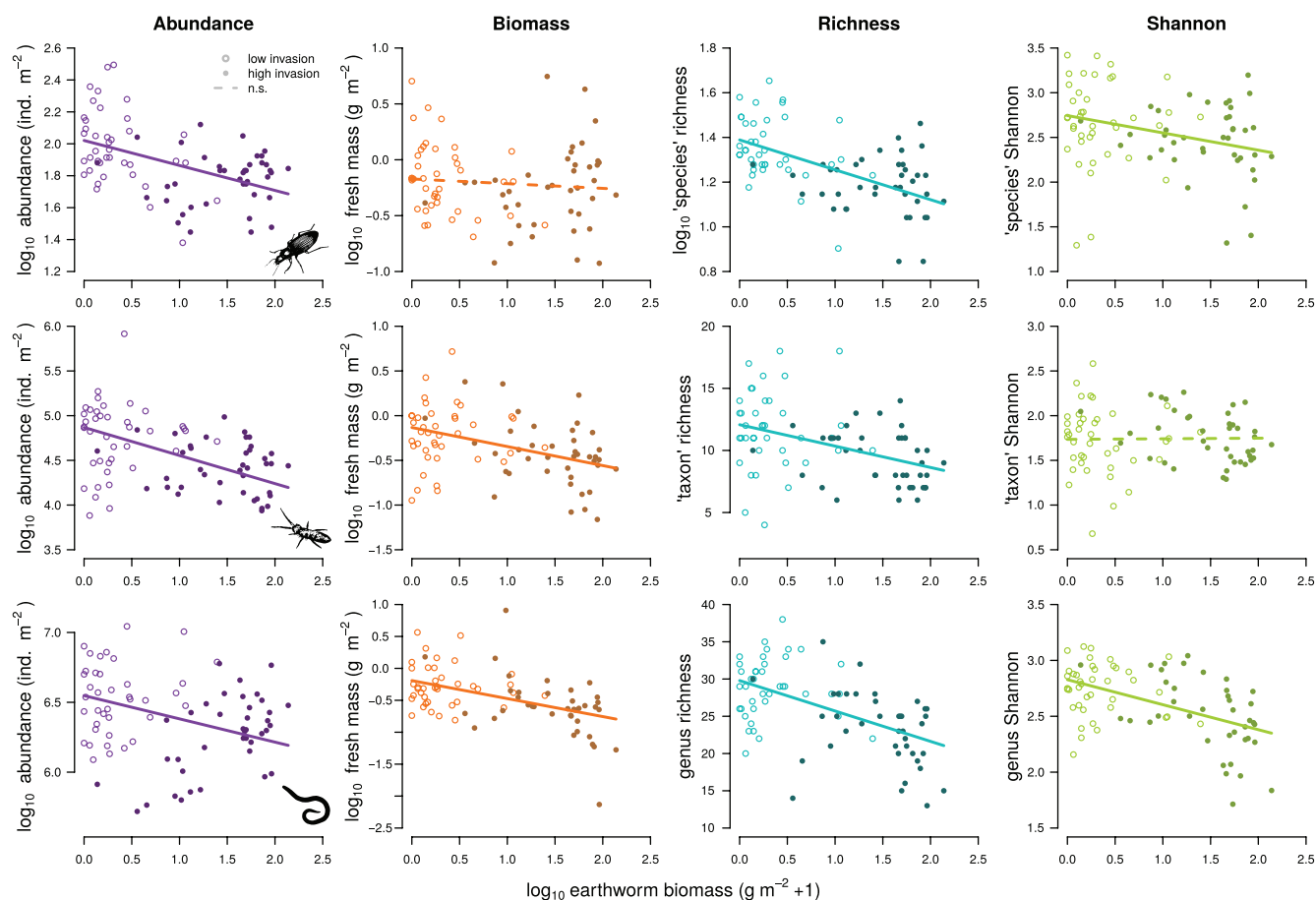
9

Figure 3. Results of linear mixed effects models testing the relationship of earthworm biomass with the abundance, biomass, richness (species, taxon and genus richness) and Shannon index (left to right columns) of soil fauna size groups (macrofauna, mesofauna, microfauna, top to bottom rows). Bright-colored, open circles show low and dark-colored, filled circles show high invasion status sites across the four northern North American forests (n = 78–80, Table 2). Dashed lines indicate non-significant results (p > 0.05). 'Forest' was treated as a random effect. Table 2 for detailed results of linear mixed effects models. Note that due to transformation of response variables, not all y-axes are on a comparable scale. Also, variables have been scaled to SI units where possible; for information on the original area of the different sampling techniques, please refer to the methods. Animal silhouettes are from PhyloPic.org (nematode) and Florian Schneider (collembola and beetle).

