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No net insect abundance and diversity declines across US Long Term Ecological Research sites

Michael S. Crossley[1]✉, Amanda R. Meier[1], Emily M. Baldwin[2], Lauren L. Berry[2], Leah C. Crenshaw[2], Glen L. Hartman[3], Doris Lagos-Kutz[3], David H. Nichols[2], Krishna Patel[2], Sofia Varriano[2], William E. Snyder[1] and Matthew D. Moran[2]

Recent reports of dramatic declines in insect abundance suggest grave consequences for global ecosystems and human society. Most evidence comes from Europe, however, leaving uncertainty about insect population trends worldwide. We used >5,300 time series for insects and other arthropods, collected over 4–36 years at monitoring sites representing 68 different natural and managed areas, to search for evidence of declines across the United States. Some taxa and sites showed decreases in abundance and diversity while others increased or were unchanged, yielding net abundance and biodiversity trends generally indistinguishable from zero. This lack of overall increase or decline was consistent across arthropod feeding groups and was similar for heavily disturbed versus relatively natural sites. The apparent robustness of US arthropod populations is reassuring. Yet, this result does not diminish the need for continued monitoring and could mask subtler changes in species composition that nonetheless endanger insect-provided ecosystem services.

Insects and other arthropods provide critical ecosystem services including pollination, natural pest control and decomposition, while influencing plant community structure and providing food to humans and other vertebrates[1]. Indeed, declines in populations of bumble bees and other pollinators endanger the production of an array of crops and reveal how dependent human society is on insects[2–5]. Thus, recent reports of sudden, dramatic drops in insect numbers[5–16] have triggered understandable fear that human-induced harm to the environment has reached a crisis point. Much evidence for what has been dubbed the 'insect apocalypse' comes from Europe[11,14], where humans have intensively managed landscapes for centuries and human population densities are particularly high. Indeed, insect declines there sometimes seem to be most rapid in the landscapes most heavily altered by human activity[12]. Other proposed drivers include relatively localized factors such as changing insecticide use patterns and artificial light pollution, and globally important factors such as climate change, nutrient dilution and increasing nitrification that presumably would reach even the most remote natural area[10,14,17]. So, depending on the underlying cause, insect decline might variously be predicted to be limited to heavily degraded landscapes (for example, ref. [12]) or reach into natural areas designated as nature preserves (for example, ref. [8]).

However, considerable scepticism has also emerged about the likelihood of the collapse of insect populations[18–20]. Critics note counter-examples where insects are relatively stable or increasing, even at sites heavily influenced by humans[20,21]. Others report apparent population rebounds through time[22]. Sometimes, sites in relatively human-disturbed areas exhibit insect populations with greater apparent stability than those in less disturbed landscapes[22], and climate change correlates with apparent declines in some cases[3] but not in others[8]. Clearly, before concluding that global insect populations are broadly in danger, we will need evidence from diverse communities of arthropods, across physically and ecologically dispersed sites that span both relatively natural and relatively human-managed landscapes, and outside of Europe[19]. This knowledge gap reflects a larger debate about what constitutes convincing evidence for global degradation of plant and animal biodiversity in the Anthropocene[23,24].

Here we utilized a geographically and taxonomically broad suite of relatively long-term datasets to search for evidence of insect decline in the United States. The US National Science Foundation initiated the establishment of a network of Long-Term Ecological Research (LTER) sites in 1980, and these now encompass a web of 25 monitoring locations across each of the country's major ecoregions (Fig. 1). Sites were chosen to reflect a diversity of habitat types in the United States and, critically, to span a range of human influence from urban (for example, within the US cities of Baltimore and Phoenix) or farmed regions (for example, the Midwest farmland aphid suction-sampling network) to those that are quite remote (for example, Arctic tundra in Alaska and Sevilleta desert/grassland in New Mexico) (Table 1). Arthropod data have been systematically collected from at least 12 different LTERs (Fig. 1) using a variety of approaches (but in a consistent way over time within each dataset; Supplementary Table 1), with some reporting multiple, separate datasets based on the taxa considered and/or method used for sampling ('Methods'). Types of arthropod data include grasshoppers per sweep in Konza Prairie (Kansas), ground arthropods per pitfall trap in Sevilleta desert/grassland (New Mexico), mosquito larvae per ovitrap in Baltimore (Maryland), pelagic macroinvertebrates per tow and crayfish per fyke net in North Temperate Lakes (Wisconsin), aphids per suction trap sample in the Midwestern United States, crab burrows per quadrat in Georgia Coastal Ecosystems, ticks per person/hour in Harvard Forest (Massachusetts), caterpillars per plot in Hubbard Brook (New Hampshire), arthropods per pitfall trap and sweep net in Phoenix metro area (Arizona) and stream insects per rock scrub in the Arctic (Alaska) (Table 1). When collecting these data, we did not discriminate based on taxa, type of

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[1]Department of Entomology, University of Georgia, Athens, GA, USA. [2]Department of Biology and Health Sciences, Hendrix College, Conway, AR, USA. [3]Agricultural Research Service, United States Department of Agriculture, Urbana, IL, USA. ✉e-mail: michael.crossley@uga.edu

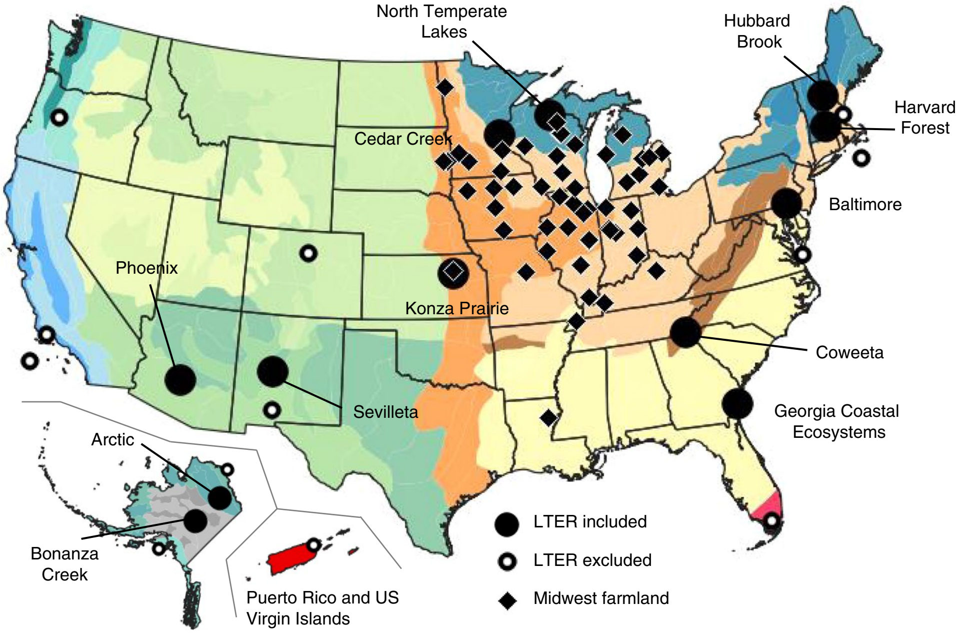

Fig. 1 | Map of LTER sites. Filled black circles represent LTER sites with arthropod data that were included in our analyses ($n=12$). White circles represent LTER sites without arthropod data or with insufficient years of tracking to estimate time trends. Black diamonds represent sites comprising Midwest farmland. Colours on the underlying map delineate ecoregions (as defined by the United States Department of Agriculture Forest Service: https://www.fs.fed.us/rm/ecoregions/products/map-ecoregions-united-states/).

Table 1 | LTER site attributes

LTER	Sites	Habitat	Taxa group	Time trends	Range
Arctic	1	Arctic tundra	Stream insects	14	1984–1998
Baltimore	1	Urban	Mosquitoes	9	2011–2015
Bonanza Creek	1	Taiga	Bark beetles	3	1975–2012
	1		Aspen leaf miner	1	2004–2015
Cedar Creek	2	Savannah/tallgrass prairie	Arthropods	940	1989–2006
	1		Grasshoppers	60	1996–2006
Phoenix	2	Urban	Ground arthropods	966	1998–2019
	1		Arthropods	312	1999–2015
Coweeta	1	Temperate deciduous forest	Aquatic invertebrates	10	1988–2006
Georgia Coastal Ecosystems	2	Salt marsh/estuary	Crabs (fiddler, burrowing)	2	2001–2018
	1		Grasshoppers	7	2007–2018
	1		Planthoppers	1	2013–2018
Harvard Forest	2	Temperate deciduous forest	Ants	88	2000–2015
	30		Ticks	115	2006–2019
Hubbard Brook	2	Temperate deciduous forest	Lepidoptera larvae	10	1986–2018
Midwest farmland	46	Row crop agriculture	Aphids	2,125	2006–2019
Konza Prairie	1	Tallgrass prairie	Gall insects	1	1988–1996
	1		Grasshoppers	54	1982–2015
North Temperate Lakes	4	Temperate lake	Pelagic/benthic macroinvertebrates	234	1981–2017
	1		Crayfish	2	1981–2017
Sevilleta	1	Desert/grassland	Grasshoppers	56	1992–2013
	1		Ground arthropods	365	1995–2004

Select attributes of LTER sites included in this study. 'Sites' refers to the number of sampling points or independent sampling methods used in an LTER. 'Taxa group' refers broadly to the types of arthropods sampled. 'Time trends' reported depends on both the number of taxa and the number of sites/methods within an LTER that met the inclusion criteria (for example, a single aphid species in Midwest farmland will have associated time trends at several suction trap sites with >3 yr of data). 'Range' refers to the first and last year of sampling included in our analysis. See Supplementary Table 1 for extended details about LTER site attributes.

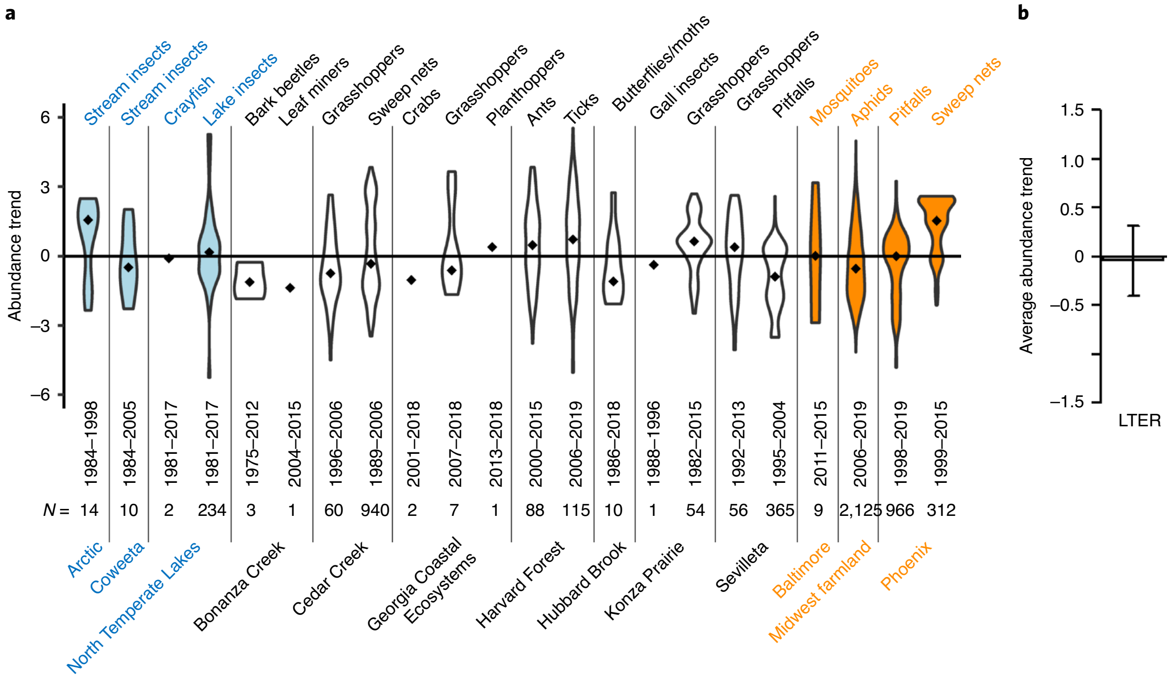

Fig. 2 | Time trends in arthropod abundance among LTERs. a, Violin plots showing the distribution of abundance trends among taxa time series. The black diamonds within boxplots depict medians. The first and last years of LTER studies as well as the number of taxa time series are included below the violin plots. Blue shading and font indicate LTER sites reporting aquatic taxa. Orange shading and font indicate LTER sites in urban or agricultural landscapes. Unfilled violin plots and black font indicate LTER sites reporting terrestrial taxa in relatively less human-disturbed habitats. Abundance trends for Midwest farmland are further separated by ecoregion in Extended Data Fig. 8. **b**, Average trend in abundance and 95% confidence intervals when trends are averaged among LTERs (d.f. = 12). Time trends were not significantly different from zero at $\alpha = 5\%$.

study or study methods, though we excluded studies outside of North America (for example, Antarctica).