macrofauna abundance, richness and Shannon index to be significantly lower in high-invasion compared to low-invasion areas (H1). While similar responses have been reported for macrofauna abundance in two different studies in North American forests (Burtis et al. 2014, McCay and Scull 2019), previous assessments of macrofauna biodiversity change (richness or diversity indices) in response to earthworm invasion are scarce. The lack of a significant difference in macrofauna biomass might have been caused by the different forests showing a variety of responses (positive, negative, neutral) for macrofauna biomass (Supporting information), partly in contrast to the abundance trend (see St John's forest). As biomass changes are dependent on abundance and body-size structure, the latter will have to be further analyzed in future studies to isolate the mechanisms of community change. Specifically, investigating whether changing biomass and body-size structure are driven by altered body size of the same species, altered relative abundance of differently-sized

species or species turnover will shed light on exactly how earthworm invasion alters soil-fauna communities.

Soil mesofauna abundance, biomass and species richness were consistently and significantly lower in high versus low invasion status areas. Responses of Shannon index varied across the four forests (Supporting information), likely causing the overall lack of a significant change. Interestingly, Bull Creek forest showed opposite mesofauna trends to the other forests (variability in Fig. 2 and Supporting information; likely driven by this forest showing an early invasion stage, low earthworm presence and very low densities of anecic species), but the overall pattern was still strong enough to result in a significant reduction of the three other biodiversity facets. Overall, our mesofauna results are in line with most previously-reported studies showing reduced mesofauna richness and density in invaded areas (Ferlian et al. 2018). Mesofauna biomass changes have rarely been studied (Ferlian et al. 2018) and, similar to the macrofauna biomass, should be

subject to future investigation of changes in body-size structure (Schlaghamersky et al. 2014).

Finally, we found clear declines in all four nematode (microfauna) biodiversity facets. Interestingly, the nematode responses were less variable across the four forests than the macro- and mesofauna responses. Whether this similarity in response across forests is driven by the native nematode communities being more comparable than the meso- and macrofauna communities remains to be investigated. Despite their impact on ecosystem functioning (Ferris 2010), their tremendous importance as a ubiquitous and globally numerically dominating soil animal group (van den Hoogen et al. 2019) and their well-known interactions with earthworms in general (Brown 1995), nematode responses to the invasion of earthworms are comparatively less-frequently studied than that of mesofauna (Migge-Kleian et al. 2006, but see Shao et al. 2017). Our finding of overall eroded nematode communities in response to earthworm invasion calls for further investigation of the specific changes in nematode community-composition. Given the variety of nematode trophic groups and their indicator role for various soil processes (Cesarz et al. 2017), further investigation of species turnover, trophic-group and body-size structure, as well as male–female ratios of nematodes in response to earthworm invasion will be important for an in-depth understanding of how future earthworm invasion will continue to affect this ubiquitous animal group and the ecosystem functions it provides. Because of the numerical and functional importance of nematodes especially in boreal systems (van den Hoogen et al. 2019) and the comparatively low biomass of earthworms in these areas combined with projected further earthworm invasions, understanding the impact of invading earthworms on nematode communities will be crucial to predict future ecosystem change.

Differences between biodiversity facets and size groups (H2)

Simultaneously assessing abundance, biomass, richness and Shannon index of macro-, meso- and microfauna across several forest systems not only provides a comprehensive overview of biodiversity change, but allowed us to systematically assess the importance of accounting for different size groups and biodiversity facets when studying consequences of earthworm invasion. Our results indicate that relationships between earthworm invasion status and soil-fauna communities are strongly affected by the choice of the investigated biodiversity facet, but not by size group (H2). Our assessment of effect sizes in biodiversity-facet responses (Fig. 2) suggests a pattern of stronger effects on soil-fauna abundance than richness and Shannon index, with biomass responding in an intermediate and more variable way. Together with the model selection approach, these results provide some indication of how the different biodiversity facets respond to earthworm invasion across size groups. Please note that differences in taxonomic resolution between size groups could not be avoided, but their impact on our results was minimized as single

taxonomists identified all samples of a given size class across all four forests. For the size groups, there was a tendency of microfauna showing stronger negative responses than macro- and mesofauna, respectively. However, the models including size group as a categorical variable were clearly inferior to that including only earthworm invasion status and biodiversity facet, and thus these results need to be interpreted with care.