The LTER arthropod data are publicly available ('Methods') but have not previously been gathered into a single dataset to be examined for evidence of broad-scale density and biodiversity changes through time (but see ref. [25]). The oldest datasets precede LTER establishment and started in the late 1800s, but data coverage becomes increasingly complete (that is, standardized and frequently sampled) from the 1980s to the present ('Methods'). Altogether, to construct our LTER arthropod abundance meta-dataset, 82,777 arthropod observations from 68 datasets were compiled into 5,375 taxa time series spanning up to 36 years, including 48 arthropod orders made up of 1 to 658 taxa in a given dataset ('Methods'). Of these, 3,412 time series were from the Midwest farmland, Phoenix and Baltimore sites most directly impacted by human development (63% of the total), while the remaining 1,963 time series (37% of the total) were from sites receiving less direct human disturbance. Of course, all sites would be expected to be affected by climate change, altered N deposition and other wide-reaching human impacts often suggested as possible drivers of insect decline[26–28]. For each time series, autoregressive models were fit using restricted maximum likelihood to estimate the change in abundance over time ('Methods'). This method yielded slopes that are interpreted as the change in the number of arthropods in units of standard deviation per unit scaled time that in turn could be used to search for general patterns of decline compared across species, datasets and sites.

Results and discussion

We found that some arthropod taxa at some sites declined in abundance through the course of their time series, while at other sites a preponderance of taxa increased or there was no clear trend towards increasing or decreasing abundance (Fig. 2a). For most datasets, the median abundance change through time was modest, lying within 1.6 standard deviations of zero net difference (Fig. 2a). Across all 5,375 time series, 1,738 (~32%) exhibited decreases greater than one standard deviation, 1,303 (24%) exhibited increases greater than one standard deviation and 2,334 (43%) did not change by more than one standard deviation. In terms of net percent change per year, 2,319 (43%) and 1,665 (~31%) trends exhibited decreases and increases greater than 1%, respectively, while 1,047 (19%) and 619 (12%) trends exhibited decreases and increases greater than 5%, respectively. Consistent with this, the average abundance trend across LTER sites broadly overlapped with zero (Fig. 2b). These patterns were similar when separating taxa into aquatic versus terrestrial arthropods (Fig. 2a) or when separately examining feeding guilds (herbivores, carnivores, omnivores, detritivores, parasites or parasitoids; Extended Data Fig. 1). Comparison of time series from sites within clearly anthropogenic landscapes with those within more natural sites suggests no overall trend of increase or decline or difference for either broad disturbance category (Fig. 2a). Four LTER sites also collected time series for insectivorous birds, and three for fish ('Methods'). We again saw no clear trend for increase or decrease through time among these vertebrates that likely rely, at least in part, on insect prey, though we note an increase in insectivorous birds at the urban Baltimore site (Extended Data Fig. 2). Our findings were remarkably robust to whether we considered the time series in the meta-datasets that spanned four or more observations through time, those that spanned at least 8 yr, or only those that spanned 15 yr and exhibited minimal temporal autocorrelation (Extended Data Fig. 3). In summary, we found no evidence of precipitous and widespread insect abundance declines in North America akin to those reported from some sites in Europe[5,6,8,10,12]. Rather, our results were broadly similar to reports for insects and other taxa, where 'winners' roughly counterbalanced 'losers'[29]. The

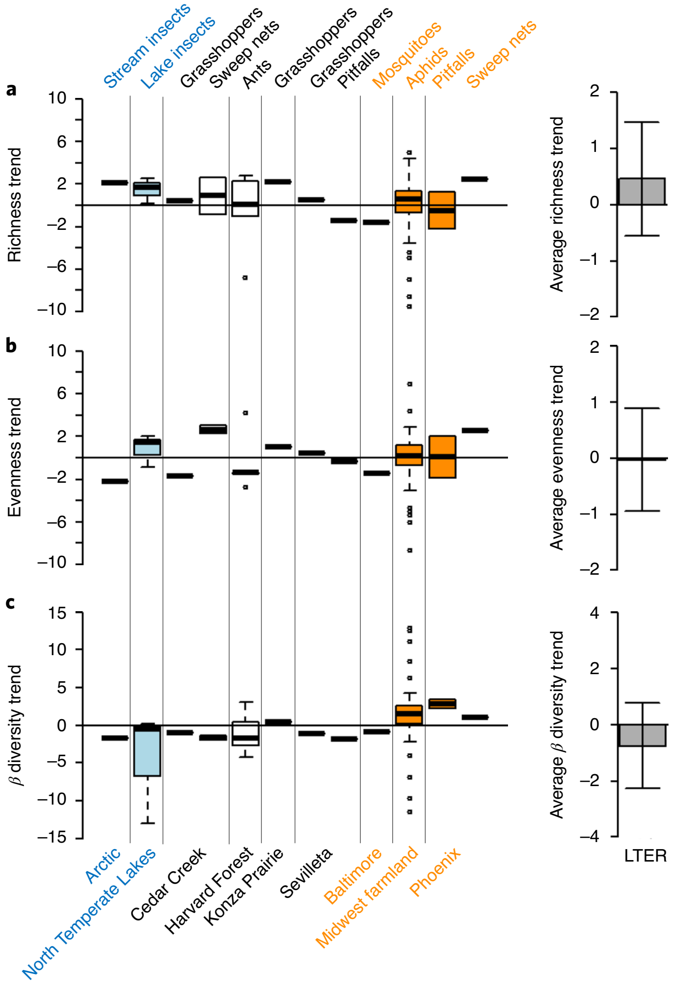

Fig. 3 | Time trends in arthropod diversity among LTERs. a–c, Species richness (rarefied) (**a**), species evenness (Pielou's index) (**b**), and β diversity (1 – Jaccard similarity index) (**c**). Boxplots depict quantiles among LTER sites. Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers) and outliers (circles). Right panels depict average change in diversity metrics and 95% confidence intervals among LTERs (d.f. = 8). Time trends were not significantly different from zero at $\alpha = 5\%$. Refer to Fig. 2 caption for description of coloured text.

lack of a clear, overall directional change in abundances was seen across habitats and feeding guilds and appeared to extend to vertebrate arthropodivores.

We examined changes in the number of species present (species richness), in the equitability of relative abundances of species (species evenness) and in species composition (β diversity, per site over time) for nine LTERs that reported time series for more than eight unambiguously identified arthropod taxa ('Methods'). For each time series of each diversity metric, we used the same autoregressive model fitting procedure as for abundance, yielding slopes that are interpreted as change in the diversity (richness, evenness and β diversity) of arthropods in units of standard deviation per unit scaled time. Degradation of species richness and evenness is known to diminish the delivery of critical insect-derived ecosystem services[30–32], while high turnover of species composition can accompany non-native species invasions and rapid environmental change[33]. We found that while species richness (Fig. 3a), evenness (Fig. 3b) and β diversity (Fig. 3c) variously increased, decreased or were unchanged through time at different LTERs, the degree of change at most sites was relatively modest and the overall mean trends across sites broadly overlapped with zero (Fig. 3). Of the 63 trends in species richness (rarefied), 15 (~24%) decreased by more than one standard deviation, 22 (~35%) increased by more than one standard deviation, and 26 (~41%) did not change by more than one standard deviation. Among trends in species evenness, 16 (~25%) were decreasing, 20 (~32%) were increasing, and 27 (~43%) did not change. Of trends in β diversity, 14 (~22%) were decreasing (that is, tending to become more similar over time), 34 (~54%) were increasing (that is, tending to become more dissimilar over time), and 15 (~24%) did not change.

Several possible explanations for the apparent overall robustness of US arthropod populations at the LTERs were considered. A particularly comprehensive study in Germany, spanning from 2008 to 2017, found the steepest arthropod declines in the landscapes most intensively affected by human activity[12], although this relationship is not consistent even across European studies (for example, refs. [5,22]). While the majority of LTER sites are located in areas of low human population density, more than half of the time series in our meta-dataset were for urban insects in Phoenix, Arizona, mosquitoes in Baltimore, Maryland and aphids across the heavily farmed US Midwest, all of which showed unchanged or slightly increasing overall insect densities, species richness and/or evenness broadly consistent with the less disturbed sites (Figs. 2 and 3). We also did not find an association between a measure of human impact (Human Footprint Index[27]) and time trends among LTER sites using random forests analysis (Extended Data Figs. 4 and 5). Indeed, none of the variables included in the random forests analysis (temperature, precipitation, LTER or start year) could reliably predict the direction or magnitude of abundance trends (Extended Data Figs. 4 and 5). A second possibility is that our meta-dataset included some time series that ended a decade or more ago, perhaps predating and masking declines that accelerated only recently[12]. However, when we divided our time series into relatively old (predating 1990) or new blocks (decades between 1990 and 2019), we did not see any detectable change in trends through time (Extended Data Fig. 6). Our meta-dataset has notable strength in that it spans several different ecoregions that are widely dispersed at a continental scale and includes species that occupy distinct habitats and with different ecological roles. Overall, our findings are most consistent with those European studies reporting decreasing insect numbers for some taxa at some sites, counterbalanced by gains or relative stability elsewhere[29], rather than providing any clear indication of widespread decline.

Recently, van Klink et al.[25] reported total abundance and/or biomass trends for insects and arachnids from 166 studies around the world, spanning as far back as 1925. This impressive dataset suggests that, globally, over the last century terrestrial insects have been steadily declining while aquatic insects have been increasing. They found that these trends were strongest in the US Midwest, with terrestrial declines and aquatic increases there the strongest contributor to overall global patterns[25]. In stark contrast, we found little consistent degradation of arthropod communities for this same region, despite sharing several LTER sites in common. Comparison of the two studies suggests several possible reasons for this apparent discrepancy. First, four of the five LTER sites included here but not in van Klink et al.[25] report increasing arthropod abundances (Supplementary Table 2), partly counterbalancing decreasing abundances found at sites included in both studies. Second, measures of total abundance across species can give particular weight to a relatively small number of numerically dominant species. For example, for Konza Prairie grasshoppers, total grasshopper abundance decreases when species are pooled[17], but this pattern is driven by falling numbers of just two

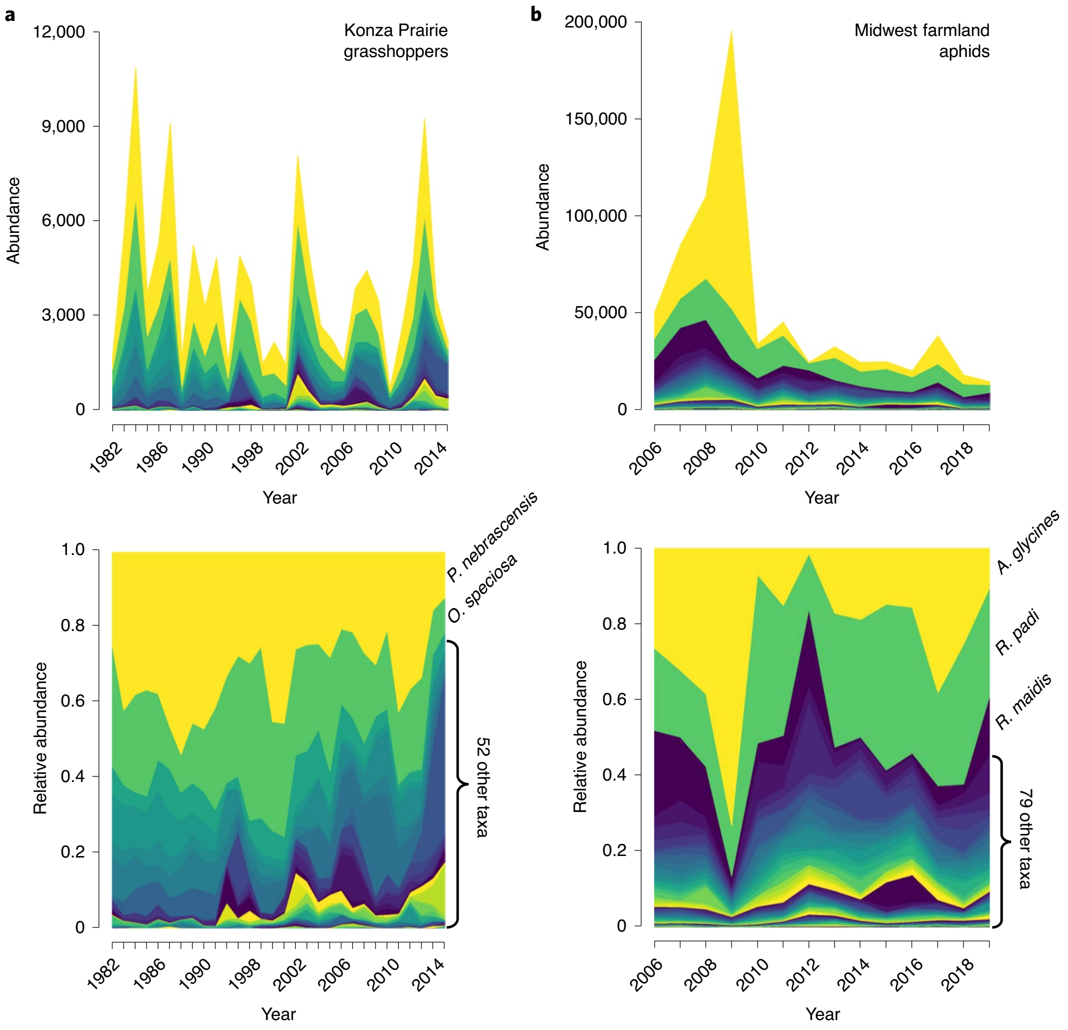

Fig. 4 | Change in relative abundance of taxa over time. a,**b**, Abundance (top) and relative (proportional) abundance (bottom) of Konza Prairie grasshoppers (1982–2015) (**a**) and Midwest farmland aphids (2006–2019) (**b**).

once-dominant species, *Phoetaliotes nebrascensis* and *Orphulella speciosa*, whereas many other formerly rare species have become more abundant and both evenness and species richness have increased (Figs. 3 and 4a). Likewise, declining total abundance among Midwest aphids reflects dropping numbers of two invasive (*Aphis glycines* and *Rhopalosiphum maidis*) and one native (*Rhopalosiphum padi*) agricultural pest species, whereas changes in abundance of the many other aphid species were variable and minor in comparison (Fig. 4b). This pattern highlights the value of reporting multiple biodiversity and abundance metrics and analysing trends at fine taxonomic level (this study) versus broad abundance measurements[8,9,25] to gain a more comprehensive picture of overall ecological health. Similarly, species richness loss was sometimes accompanied by gains in evenness (Extended Data Fig. 7; one Cedar Creek sweep net and two Midwest farmland sampling points) or vice versa (Extended Data Fig. 7; Arctic stream insects, Cedar Creek grasshoppers, Harvard Forest ants and three Midwest farmland sampling points), indicating that degradation in one aspect of biodiversity does not necessarily mean a wholesale decline. Finally, the coverage of the LTER data is greatest only in the last few decades, a period where van Klink et al.[25] found attenuation of the stronger trends seen in earlier time series.

On the surface, our finding of no overall net change in arthropod abundance and biodiversity may seem reassuring, but reasons for concern remain. Particular insect species that we rely on for the key ecosystem services of pollination, natural pest control and decomposition remain unambiguously in decline in North America[14,34–36]. We know that shifts in species composition can impact ecosystem function even when overall biodiversity and abundance remain unchanged[37]. Indeed, at least two of the LTER sites were dominated by relatively recently arrived invasive species: soybean aphid (*Aphis glycines*), which has been a major component of Midwest aphid communities (though note the increasing numerical dominance of the native bird cherry-oat aphid, *Rhopalosiphum padi*), and Asian tiger mosquito (*Aedes albopictus*), which is found in the Baltimore, Maryland mosquitoes data (Fig. 5a,b). Yet, the changes in the abundance of these invasive species mirrored large fluctuations in native species within less disturbed sites (Fig. 5c,d), and their net effects on the structure of surrounding arthropod communities, if any, remain unclear. Changes in food web structure can also have important ecosystem consequences[30], and the LTER data did not include information on trophic connections. Finally, several sites showed declines in abundance and biodiversity through time (for example, ground-dwelling arthropods at the southwest desert Sevilleta site; Figs. 2 and 3) that may indicate worrying ecological degradation at those particular locations. We note, however, that recent trends might obscure past population fluctuations or even increases, as has been found in deeper time series[22].

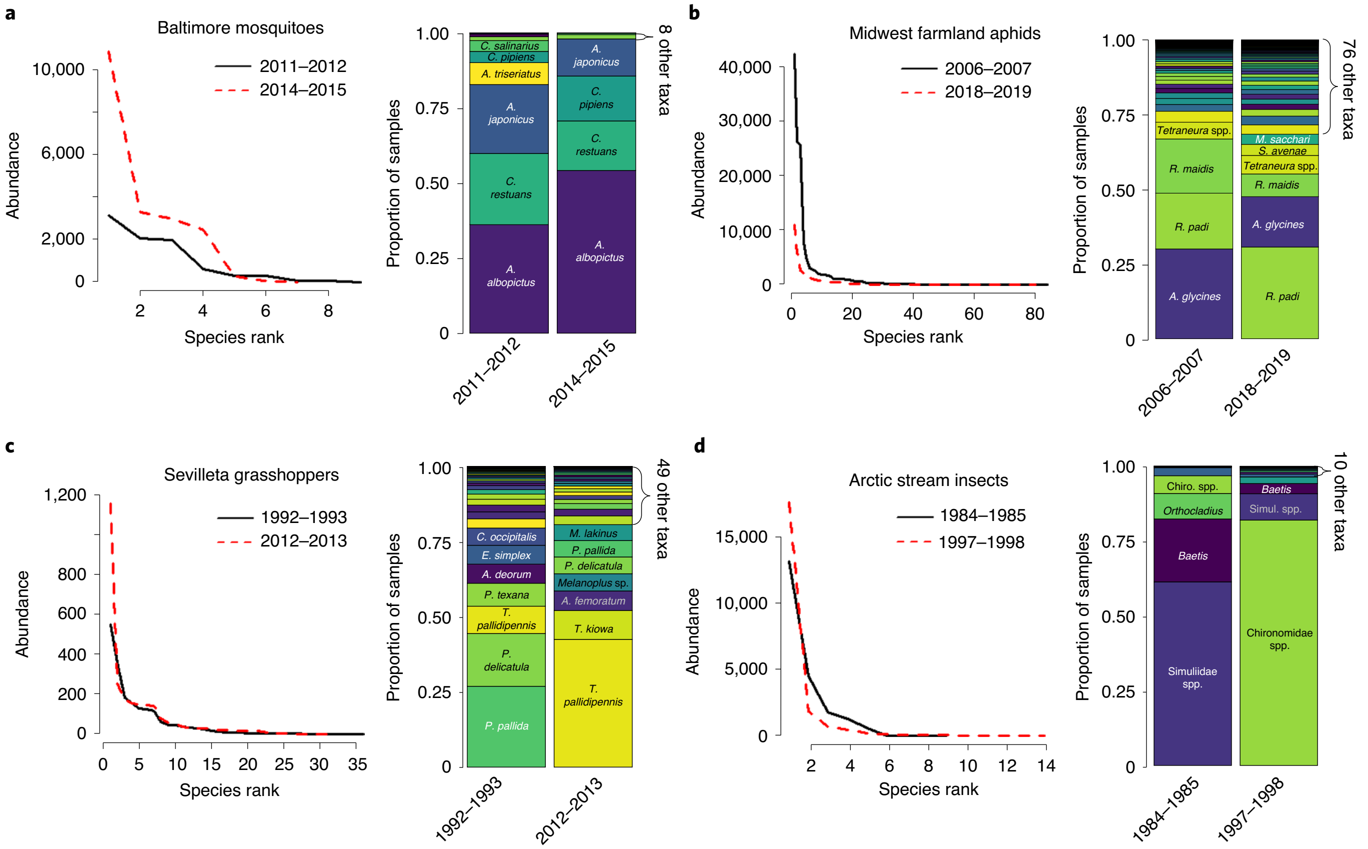

Fig. 5 | Comparison of species rank abundance and community composition. a–d, Changes between the first and last two years of each study at representative LTER sites located in highly human-modified (**a**,**b**) and natural (**c**,**d**) areas: Baltimore (Maryland), documenting 12 mosquito species between 2011 and 2015 (**a**), Midwest farmland (11 Midwestern states), including 82 aphid taxa between 2006 and 2019 (**b**), Sevilleta desert/grassland (New Mexico), documenting 31 grasshopper species between 1992 and 2013 (**c**) and Arctic tundra (Alaska), documenting 14 aquatic insect genera/families (**d**). *A.*, *Aedes*; *C.*, *Culex* (**a**). *A.*, *Aphis*; *M.*, *Melanaphis*; *R.*, *Rhopalosiphum*; *S.*, *Sitobion* (**b**). *A. deorum*, *Ageneotettix deorum*; *A. femoratum*, *Aulocara femoratum*; *C.*, *Cordillacris*; *E.*, *Eritettix*; *M.*, *Melanoplus*; *P. delicatula*, *Psoloessa delicatula*; *P. texana*, *Psoloessa texana*; *P. pallida*, *Paropomala pallida*; *T. kiowa*, *Trachyrhachis kiowa*; *T. pallidipennis*, *Trimerotropis pallidipennis* (**c**). Chiro., Chironomidae; Simul., Simuliidae (**d**).

There is no doubt that the near-wholesale conversion of Midwestern US prairies to agricultural fields has dramatically altered insect communities. For example, North American tallgrass prairies have been reduced over 90% in the last 150 years[38], certainly reducing the abundance of arthropods in these habitats on a continental scale. Yet, at a protected tallgrass site in the Flint Hills (the largest block of surviving tallgrass prairie), we found that arthropod species did not show dramatic losses, a pattern indicative of local stability (but see ref. [17]). The emerging 'insect apocalypse' narrative focuses on a recent, sudden and dramatic degradation of insect communities that compounds past changes that probably occurred during past habitat conversion. For the sites we studied though, this degradation was not apparent.

Separating natural year-to-year density variation from that driven by emerging human impacts is a challenge for many species of conservation concern[39] but is particularly daunting for arthropods with relatively high species and functional diversity and high reproductive potential. After all, insects can undergo dramatic increases and declines through time among particular taxa at particular sites even without new human-derived drivers[40]. Vigilance against emerging broad declines will benefit from expansive monitoring networks that collect environmental data and welcome contributions from citizen scientists (for example, refs. [41,42]), greater monitoring of arthropod communities outside of Europe[14], a broader search for historical descriptions of insect communities at spatially dispersed and ecologically different sites that can be resampled (for example, ref. [43]) and an improved theoretical understanding of how to definitively isolate changes in the underlying causes of population dynamics (for example, ref. [40]). These components will be needed to decide between relatively focused conservation schemes aimed at particular at-risk species or sites versus the need for much broader socio-environmental change at a global scale, when seeking to maintain robust insect communities[44].

The recent avalanche of studies reinforcing or critiquing the 'insect apocalypse' narrative echoes a broader discussion about global biodiversity change and ecosystem functioning[45], which is itself contentious[23,24]. Despite agreement that Anthropocene forces threaten biodiversity, evidence of wholesale declines remains elusive[21,46–48]. Sceptics of biodiversity meta-analyses argue that conclusions of no net biodiversity change are reached because available data are neither globally representative nor of sufficient duration to refute the axiom of global biodiversity declines[23]. While acknowledging the need for more spatiotemporally extensive biodiversity monitoring, we contend that timely, cautious interpretations of findings from imperfect data will be more fruitful than dismissing them altogether. Our synthesis of US LTER arthropod trends shares many weaknesses with previous datasets, but the broad representation of taxa, habitats, feeding guilds and sampling methods makes our data well suited to detect any broad decline in arthropod biodiversity. Though the implications of species turnover for ecosystem services remain to be examined, our data clearly indicate no evidence of wholesale declines in arthropod abundance and diversity in the United States.

Methods

Data sources. We visited the website of each of the US National Science Foundation's LTER sites to search for publicly available data files reporting the tracking of arthropod populations through time. When collecting these data, we did not discriminate based on taxa, type of study or study methods, though we excluded studies outside of North America (for example, Antarctica). We included studies that were terrestrial, freshwater and estuarine, but excluded exclusively marine studies. Source data varied widely in their formats, including, for example, grasshoppers per sweep in Konza Prairie (Kansas), ground arthropods per pitfall trap in Sevilleta desert/grassland (New Mexico), mosquito larvae per ovitrap in Baltimore (Maryland), pelagic macroinvertebrates per tow and crayfish per fyke net in North Temperate Lakes (Wisconsin), crab burrows per quadrat in Georgia Coastal Ecosystems, ticks per person/hour in Harvard Forest (Massachusetts), caterpillars per plot in Hubbard Brook (New Hampshire), arthropods per pitfall and sweep net in Phoenix (Arizona) and stream insects per rock scrub in the Arctic (Alaska) (Table 1). We acknowledge that some datasets, such as Harvard Forest ticks, Konza Prairie gall insects and Georgia Coastal Ecosystems crab burrows, more directly measure arthropod activity, but they are nonetheless included in our abundance time series meta-dataset. In addition, data on aphid abundance in the Midwest from 2006 to 2019 were obtained from the Suction Trap Network website (https://suctiontrapnetwork.org/), which is supported by the University of Georgia Center for Invasive Species and Ecosystem Health ('Bugwood Center') as part of the Southern IPM Center's IPM Information Supplement funded by the United States Department of Agriculture National Institute of Food and Agriculture. The Midwest Suction Trap Network included data from an additional 46 sites representing 11 Midwestern states. This trap network documented 152 aphid species as of 2020[49], 82 of which were identified consistently over time among sampling points that met our inclusion criteria. We refer to these data as 'Midwest farmland' throughout.