Given the importance of different trophic groups and biodiversity facets for ecosystem functioning and stability (Craven et al. 2018, Delgado-Baquerizo et al. 2020), our results call for a more-widespread adoption of simultaneously assessing several biodiversity facets rather than focusing on single aspects of biodiversity. Different biodiversity facets not only vary in their importance for ecosystem processes, but also in how they respond to ecosystem change (Jochum et al. 2017). External stressors affecting communities do so via multiple pathways, thereby affecting abundance, and biomass differently, e.g. if body mass is affected (Yin et al. 2020). Furthermore, abundance and richness responses to stressors might differ depending on whether these stressors first reduce overall density of organisms or impact e.g. rare species more strongly. Finally, in comparison to species richness, Shannon index contains information on species evenness (Magurran 2004) and thus depends on relative responses of these two aspects. As such, focusing on only one biodiversity facet when studying biodiversity change could lead to over- or underestimating the overall impact of global-change drivers and ecosystem consequences (Spaak et al. 2017). In our case, focusing on only Shannon index to compare communities would suggest a comparatively weak impact of earthworm invasion while focusing on just abundance would suggest a comparably strong impact. Similarly, in our study system, studying only macrofauna responses to earthworm invasion, would have led to a much more variable picture than studying microfauna responses. We therefore advocate choosing a relatively broad approach including several size groups and biodiversity facets when aiming to draw general conclusions of global-change impacts on ecosystems. The combination of such broad approaches with in-depth analyses on changes in community composition, body-size and trophic structure across and within size groups and taxa will then provide the detailed picture needed to predict future ecosystem responses to global change.

Effects of earthworm-invasion intensity (H3)

Our analysis of earthworm-biomass effects revealed that increasing invasion intensity was negatively related to soil-fauna communities across biodiversity facets and size groups (H3). Overall, the effects were remarkably similar to the invasion-status results (H1) in that all responses were statistically significant except for the responses of macrofauna biomass and mesofauna Shannon index. By going beyond analyzing categorical invasion status and deliberately including invasion intensity, these results provide novel insights into how earthworm invasion impacts soil fauna after an invasion has been started and how much they change

over the gradient of invasive earthworm biomass. Our results nicely complement earlier studies investigating the impact of earthworm-invasion intensity on ecosystem processes such as, for example, nutrient cycling and leaf-litter decomposition (Suárez et al. 2006, Crumsey et al. 2013, Chang et al. 2016, Mahon et al. 2020), by extending these findings to the responses of the soil-fauna communities which are responsible, at least in part, for the change in ecosystem processes. Interestingly, some of these changes in relation to earthworm-invasion intensity appear to happen at relatively low earthworm biomass levels (e.g. macrofauna abundance and richness, Supporting information), thus indicating that, at least for some combinations of soil-fauna biodiversity facet and size group, earthworms begin to have impacts as soon as they appear and it is not necessarily the high earthworm biomass building up over time that will trigger ecosystem change. Given the importance of macrofauna decomposers for decomposition processes (Handa et al. 2014) and the expected impact of changing the abundance of high trophic levels on ecosystem processes such as energy flux through trophic networks (Barnes et al. 2014, 2018), our results suggest that strong ecosystem change is likely to appear even at relatively low levels of invasion intensity. Please note that as we do not have information on the exact invasion history of our research plots, and because previous research has shown earthworm invasion to often come with very high earthworm abundance in an initial wave (Eisenhauer et al. 2011), but much lower abundances later on, we cannot entirely rule out the possibility that some of these low earthworm biomass plots could represent late invasion stages.

Broader context and outlook

Our results show that earthworm invasion affects soil-fauna communities. However, the soil fauna is neither an isolated player responding to such a perturbation, nor does it affect ecosystem functioning or multifunctionality without complex interactions with other organism groups and abiotic changes in the environment (Frelich et al. 2019). While responses of the abiotic environment in the soil (Ferlian et al. 2020), as well as plants (Craven et al. 2017) and microbes (Ferlian et al. 2018) have been studied, they are typically assessed in isolation (but see Eisenhauer et al. 2007). Consequently, the complex interrelationships of changes in abiotic ecosystem properties and the different trophic groups and how they interactively affect the functioning of below- and aboveground systems are far from being well understood. We report significant differences in soil abiotic properties such as carbon, nitrogen and water content, and the thickness of the humus layer between low- and high-invasion status areas (Supporting information). These changes in soil abiotic conditions likely mediate the effect of invasive earthworms on animal, plant and microbe communities. Consequently, we advocate for more comprehensive investigations of how earthworm-invasion induced changes in soil fauna are related to changes in abiotic properties and changes at other trophic levels and how the altered biodiversity of multiple taxa affects ecosystem processes

and multifunctionality (Soliveres et al. 2016, Delgado-Baquerizo et al. 2020). In addition, recent research connecting multitrophic biodiversity to multitrophic ecosystem functioning in the form of energy flux through trophic networks (Barnes et al. 2018) shows that combining community data with metabolic theory and food-web theory can provide valuable further insights into how global-change induced shifts in communities affect ecosystem functioning (Barnes et al. 2014, Schwarz et al. 2017). Consequently, future studies combining data on soil-fauna responses to earthworm invasion with data from other trophic levels and the abiotic environment to assess the consequences for the energetic backbone of affected ecosystems (Potapov et al. 2019) are urgently needed to gain a more comprehensive understanding of the complex changes following earthworm invasion (Eisenhauer et al. 2019). In addition, there is a multitude of other global-change drivers interacting with biological invasions in driving community and ecosystem change (Phillips et al. 2019a). Recent research suggests climate to be more important than soil properties for earthworm distribution (Phillips et al. 2019b). Thus, climate change is likely to shift the potential distribution of areas suitable for earthworms and potentially facilitate future earthworm invasion (Hendrix et al. 2008), which, especially in high latitudes, might have profound impacts on carbon release from soils (Cameron et al. 2015). As such, to better understand and ultimately mitigate the negative consequences, we need to strive for a more comprehensive understanding of the globally-revelant phenomenon of earthworm invasions.

Code and data accessibility

R-code and datasets are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.0p2ngf20r> (Jochum et al. 2021).

Author contributions

Malte Jochum: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Software (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Olga Ferlian**: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Investigation (equal); Methodology (equal); Project administration (supporting); Software (supporting); Writing – review and editing (equal). **Madhav P. Thakur**: Conceptualization (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Marcel Ciobanu**: Investigation (equal); Writing – review and editing (equal). **Bernhard Klarner**: Investigation (equal); Writing – review and editing (equal). **Jörg-Alfred Salamon**: Investigation (equal); Writing – review and editing (equal). **Lee E. Frelich**: Resources (supporting); Writing – review and editing (equal). **Ed A. Johnson**: Resources (supporting); Writing – review and editing (equal). **Nico Eisenhauer**: Conceptualization (lead); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Methodology (lead); Project administration (lead); Resources (lead); Supervision (lead); Writing – review and editing (equal).

References

Bardgett, R. D. and van der Putten, W. H. 2014. Belowground biodiversity and ecosystem functioning. – Nature 515: 505–511.

Barnes, A. D. et al. 2014. Consequences of tropical land use for multitrophic biodiversity and ecosystem functioning. – Nat. Comm. 5: 5351.

Barnes, A. D. et al. 2016. Species richness and biomass explain spatial turnover in ecosystem functioning across tropical and temperate ecosystems. – Phil. Trans. R. Soc. B 371: 20150279.

Barnes, A. D. et al. 2018. Energy flux: the link between multitrophic biodiversity and ecosystem functioning. – Trends Ecol. Evol. 33: 186–197.

Barton, K. 2015. MuMIn: multi-model inference. – R packag. ver. 1.15.1.

Blouin, M. et al. 2013. A review of earthworm impact on soil function and ecosystem services. – Eur. J. Soil Sci. 64: 161–182.

Bohlen, P. J. et al. 2004. Non-native invasive earthworms as agents of change in northern temperate forests. – Front. Ecol. Environ. 2: 427–435.

Bøhn, T. et al. 2008. Competitive exclusion after invasion? – Biol. Invas. 10: 359–368.