Source data included various inconsistencies, for example, in how a single species' name was designated. To create a single data file with consistent formatting for analysis, we processed each data file in R 3.6.2[50], used automated workflows to identify and correct inconsistencies/errors, and extracted a consistent set of variables (species/taxon code, site/sampling method, year and abundance/count) for estimation of abundance time trends, calculation of diversity metrics and estimation of diversity time trends ('Analysis'). We combined data for each arthropod species across sampling points within LTERs if sampling methods were consistent and if the arthropods inhabiting sample points could reasonably be considered a single population or meta-population, such that we could obtain one trend per species per site. Summing arthropod abundances across sample points minimized non-independence of species counts within LTERs and improved estimates of trends in species abundance over time. Two LTER databases (Harvard Forest and North Temperate Lakes) contained data from sample points that utilized different collection methods (for example, pitfall trap versus litter bag); in these cases, we treated species abundance according to each method as separate time series. Because abundance data were ultimately natural log- and Z-transformed and used to estimate time trends measured in units of standard deviation ('Analysis'), we did not standardize reported arthropod abundances by sampling effort. Indeed, due to the variability in how source data were reported (Supplementary Table 1), arriving at comparable, sampling effort-standardized measures of arthropod abundance among LTERs would not be possible.

In total, arthropod data curation compiled 82,777 arthropod observations from 68 sample points, yielding 6,501 abundance time series; 4,310 time series came from 12 LTERs (and from a total of 22 sample points within these LTERs; Supplementary Table 1), and 2,191 time series came from 46 sample points within Midwest farmland. However, we present results from analyses with 5,375 species time series (3,250 from LTERs and 2,125 from the Midwest farmland) that meet our criteria for inclusion in the study ('Analysisrsquo; and Supplementary Table 1). Curated data on arthropod abundances and time trends ('Analysis') as well as R code that can be used to replicate our data curation and analysis are available at Dryad (https://doi.org/10.5061/dryad.cc2fqz645).

We also utilized bird and fish community samples that were taken in association with (that is, found in the same general location of) arthropod samples. These data were available from four LTER sites for birds and three sites for fish, representing 775 and 171 species time series, respectively. Our goal was to determine whether birds and fish, organisms that often feed on arthropods, exhibited density changes alongside any found for local arthropods.

Data classification. Using literature searches, we classified each arthropod species within each study according to taxonomic classification (order level), habitat (aquatic or terrestrial) and feeding guild. Many insects have both an aquatic and terrestrial stage of development, creating a complexity in how to classify their habitat and, in many cases, their feeding guild. We therefore classified each species according to the habitat from which the specimen was collected (for example, dragonflies reported from sweep-net sampling of prairie plants were classified as terrestrial). For feeding guilds, we assigned each species one of the following: herbivore, carnivore, detritivore, omnivore, parasitoid and parasite (or 'none' if the life stage collected does not feed). As in the habitat classification, we also classified feeding by the habitat-specific stage collected. For example, Chironomidae adults collected in a terrestrial study would have feeding classified as 'herbivore' since many are nectarivorous (or none for some species), while the larvae collected in an aquatic study would have feeding classified as 'omnivore' (unless there was a different species-specific feeding strategy). Feeding guild assignments were based on their general feeding behaviour, and we recognize that in some cases there could be debate about our assignments.

For birds, all species that are obligate or facultative arthropodivores (insectivores and/or crustaceovore[51]) were included in the analysis ($n = 50$). Birds that do not typically feed on arthropods were excluded. Fish are highly variable in their feeding, depending on life stage, so we did not exclude any fish species from the population analysis. Changes in bird and fish abundance over time were estimated using the same procedure as for arthropods ('Analysis').

Analysis. For each taxon time series, we estimated a temporal trend using an autoregressive model fit using restricted maximum likelihood[52]. Before fitting models, we scaled time such that the distance between consecutive years was equal and spanned 0 to 1, and we Z-transformed the natural log of species counts. Resulting trends can be interpreted as the change in species abundance in standard deviations per unit scaled time. Our autoregressive models also estimated the temporal autocorrelation coefficient, b, which was used to remove time series whose trends could not be well estimated due to high temporal autocorrelation. We filtered time series based on three levels of stringency in quality criteria, and examined whether our degree of filtering stringency altered median trends among LTER sites. Our relaxed criteria required at least four years of counts, one of which had to be non-zero ($n = 5,328$ out of 6,501 trends remained). Moderate criteria required at least 8 years of counts, of which 4 had to be non-zero ($n = 2,266$ trends remained). Strict criteria required at least 15 years of counts, of which 10 had to be non-zero, and that temporal autocorrelation be <1 ($n = 308$ trends remained). Because LTER site median time trends were insensitive to filtering stringency by these criteria (Extended Data Fig. 3), we present results from the relaxed criteria that retained abundance time trends for the most taxa and that were most inclusive of large trends. We present overall patterns in time trends among LTERs and sample points within LTERs in terms of percentiles. Because Midwest farmland spanned several ecoregions, we further separate aphid abundance trends from this dataset by ecoregion (Extended Data Fig. 8). We use a one-sample T test (using the t.test R function) to test whether mean trends among LTERs are different from zero at $\alpha = 5\%$. For this analysis, we grouped datasets by LTER (d.f. = 12) or site–taxa group (d.f. = 22) (Table 1). We note that no means were significantly different from zero at $\alpha = 5\%$ (Supplementary Table 1). To represent trends in terms of net percent change per year, we regressed $\log_{10}$-transformed abundance on year (scaled between 0 and the length of the time series); because the slope of this regression represents proportional change, we calculated percent change as the slope multiplied by 100.

We evaluated the importance of LTER and taxon attributes in predicting the direction and magnitude of time trends using random forests analysis[53]. Random forests analysis uses machine learning to classify observations according to suites of associated variables and attempts to minimize the classification error by integrating outcomes across many decision trees. Relevant to our analysis, the importance of variables for increasing prediction accuracy can then be assessed. Predictor variables in our analysis included taxon attributes (feeding guild and terrestrial/aquatic habitat) and LTER attributes (LTER, start year, mean annual temperature (1970–2000), mean cumulative annual precipitation (1970–2000) and Human Footprint Index (average of data in available years between 1993 and 2009)). Temperature and precipitation variables were obtained from WorldClim climate rasters[54], and the Human Footprint Index was obtained from Venter et al.[55,56]; values were associated with LTERs by averaging raster pixel values that were within 10 km of the LTER central coordinates (External Database S3). Comparison of the distribution of Human Footprint Index values across the United States with the distribution of values among LTER sites suggests that LTER sites span a range levels of human disturbance (Extended Data Fig. 9). The response variable was the slope of time trends, treated as a continuous variable ($n = 5,328$) or as categorically high versus low (magnitude of slope exceeding two standard deviations per unit scaled time; $n = 1,318$). We trained the random forests classifier with a random sample of half of the time trends. Decision trees were constrained to use five of the seven predictor variables. The random forests algorithm was implemented using the randomForest R package[57]. The importance of predictor variables was then assessed by examining the decrease in prediction accuracy (increase in mean square error) when a variable was excluded from decision trees. Results from this analysis suggested that the start year of time series best predicted whether a trend was increasing or decreasing, improving the random forests prediction accuracy threefold more than other predictors (feeding guild, terrestrial/aquatic habitat, LTER, mean annual temperature, cumulative annual precipitation and Human Footprint Index), none of which appreciably increased prediction accuracy (Extended Data Figs. 4 and 5). This result was consistent whether random forests were predicting time trends as a continuous or categorical variable (considered strongly increasing or decreasing if the change in abundance was greater than two standard deviations per unit scaled time). Examination of arthropod abundance time trends along a gradient of starting years, however, revealed no consistent directional effect of starting year (Extended Data Fig. 4). In addition, predictor variables together only explained 19% of the variance in time trends, and prediction error rates were as high as 36%, suggesting that the random forests classifier could not reliably predict the magnitude or direction of arthropod abundance time trends.

Because our meta-dataset included some time series that ended a decade or more ago and potentially predated or masked declines that accelerated only recently, we compared abundance trends among LTER sites where sampling start years were earlier than 1990, spanned 1990–2000, spanned 2000–2010, or were after 2010. Still, average abundance trends did not differ significantly from zero at $\alpha = 5\%$ (Extended Data Fig. 6). Results were the same when trends were grouped according to final sampling years (except that no final sampling years predated 1990) (Supplementary Table 3).

We estimated taxa richness, evenness and β diversity, per LTER that had at least nine unambiguously identified taxa reported over the course of the study ($n = 9$). Rarefied taxa richness (S') was estimated using the rarefy function in the vegan R package[58], and evenness was calculated using Pielou's Evenness Index. To check the consistency of richness and evenness metrics, we calculated dominance as the proportional abundance of the most abundant taxon at a site in a given year (Extended Data Fig. 7). We calculated β diversity (differentiation in species composition) per LTER over time using three metrics: Jaccard[59], β_{-2} (ref. [60]) and Bray–Curtis distance[61,62]. Jaccard and β_{-2} use presence/absence data and differ in their sensitivity to species gain or loss: Jaccard considers only the proportion of species shared between communities, whereas β_{-2} incorporates information about the proportion of species that are unique to either community[62]. Bray–Curtis distance is a multivariate measure of β diversity, incorporating species abundance data. We calculated β diversity indices using the 'betadiver' and 'vegdist' functions in the vegan R package. Differences in β diversity results were slight (Extended Data Fig. 10), and results with the Jaccard index are presented (Fig. 3c). Changes in diversity over time were assessed using the same autoregressive model fitting approach as was used for species abundance.

To test whether changes in richness, evenness and β diversity were associated with increases in invasive species, we generated species rank abundance curves (sensu ref. [12]) and identified species whose abundance increased (or decreased) over the course of each time series, specifically focusing on species whose relative abundance changed substantially in the last two years compared with the first two years of each study (only studies with >4 years were included in this analysis). We focused on taxa exhibiting substantial changes in the beginning and end of each study to examine whether invasive taxa were becoming dominant (potentially at the expense of native taxa) or whether generalists were replacing specialists. We did not see evidence of increasing dominance of invasive taxa, though generalists were among the most abundant taxa at some sites (for example, Fig. 4c).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study (curated arthropod abundances and estimated time trends) are available at the Dryad Data Repository (https://doi.org/10.5061/dryad.cc2fqz645).

Code availability

The R code used to curate and analyse data are available at the Dryad Data Repository (https://doi.org/10.5061/dryad.cc2fqz645).

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Author contributions

M.S.C., A.R.M., W.E.S. and M.D.M. conceived of the idea for the paper, and M.S.C. and A.R.M. conducted analyses; M.S.C., A.R.M., W.E.S., M.D.M., E.M.B., D.L.-K., G.L.H., L.L.B., L.C.C., D.H.N., K.P. and S.V. assisted with data collection and curation; M.S.C., W.E.S. and M.D.M. primarily wrote the paper, although all authors contributed to the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-020-1269-4.

Supplementary information is available for this paper at https://doi.org/10.1038/s41559-020-1269-4.

Correspondence and requests for materials should be addressed to M.S.C.

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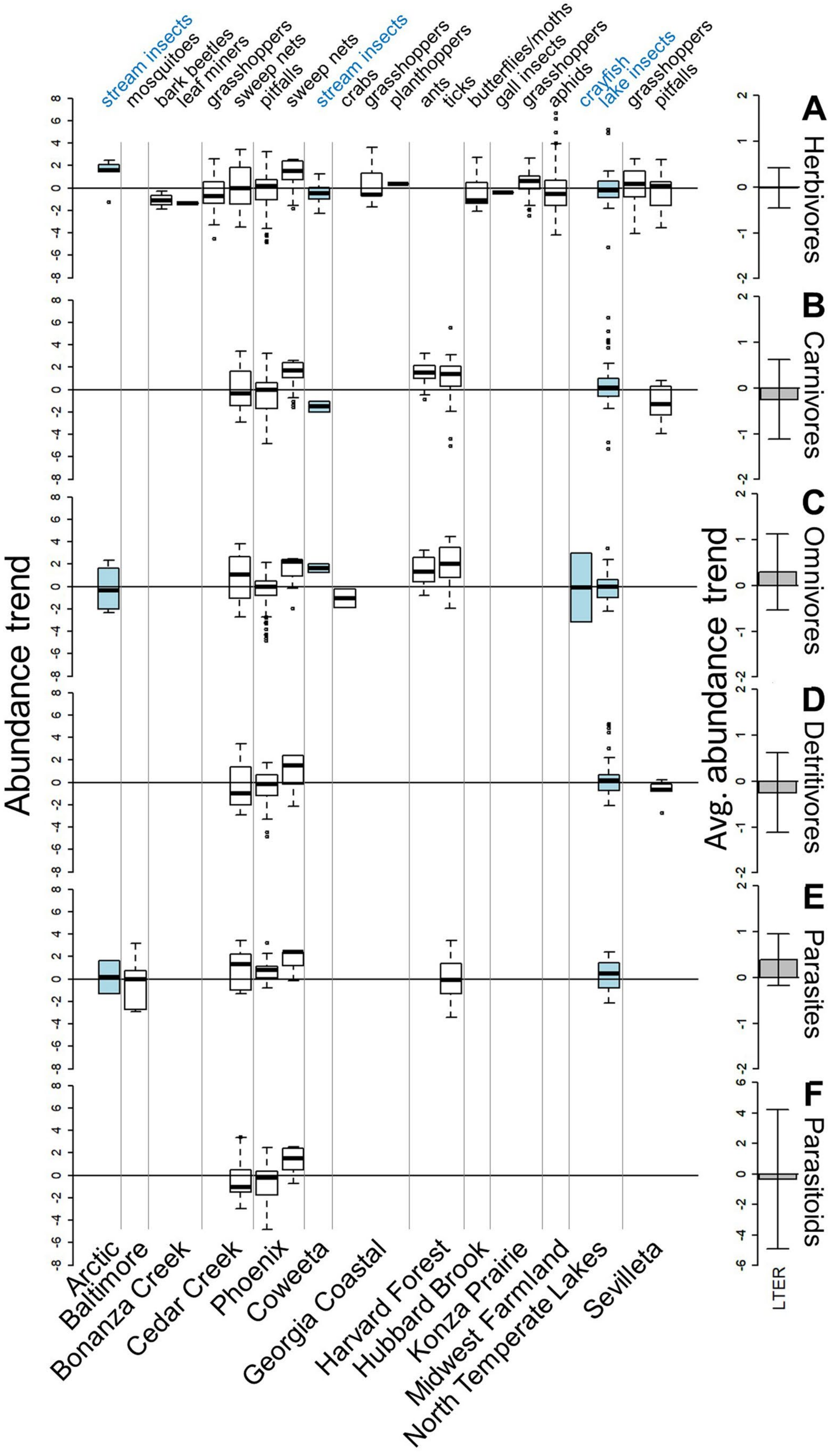
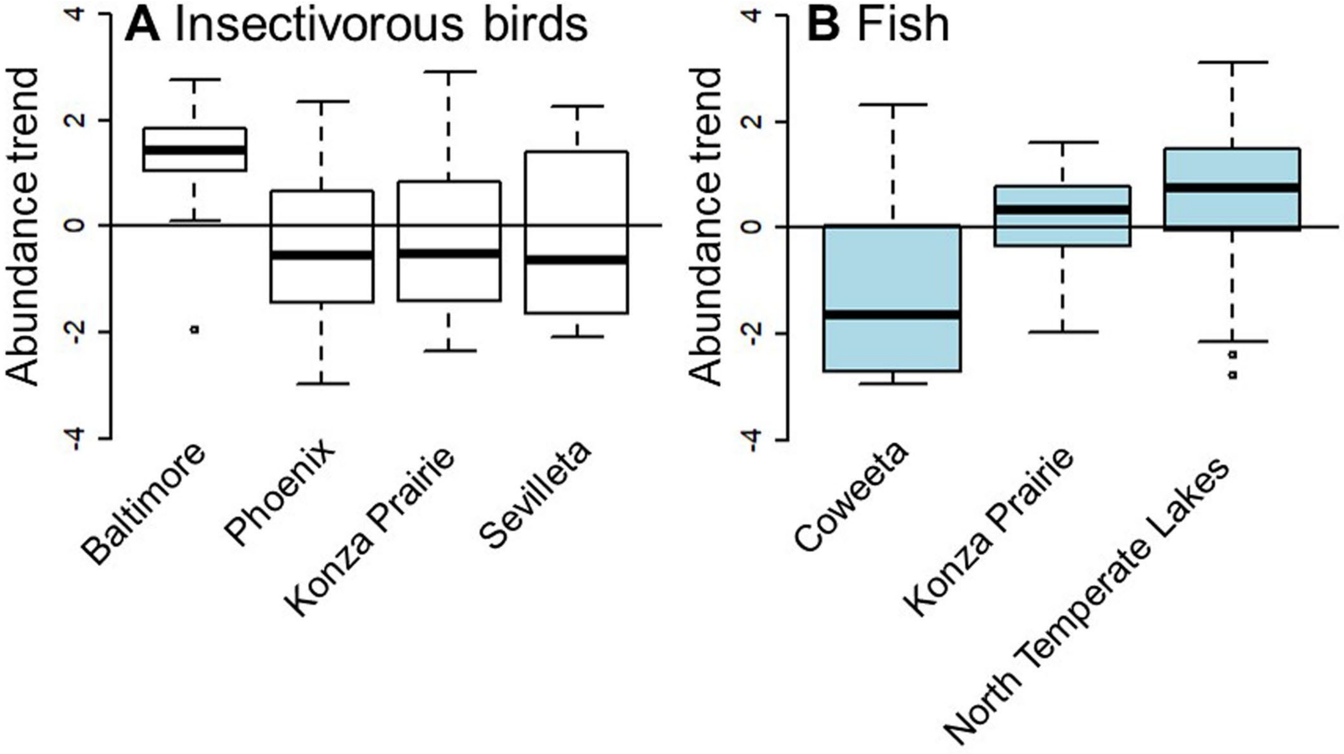
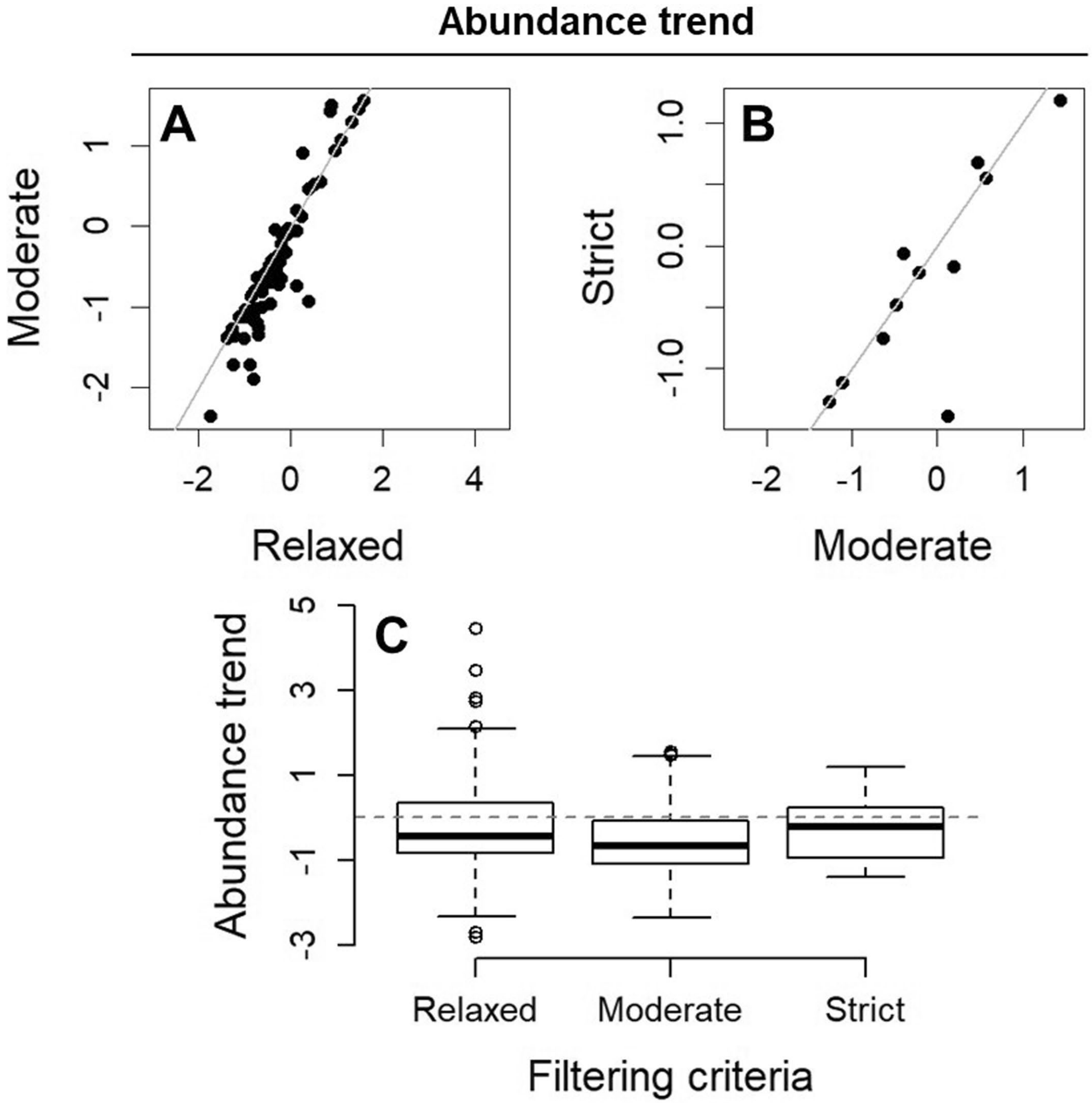


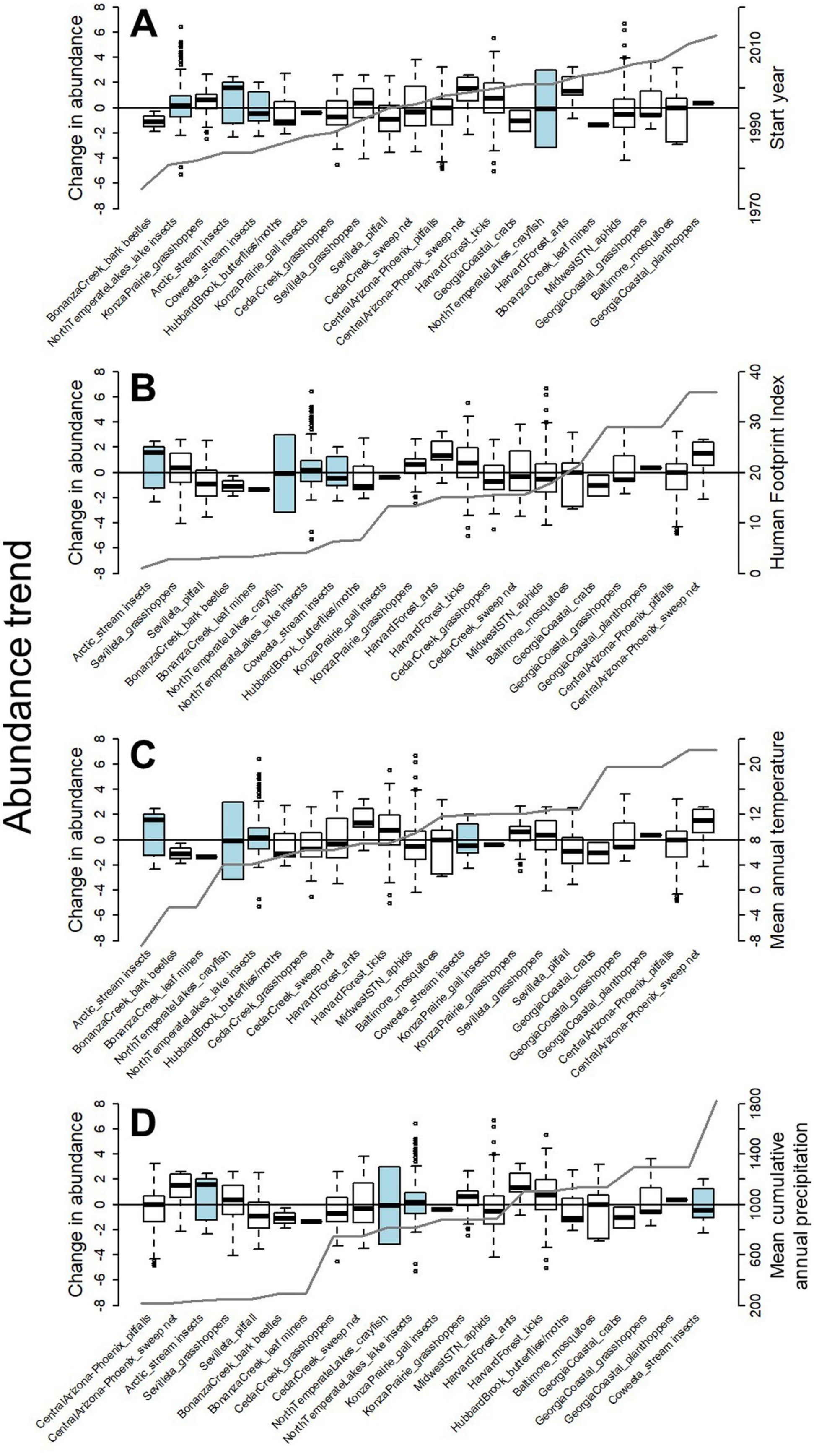
Extended Data Fig. 1 | Time trends in abundance of arthropod feeding groups among LTERs. (**a**) herbivores, (**b**) carnivores, (**c**) omnivores, (**d**) detritivores, (**e**) parasites, and (**f**) parasitoids. Right panels depict average change in diversity metrics and 95% confidence intervals among LTERs. Blue shading and font indicate LTER sites reporting aquatic taxa.