Bongers, T. 1994. De nematoden van Nederland: een identificatietabel voor de in Nederland aangetroffen zoetwater- en bodembewonende nematoden. – Koninklijke Nederlandse Natuurhistorische Vereniging.

Brown, G. G. 1995. How do earthworms affect microfloral and faunal community diversity? – Plant Soil 170: 209–231.

Burtis, J. C. et al. 2014. Impact of invasive earthworms on *Ixodes scapularis* and other litter-dwelling arthropods in hardwood forests, central New York State, USA. – Appl. Soil Ecol. 84: 148–157.

Cameron, E. K. et al. 2015. Modelling interacting effects of invasive earthworms and wildfire on forest floor carbon storage in the boreal forest. – Soil Biol. Biochem. 88: 189–196.

Cameron, E. K. et al. 2018. Global gaps in soil biodiversity data. – Nat. Ecol. Evol. 2: 1042–1043.

Cameron, E. K. et al. 2019. Global mismatches in aboveground and belowground biodiversity. – Conserv. Biol. 33: 1187–1192.

Canty, A. and Ripley, B. 2020. boot: Bootstrap R (S-Plus) functions. – R package ver. 1.3-25.

Cesarz, S. et al. 2017. Plant species richness sustains higher trophic levels of soil nematode communities after consecutive environmental perturbations. – Oecologia 184: 715–728.

Cesarz, S. et al. 2019. Testing soil nematode extraction efficiency using different variations of the Baermann funnel method. – Soil Org. 91: 61–72.

Chang, C.-H. et al. 2016. Species-specific effects of earthworms on microbial communities and the fate of litter-derived carbon. – Soil Biol. Biochem. 100: 129–139.

Christiansen, K. and Bellinger, P. 1998. The Collembola of North America north of the Rio Grande. – Grinnell College.

Craven, D. et al. 2017. The unseen invaders: introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). – Global Change Biol. 23: 1065–1074.

Craven, D. et al. 2018. Multiple facets of biodiversity drive the diversity–stability relationship. – Nat. Ecol. Evol. 2: 1579–1587.

Crumsey, J. M. et al. 2013. Community-specific impacts of exotic earthworm invasions on soil carbon dynamics in a sandy temperate forest. – Ecology 94: 2827–2837.

Csuzdi, C. et al. 2017. Molecular phylogeny and systematics of native North American lumbricid earthworms (Clitellata: Megadrili). – PLoS One 12(8): e0181504.

Delgado-Baquerizo, M. et al. 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. – Nat. Ecol. Evol. 4: 210–220.

Díaz, S. et al. 2018. Assessing nature's contributions to people. – Science 359: 270–272.

Edwards, C. 2004. Earthworm ecology. – CRC Press.

Ehrenfeld, J. G. and Scott, N. 2001. Invited feature: invasive species and the soil: effects on organisms and ecosystem processes. – Ecol. Appl. 11: 1259–1260.

Eisenhauer, N. 2010. The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarthropods. – Pedobiologia 53: 343–352.

Eisenhauer, N. and Eisenhauer, E. 2020. The 'intestines of the soil': the taxonomic and functional diversity of earthworms – a review for young ecologists. – EcoEvoRxiv doi: 10.32942/osf.io/tfm5y.

Eisenhauer, N. et al. 2007. Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. – Soil Biol. Biochem. 39: 1099–1110.

Eisenhauer, N. et al. 2011. The wave towards a new steady state: effects of earthworm invasion on soil microbial functions. – Biol. Invas. 13: 2191–2196.

Eisenhauer, N. et al. 2019. Ecosystem responses to exotic earthworm invasion in northern North American forests. – Res. Ideas Outcomes 5: e34564.

Ellison, A. M. 2010. Partitioning diversity. – Ecology 91: 1962–1963.

Estes, J. A. et al. 2011. Trophic downgrading of planet earth. – Science 333: 301–306.

Ferlian, O. et al. 2018. Invasive earthworms erode soil biodiversity: a meta-analysis. – J. Anim. Ecol. 87: 162–172.

Ferlian, O. et al. 2020. Soil chemistry turned upside down: a meta-analysis of invasive earthworm effects on soil chemical properties. – Ecology 101: e02936.

Ferris, H. 2010. Contribution of nematodes to the structure and function of the soil food web. – J. Nematol. 42: 63–7.

Frelich, L. E. et al. 2019. Side-swiped: ecological cascades emanating from earthworm invasions. – Front. Ecol. Environ. 17: 502–510.

Guerra, C. A. et al. 2020. Blind spots in global soil biodiversity and ecosystem function research. – Nat. Comm. 11: 3870.

Gunn, A. 1992. The use of mustard to estimate earthworm populations. – Pedobiologia 36: 65–67.

Handa, I. T. et al. 2014. Consequences of biodiversity loss for litter decomposition across biomes. – Nature 509: 218–221.

Hendrix, P. F. and Bohlen, P. J. 2002. Exotic earthworm invasions in North America: ecological and policy implications. – Bioscience 52: 801.

Hendrix, P. F. et al. 2008. Pandora's box contained bait: the global problem of introduced earthworms. – Annu. Rev. Ecol. Evol. Syst. 39: 593–613.

Hilton, D. and Reynolds, J. 1983. The earthworm (Oligochaeta: Lumbricidae) fauna of Cypress Hills Provincial Park, Alberta. – Megadrilogica 4: 91–92.

Hines, J. et al. 2015. Towards an integration of biodiversity–ecosystem functioning and food web theory to evaluate relationships between multiple ecosystem services. – Elsevier.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. – Scand. J. Stat. 6: 65–70.

Ikeda, H. et al. 2020. A comparison of latitudinal species diversity patterns between riverine and terrestrial earthworms from the North American temperate zone. – J. Biogeogr. 47: 1373–1382.

PBES 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (Díaz, S. et al., eds). – IPBES secretariat, Bonn, Germany.

James, S. and Hendrix, P. 2004. Invasion of exotic earthworms into North America and other regions. – In: Earthworm ecology. CRC Press, pp. 75–88.

Jochum, M. et al. 2017. Resource stoichiometry and availability modulate species richness and biomass of tropical litter macroinvertebrates. – J. Anim. Ecol. 86: 1114–1123.

Jochum, M. et al. 2021. Data from: Earthworm invasion causes declines across soil fauna size classes and biodiversity facets in northern North American forests. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.0p2ngf20r>.

Jones, C. et al. 1996. Organisms as ecosystem engineers. – In: Ecosystem management. Springer, pp. 130–147.

Jost, L. 2006. Entropy and diversity. – Oikos 113: 363–375.

Jouquet, P. et al. 2006. Soil invertebrates as ecosystem engineers: intended and accidental effects on soil feedback loops. – Appl. Soil Ecol. 32: 153–164.

Lefcheck, J. S. 2016. piecewiseSEM: piecewise structural equation modelling in r for ecology, evolution and systematics. – Methods Ecol. Evol. 7: 573–579.

Macfadyen, A. 1961. Improved funnel-type extractors for soil arthropods. – J. Anim. Ecol. 30: 171–184.

Magurran, A. 2004. Measuring biological diversity. – Blackwell.

Mahon, M. B. et al. 2020. Interactive effects of white-tailed deer, an invasive shrub and exotic earthworms on leaf litter decomposition. – Ecosystems 23: 1523–1535.

Maxwell, S. L. et al. 2016. Biodiversity: the ravages of guns, nets and bulldozers. – Nature 536: 143–145.

McCay, T. S. and Scull, P. 2019. Invasive lumbricid earthworms in northeastern North American forests and consequences for leaf-litter fauna. – Biol. Invas. 21: 2081–2093.

McCay, T. S. et al. 2017. Distribution and habitat of the endemic earthworm *Eisenoides lonnbergi* (Michaelsen) in the northeastern United States. – Northeast. Nat. 24: 239–248.

Mercer, R. D. et al. 2001. Invertebrate body sizes from Marion Island. – Antarct. Sci. 13: 135–143.

Migge-Klein, S. et al. 2006. The influence of invasive earthworms on indigenous fauna in ecosystems previously uninhabited by earthworms. – Biol. Invas. 8: 1275–1285.

Moran, M. D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. – Oikos 100: 403–405.

O'Loughlin, L. S. and Green, P. T. 2017. Secondary invasion: when invasion success is contingent on other invaders altering the properties of recipient ecosystems. – Ecol. Evol. 7: 7628–7637.

Oksanen, J. et al. 2016. Vegan: community ecology package. – R package ver. 2.5-7.

Phillips, H. R. P. et al. 2017. Red list of a black box. – Nat. Ecol. Evol. 1: 1.