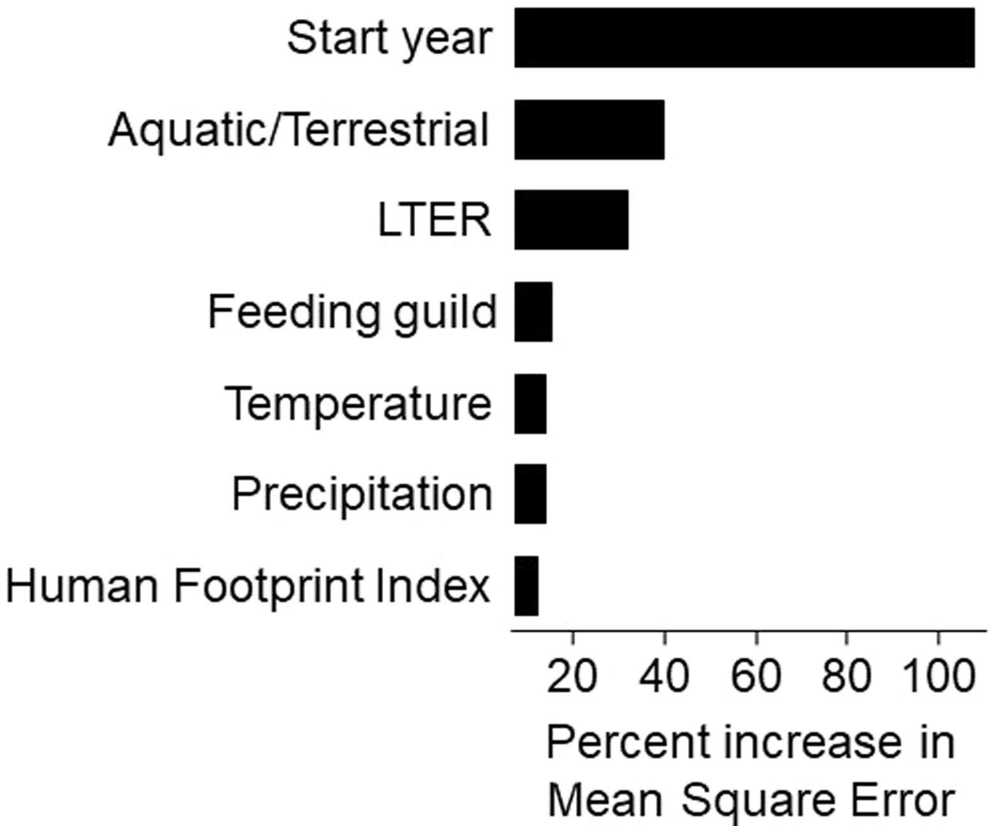
Extended Data Fig. 2 | Time trends in insectivorous bird (a) and fish (b) abundance among LTERs. Boxplots depict medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers), and outliers (circles).



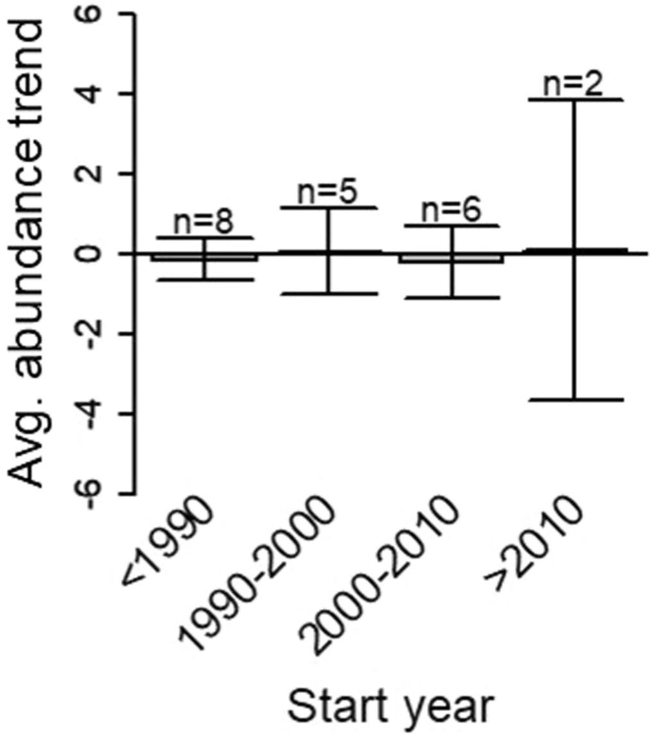
Extended Data Fig. 3 | Sensitivity analysis on stringency of time series quality filtering. Abundance trends of all taxa under (**a**) moderate vs. relaxed time series filtering criteria and (**b**) strict vs. moderate filtering criteria. (**c**) Boxplots of abundance trends under relaxed, moderate, and strict timer series filtering criteria. Relaxed criteria required at least four years of counts, one of which had to be non-zero (n = 5,328 out of 6,501 trends remained). Moderate criteria required at least eight years of counts, of which four had to be non-zero (n = 2,266 trends remained). Strict criteria required at least 15 years of counts, of which 10 had to be non-zero, and that temporal autocorrelation be < 1 (n = 308 trends remained).



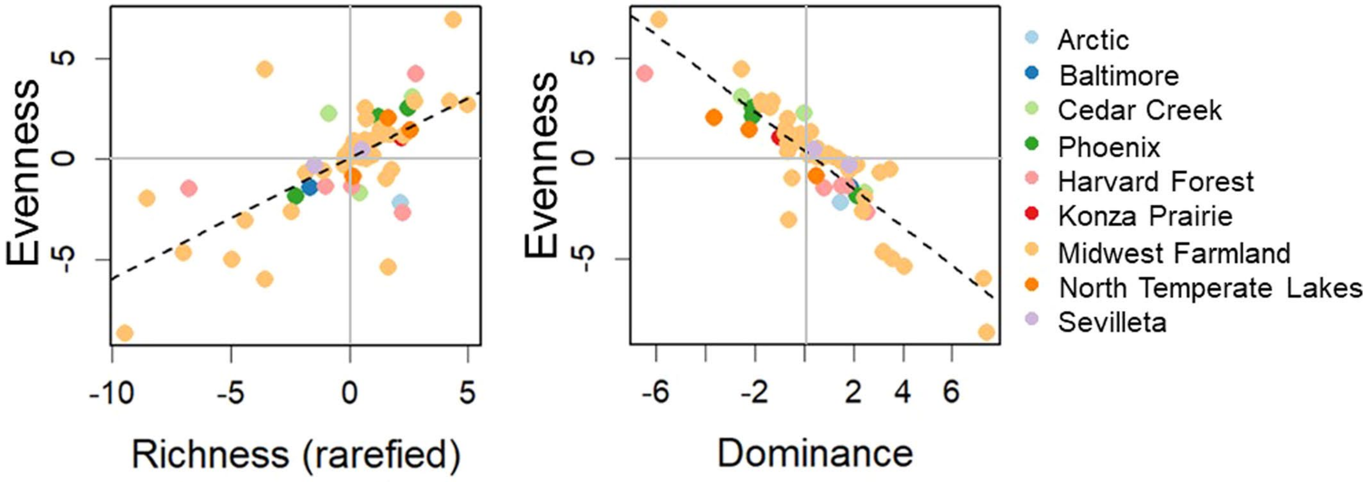
Extended Data Fig. 4 | Explanatory variables overlaid on (sorted) time trends in arthropod abundance among LTERs. (**a**) Start year of LTER site sampling. (**b**) Human Footprint Index associated with LTER site. The average HFI value for locations within the US is 7; LTER sites ranged from 1 to 38. (**c**) Mean annual temperature at LTER sites. (**d**) Mean cumulative annual precipitation at LTER sites.



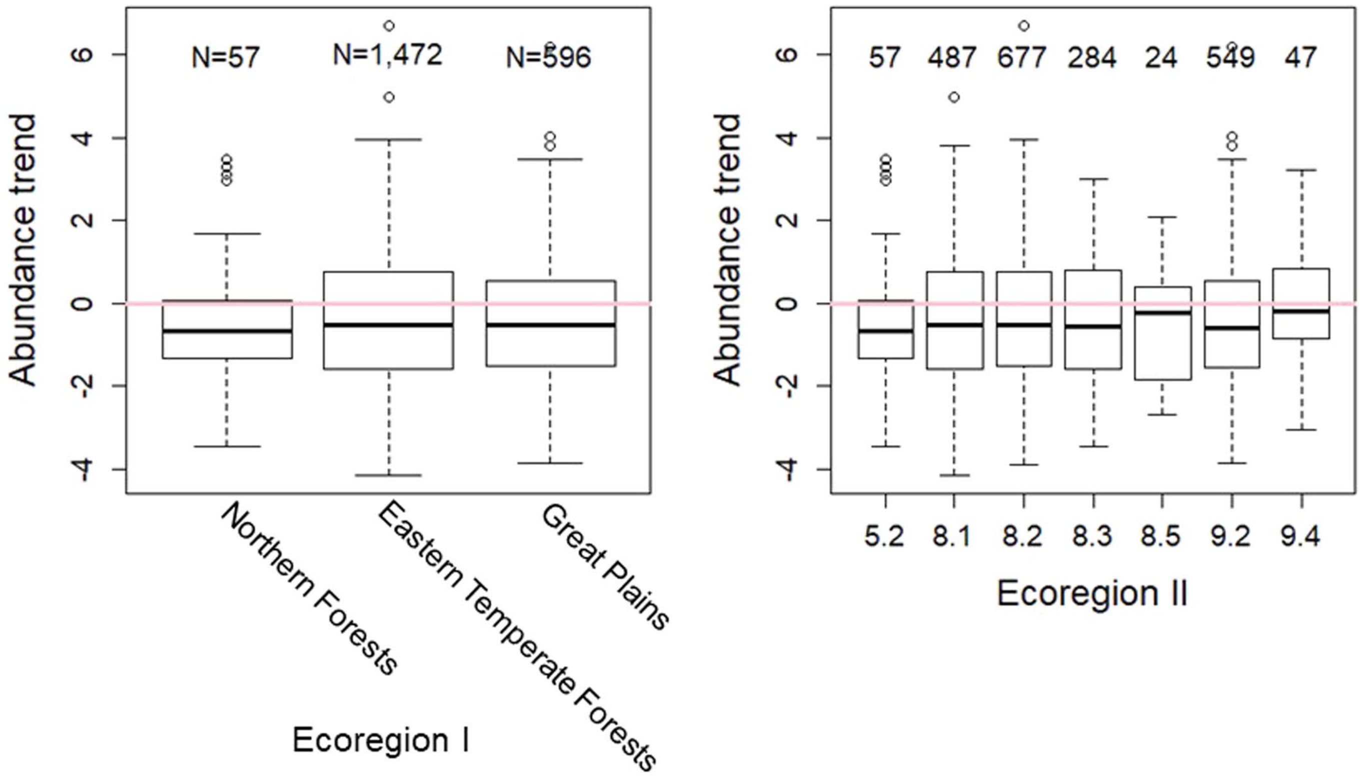
Extended Data Fig. 5 | Importance of explanatory variables in predicting time trends of arthropod abundance. Contribution of each variable to the accuracy of the Random Forests classifier, defined as the percent increase in Mean Square Error (decrease in accuracy) when the variable was excluded from decision trees.