Phillips, H. R. P. et al. 2019a. The effects of global change on soil faunal communities: a meta-analytic approach. – Res. Ideas Outcomes 5: e36427.

Phillips, H. R. P. et al. 2019b. Global distribution of earthworm diversity. – Science 366: 480–485.

Pinheiro, J. et al. 2014. Nlme: linear and nonlinear mixed effects models. – R package ver. 3.1-152.

Potapov, A. M. et al. 2019. Linking size spectrum, energy flux and trophic multifunctionality in soil food webs of tropical land-use systems. – J. Anim. Ecol. 88: 1845–185965-2656.13027.

Reid, W. V. et al. 2005. Millennium ecosystem assessment (WR Institute, Ed.). – World Resources Inst., Washington DC.

Rillig, M. C. et al. 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. – Science 366: 886–890.

Sala, O. E. et al. 2000. Global biodiversity scenarios for the year 2100. – Science 287: 1770–1774.

Savigny, J. C. 1826. - In: Cuvier, G. (ed.), Analyse des Travaux de l'Academie royale des Sciences, pendant l'anne´e 1821, partie physique. Mem. l'Acad. Sci. l'Inst. de France Paris, pp. 176−184.

Scheu, S. and McLean, M.-A. 1993. The earthworm (Lumbricidae) distribution in Alberta (Canada). – Megadrilogica 4: 175–185.

Schlaghamersky, J. et al. 2014. Earthworm invasion alters enchytraeid community composition and individual biomass in northern hardwood forests of North America. – Appl. Soil Ecol. 83: 159–169.

Schwarz, B. et al. 2017. Warming alters the energetic structure and function but not resilience of soil food webs. – Nat. Clim. Change 7: 895–900.

Shao, Y. et al. 2017. Nitrogen deposition cancels out exotic earthworm effects on plant-feeding nematode communities. – J. Anim. Ecol. 86: 708–717.

Simberloff, D. and Von Holle, B. 1999. Positive interactions of nonindigenous species: invasional meltdown? – Biol. Invas. 1: 21–32.

Simberloff, D. et al. 2013. Impacts of biological invasions: what's what and the way forward. – Trends Ecol. Evol. 28: 58–66.

Sohlström, E. H. et al. 2018. Applying generalized allometric regressions to predict live body mass of tropical and temperate arthropods. – Ecol. Evol. 8: 12737–12749.

Soliveres, S. et al. 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. – Nature 536: 456–459.

Spaak, J. W. et al. 2017. Shifts of community composition and population density substantially affect ecosystem function despite invariant richness. – Ecol. Lett. 20: 1315–1324.

Stevens, R. D. and Tello, J. S. 2014. On the measurement of dimensionality of biodiversity. – Global Ecol. Biogeogr. 23: 1115–1125.

Straube, D. et al. 2009. Nonlinearity of effects of invasive ecosystem engineers on abiotic soil properties and soil biota. – Oikos 118: 885–896.

Suárez, E. R. et al. 2006. Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. – Ecol. Appl. 16: 154–165.

Thakur, M. P. et al. 2020. Towards an integrative understanding of soil biodiversity. – Biol. Rev. 95: 350–364.

van den Hoogen, J. et al. 2019. Soil nematode abundance and functional group composition at a global scale. – Nature 572: 194–198.

Van Groenigen, J. W. et al. 2014. Earthworms increase plant production: a meta-analysis. – Sci. Rep. 4: 1–7.

Veresoglou, S. D. et al. 2015. Extinction risk of soil biota. – Nat. Comm. 6: 8862.

Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor. – J. Stat. Softw. 36: 1–48.

Voigt, W. et al. 2007. Using functional groups to investigate community response to environmental changes: two grassland case studies. – Global Change Biol. 13: 1710–1721.

Wardhaugh, C. W. 2013. Estimation of biomass from body length and width for tropical rainforest canopy invertebrates. – Aust. J. Entomol. 52: 291–298.

Wardle, D. A. et al. 2011. Terrestrial ecosystem responses to species gains and losses. – Science 332: 1273–1277.

Weigmann, G. 2006. Hornmilben (Oribatida). Acari, Actinachaetida. Neubearbeitung der Hornmilben Deutschlands. – Goecke & Evers.

Yin, R. et al. 2020. Climate change and intensive land use reduce soil animal biomass through dissimilar pathways. – eLife 9: e54749.