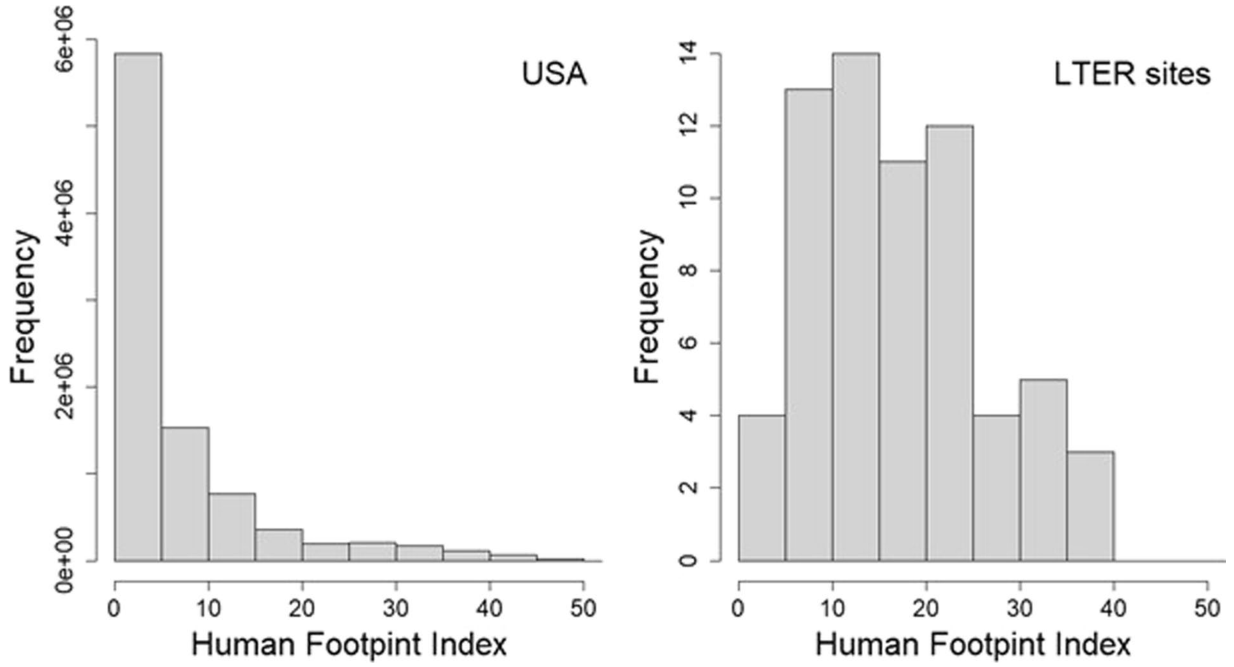
Extended Data Fig. 6 | Time trends in arthropod abundance, average among studies with similar start years. Abundance trends are averaged among LTERs where sampling start years were earlier than 1990, spanned 1990–2000, spanned 2000–2010, or were after 2010. Results were the same when trends were grouped according to final sampling years (except that no final sampling years predated 1990).



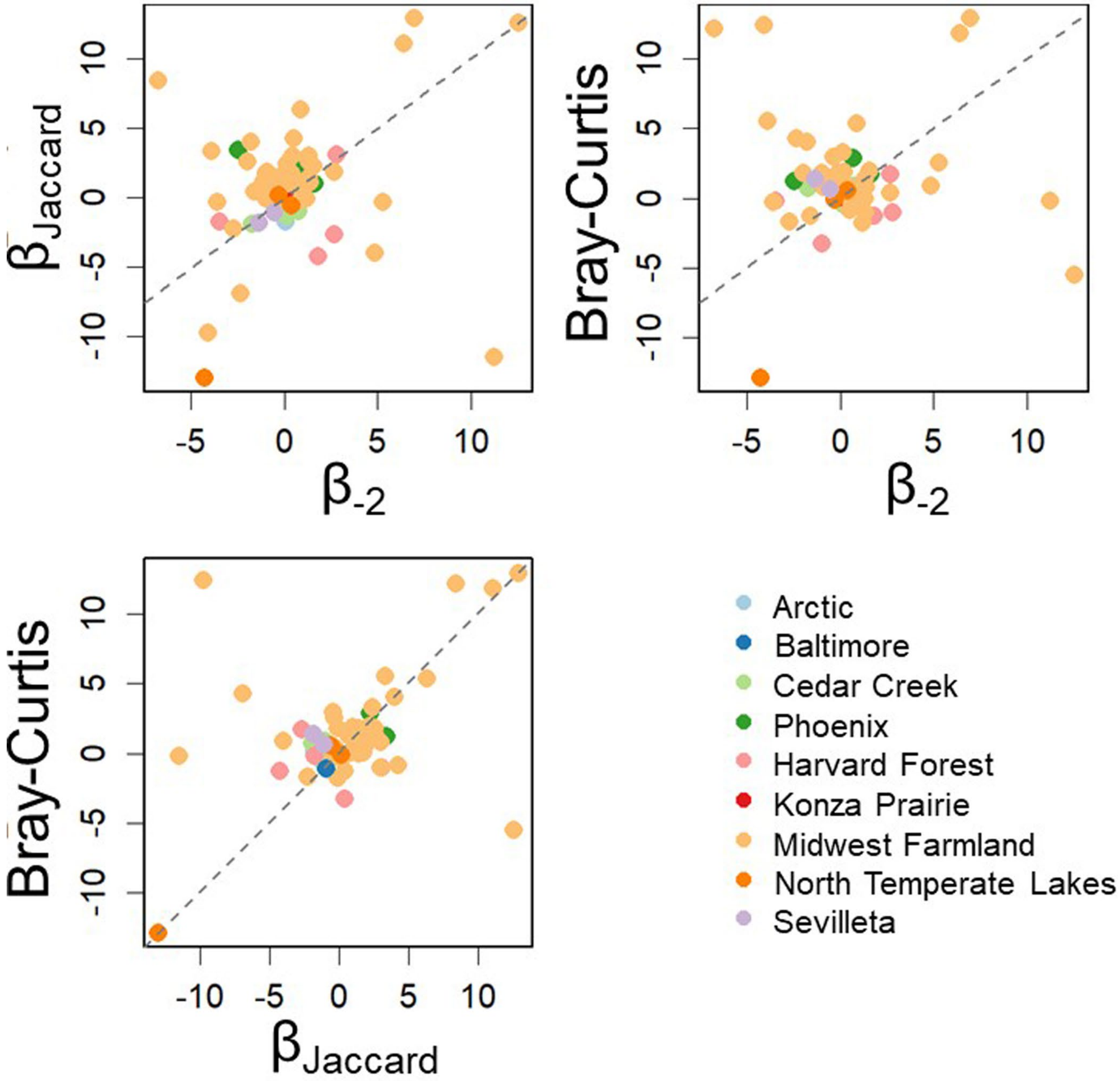
Extended Data Fig. 7 | Relationships among temporal trends in α diversity metrics. Dots represent the change over time of a diversity metric at an LTER site. Species evenness was calculated as Pielou's Evenness Index, and dominance represents the proportional frequency of the most abundant taxon. Light gray lines divide each plot into quadrants to help visualize sites where the sign of change in diversity metrics was similar (top right, bottom left) or opposite (top left, bottom right). Black dashes denote the line of best fit. Slopes are significant at the α = 5% level, R2 = 0.36 for evenness vs. richness, and R2 = 0.68 for evenness vs. dominance.



Extended Data Fig. 8 | Time trends in Midwest Farmland aphid abundance 2006–2019. Left panel depicts abundance trends separated by ecoregion level I. Right panel depicts abundance trends separated by ecoregion level II. Boxplots depict quantiles among LTER sites. Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers), and outliers (circles).



Extended Data Fig. 9 | Human Footprint Index values in the USA (left panel) and among LTER sites (right panel).



Extended Data Fig. 10 | Relationships among temporal trends in β diversity metrics. Dots represent the change over time of a diversity metric at an LTER site. The grey dashed line denotes the 1:1 line.

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n/a	Confirmed	
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☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All data used in this study are publicly available, and were collected as *.csv and *.txt files. Data sources are clearly defined in the manuscript methods and supplementary information files. Data curation was done in R 3.6.2. using custom code available in the supplementary information files.
Data analysis	All data analysis was done in R 3.6.2, using custom code (available in the supplementary information files) and R packages available in CRAN (as indicated in the manuscript)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data supporting the findings of this study (curated arthropod abundances and estimated time trends) are available at Dryad Data Repository https://doi.org/10.5061/dryad.cc2fqz645.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We utilized a geographically and taxonomically broad suite of relatively long-term datasets available through the U.S. National Science Foundation network of Long-Term Ecological Research (LTER) sites established in 1980 to examine trends in arthropod abundance and diversity.
Research sample	Altogether, our LTER arthropod abundance meta-dataset compiled 82,777 arthropod observations from 68 datasets into 5,375 taxa time series that spans up to 36 years and is comprised of 48 arthropod orders made up of 1 to 658 taxa in a given dataset.
Sampling strategy	All data were gathered from public LTER data repositories. The only restriction was that LTER data must contain records of arthropod abundance.
Data collection	Data were downloaded from public LTER data repositories as *.csv or *.txt files.
Timing and spatial scale	Data span 1975-2019, the entire U.S. (from Alaska to Georgia, New Hampshire to Arizona).
Data exclusions	Non-arthropod LTER data were not included, except for birds and fish when available alongside arthropod data.
Reproducibility	R code used to curate and analyze data are available in the supplementary information files.
Randomization	Randomization is not relevant to this study, because we are observing trends among sites over time.
Blinding	Blinding is not relevant to this study, because human subjects were not involved.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

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Materials & experimental systems

n/a	Involved in the study	
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology
☒	☐	Animals and other organisms
☒	☐	Human research participants
☒	☐	Clinical data

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
☒	☐	MRI-based neuroimaging

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https://doi.org/10.1038/s41559-021-01424-0

Studies of insect temporal trends must account for the complex sampling histories inherent to many long-term monitoring efforts

Ellen A. R. Welti[1]✉, Anthony Joern[2], Aaron M. Ellison[3], David C. Lightfoot[4], Sydne Record[5], Nicholas Rodenhouse[6], Emily H. Stanley[7] and Michael Kaspari[8]

ARISING FROM M. S. Crossley et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-020-1269-4 (2020).

Crossley et al.[1] examine patterns of change in insect abundance and diversity across US Long-Term Ecological Research (LTER) sites, concluding a "lack of overall increase or decline". This is notable if true, given mixed conclusions in the literature regarding the nature and ubiquity of insect declines across regions and insect taxonomic groups[2–6]. The data analysed, downloaded from and collected by US LTER sites, represent unique time series of arthropod abundances. These long-term datasets often provide critical insights, capturing both steady changes and responses to sudden unpredictable events. However, a number of the included datasets are not suitable for estimating long-term observational trends because they come from experiments or have methodological inconsistencies. Additionally, long-term ecological datasets are rarely uniform in sampling effort across their full duration as a result of the changing goals and abilities of a research site to collect data[7]. We suggest that Crossley et al.'s results rely on a key, but flawed, assumption that sampling was collected "in a consistent way over time within each dataset". We document problems with data use prior to statistical analyses from eight LTER sites due to datasets not being suitable for long-term trend estimation and not accounting for sampling variation, using the Konza Prairie (KNZ) grasshopper dataset (CGR022) as an example.

Unsuitable datasets to estimate long-term observational trends

Several of the LTER datasets included in Crossley et al. document experiments that either have confounding treatment effects or are too variable in sampling methods to allow for comparison of samples across time. Additionally, in one case, lepidopteran outbreak dynamics with long intervals (10–13 years) at Hubbard Brook limit the power to detect meaningful trends without extremely long-term data[8]. Datasets from Cedar Creek include arthropods collected in plots with nitrogen addition, herbivore exclosures and manipulated plant diversity. All three of the datasets from Harvard Forest included in Crossley at al.'s analysis have large methodological inconsistencies over time and one dataset documents ants collected in a canopy manipulation experiment, including one treatment where trees were girdled to simulate hemlock woolly adelgid (*Adelges tsugae*) infestation of the hemlock trees years prior to the arrival of the invasive insect to the area. One dataset from North Temperate Lakes documents the responses of two crayfish species in a lake where one species was being experimentally removed. With a few exceptions for partial components of these datasets (for example, control plots in the arce153 Cedar Creek dataset), these data are inappropriate for estimation of long-term observational species trends.

Not accounting for sampling variation and Konza grasshoppers as a case in point

The KNZ CGR022 dataset documents grasshopper species abundances on 15 KNZ watersheds and spans 1982 to present (up to 2015 included in Crossley et al.). Crossley et al. analyse time series of individual species from each dataset (the number of 'time trends' in their Table 1). However, regardless of variant sampling effort, they regularly sum all individuals within LTER datasets to yield a single value of abundance for a given species and year. This is the case for KNZ grasshoppers and most other included datasets (number of 'sites' in their Table 1). Importantly, sampling effort at KNZ and other LTER sites was not constant. At KNZ, variation occurred in the number of samples per watershed and the number of watersheds in which grasshoppers were collected per year (Fig. 1). Most notably, six bison-grazed watersheds were added to KNZ sampling in 2002. Changes in sample numbers over time are documented in the online metadata (http://lter.konza.ksu.edu/content/cgr02-sweep-sampling-grasshoppers-konza-prairie-lter-watersheds).

Not accounting for sampling effort and data structure causes errors in trend estimates (see also Supplementary Information and Supplementary Fig. 1). At KNZ, bison-grazed watersheds support higher grasshopper abundances and species richness[9,10]. In a recent analysis using the CGR022 dataset, to account for this change in sampling effort, only data collected in the same years from watersheds were combined (for example, by splitting samples from grazed watersheds into a separate time series) and abundances within each watershed and year were divided by the number of samples. Analysis of the data structured in this way showed a >2% annual decline in grasshopper abundance, with only one common species increasing[11]. Crossley et al., in contrast, report that most grasshopper species increased in abundance from 1982 to 2015. Crossley et al.

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[1]Senckenberg Research Institute and Natural History Museum Frankfurt, Gelnhausen, Germany. [2]Division of Biology, Kansas State University, Manhattan, KS, USA. [3]Harvard Forest, Harvard University, Petersham, MA, USA. [4]Museum of Southwestern Biology, Biology Department, University of New Mexico, Albuquerque, NM, USA. [5]Department of Biology, Bryn Mawr College, Bryn Mawr, PA, USA. [6]Biological Sciences, Wellesley College, Wellesley, MA, USA. [7]Center for Limnology, University of Wisconsin-Madison, Madison, WI, USA. [8]Geographical Ecology Group, Department of Biology, University of Oklahoma, Norman, OK, USA. ✉e-mail: ellen.welti@senckenberg.de

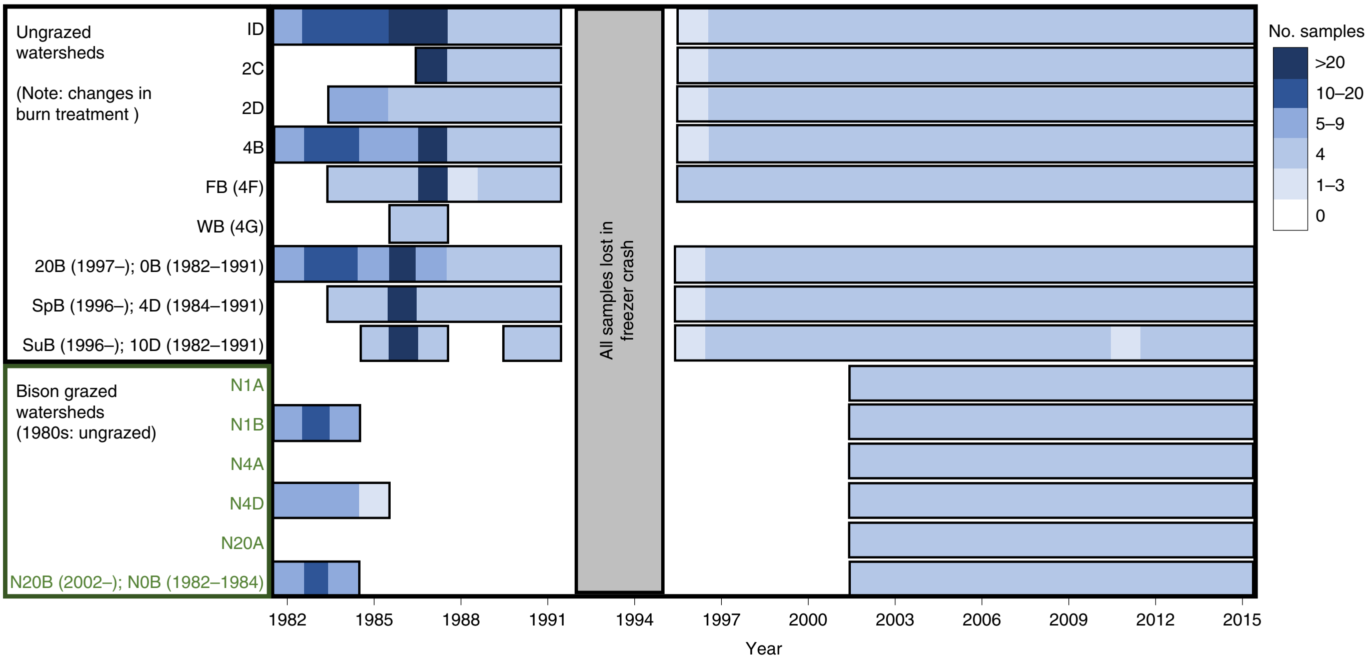

Fig. 1 | The complex history of sampling of the KNZ grasshopper dataset. The KNZ grasshopper dataset (CGR022) exhibits high variance both in number of watersheds sampled per year (number of bars per year) and number of samples collected within each watershed each year (depicted in colour). Other complexities include the tragic loss of four years (1992–1995) of sampling due to a freezer crash, changes in sampling month, changes in watershed burn frequencies and the reintroduction of bison in the 1990s to six of the later-sampled watersheds.

note the discrepancy with both this study[11] and another[3], and suggest it is "driven by falling numbers of just two once-dominant species…whereas many other formerly rare species have become more abundant and both evenness and species richness have increased". However, we believe the discrepancy arises because Crossley et al. did not account for variable sampling effort, including KNZ's incorporation of additional, more diverse grazed habitats midway in the time series. Similar errors, where data structure was not accounted for, are evident in 17 of the 19 datasets that we examined and were included in Crossley et al.'s results.

Conclusion

We have thus far been able to confirm issues with data from 8 of the 13 LTER sites (comprising 60% of Table 1's 'time trends') included in Crossley et al. We note that this is not a comprehensive assessment, as we have included errors only from datasets for which either we ourselves are the principal investigators or we have been able to confirm with the corresponding LTER principal investigators and information managers. The eight sites are: Baltimore, Cedar Creek, Central Arizona–Phoenix, Harvard Forest, Hubbard Brook, Konza Prairie, North Temperate Lakes and Sevilleta. We provide details on dataset unsuitability, mistakes in not accounting for sampling effort and several coding errors in the Supplementary Information.

Given these mistakes, we urge scepticism regarding Crossley et al.'s general conclusion of no net decline in insect abundances at US LTER sites in recent decades. Although their goal is laudable, both the use of unsuitable datasets and not taking sampling effort into account generate erroneous estimates of population change. Recently, a study reporting widespread collapse of rainforest insect populations at the LTER Luquillo site necessitated a similar correction[5]. We echo those authors, when they suggest that scientists can avoid errors by reading corresponding metadata. Contacting the data providers/field biologists in advance (or even including them as authors) is an additional good practice that ensures appropriate use of the data. Like the ecology they document, it is important to take into account that long-term monitoring efforts by LTERs and similar institutions are themselves complex and full of history.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

KNZ grasshopper abundance data are available from the Long-Term Ecological Research Data Portal (https://doi.org/10.6073/pasta/7b2259dcb0e499447e0e11dfb562dc2f). Citations for the additionally described LTER datasets are provided in the Supplementary Information.

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Author contributions

E.A.R.W., S.R., A.J. and M.K. conceived the idea for the paper. E.A.R.W. wrote the first draft. A.M.E., D.C.L., S.R., N.R. and E.H.S. identified further errors in the Crossley et al. online data. All authors significantly contributed to revisions.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41559-021-01424-0.

Correspondence and requests for materials should be addressed to E.A.R.W.

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Corresponding author(s): Ellen A. R. Welti

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☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
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☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
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☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Software and code

Policy information about availability of computer code

Data collection	N/A
Data analysis	N/A

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Policy information about availability of data

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

No new data was collected. Links to the datasets referred to are included in the Supplementary Information.

Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	A re-evaluation of the analysis of Crossley et al. 2020.
Research sample	No new data was collected. Links to the datasets referred to are included in the Supplementary Information.
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	Links to the datasets and their metadata are listed in the Supplementary Information.
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☒ No

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Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

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M. S. Crossley et al. reply

Michael S. Crossley[1]✉, William E. Snyder[1] and Matthew D. Moran[2]

REPLYING TO E. A. R. Welti et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-021-01424-0 (2021)
REPLYING TO M. Desquilbet et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-021-01427-x (2021)

Our recent study showing no broad-scale declines in arthropods at Long Term Ecological Research (LTER) sites across the USA has garnered critiques from peers. Welti et al.[1] note that the LTER sites have complex management histories, and describe instances where we failed to correct for changes in sampling intensity through time. Using their critique as a guide, we re-curated the LTER metadata to maintain per-sample (for example, per sweep, pitfall trap and so on) arthropod numbers. We then repeated the analyses of abundance and biodiversity trends for the arthropod taxa and sites described in Crossley et al.[2], using several different approaches to generate trends for each taxon and site. Results were generally similar to our original findings, with broad variation in abundance and biodiversity trends among taxa and sites, but no clear overall pattern of abundance or biodiversity changes, supporting our original conclusion that LTER data do not show evidence of an "insect apocalypse". Desquilbet et al.[3] raise additional concerns that pertain to two aspects of our original study: selection criteria for studies included; and analysis. We find that the criticism of time series included is unwarranted, because the data from the Midwest Suction Trap Network are curated by an LTER and our inclusion of non-insect arthropods was intentional and clearly stated in the manuscript. The criticisms of our analysis are more substantial, but mostly represent ongoing debate on how to analyse time series data and what criteria should be utilized to include a time series. We note that within our publication, we address this uncertainty in several places and point out that changing criteria for time series inclusion has little effect on our results. We find that the criticisms of Desquilbet et al.[3] raise some important questions, but mostly reflect differences in opinion and not substantial flaws in our analysis and interpretation.

Welti et al.[1] begin their critique by noting that sampling intensity varied at several LTERs through time, and that Crossley et al.[2] failed to account for these changes when summing to generate taxon abundance trends. They also noted one instance (crayfish in North Temperate Lakes) where a coding error removed several time trends from analysis. We re-curated the metadata to correct these errors. Then, we repeated abundance and biodiversity trend analyses after collapsing abundances into a single trend per taxon (Methods). We found broad variability among taxa and sites in whether arthropods were decreasing or increasing through time (Fig. 1a), yielding a net trend whose distribution overlapped with zero (Fig. 1b). Likewise, sites varied in whether the various biodiversity metrics were showing gains or declines, yielding no net directional trend across sites (Fig. 2a). These results are generally consistent with the findings of Crossley et al.[2].

Second, Welti et al.[1] note that a simple summing across subplots at each LTER site may have masked important plot-specific differences in abundance trends that, had they been considered, would have revealed general arthropod declines. To address this possibility, we calculated separate abundance trends for each species, at each subsite, before averaging these values to arrive at the single species-specific abundance trend per site. The revised dataset contained many more subsites within each main site, such that the overall number of abundance trends considered in the meta-analysis increased ~80%. We also separately calculated biodiversity trends for each subsite. Here again, results were broadly consistent with those in Crossley et al.[2], with broad variability in trends by species and site but no clear overall directional trend (Figs. 1c,d and 2b).

Third, Welti et al.[1] suggest that three datasets are not appropriate for estimation of long-term arthropod trends because they involve observations made in experimentally manipulated plots (Cedar Creek arthropod sweep datasets acre153 and aage120) or physical removal of the focal taxon (North Temperate Lakes crayfish dataset knb-lter-ntl.217.9). While we consider these instances to be a special case where drivers of arthropod abundance change are potentially well known and not grounds to exclude trend data from our meta-analysis, a reanalysis of abundance trends after excluding these data did not change overall results (Extended Data Fig. 1).

Finally, since our paper was published, Didham et al.[4] have suggested that time series that include at least 10 points may provide the most reliable measure of arthropod abundance change through time. Thus, we again repeated our analyses using only those subsites that included 10 or more data points. This process removed one LTER site (Baltimore) altogether, and several subdatasets at particular sites (Fig. 1e). However, the general patterns were similar to those generated with other data treatments, with variability in abundance (Fig. 1f) and biodiversity (Extended Data Fig. 2) trends but no clear overall directional change across sites. Despite a consistent finding of broad variability in trends, we note that a proportion of abundance trends changed sign after standardizing taxa counts by sampling intensity and accounting for subsite structure (Fig. 3). However, a roughly equal proportion of changing trends switched from positive to negative and vice versa, suggesting no upward bias in abundance trend estimates in our original analysis (for example, 19% and 17% changed to decreasing or increasing, respectively, after accounting for sampling effort).

A key challenge when searching for evidence of recent declines in many animal and plant groups is that it often is necessary to rely on data collected for other purposes. We acknowledge that there may be longer-term periodicity in arthropod trends, as elegantly described at several of the LTER sites[5,6], that make it difficult to isolate any recent arthropod declines. However, autoregressive models are capable of detecting general declines embedded within data that show periodicity[7]. Last, we found that net abundance trends across our many different analysis approaches were consistently weakly

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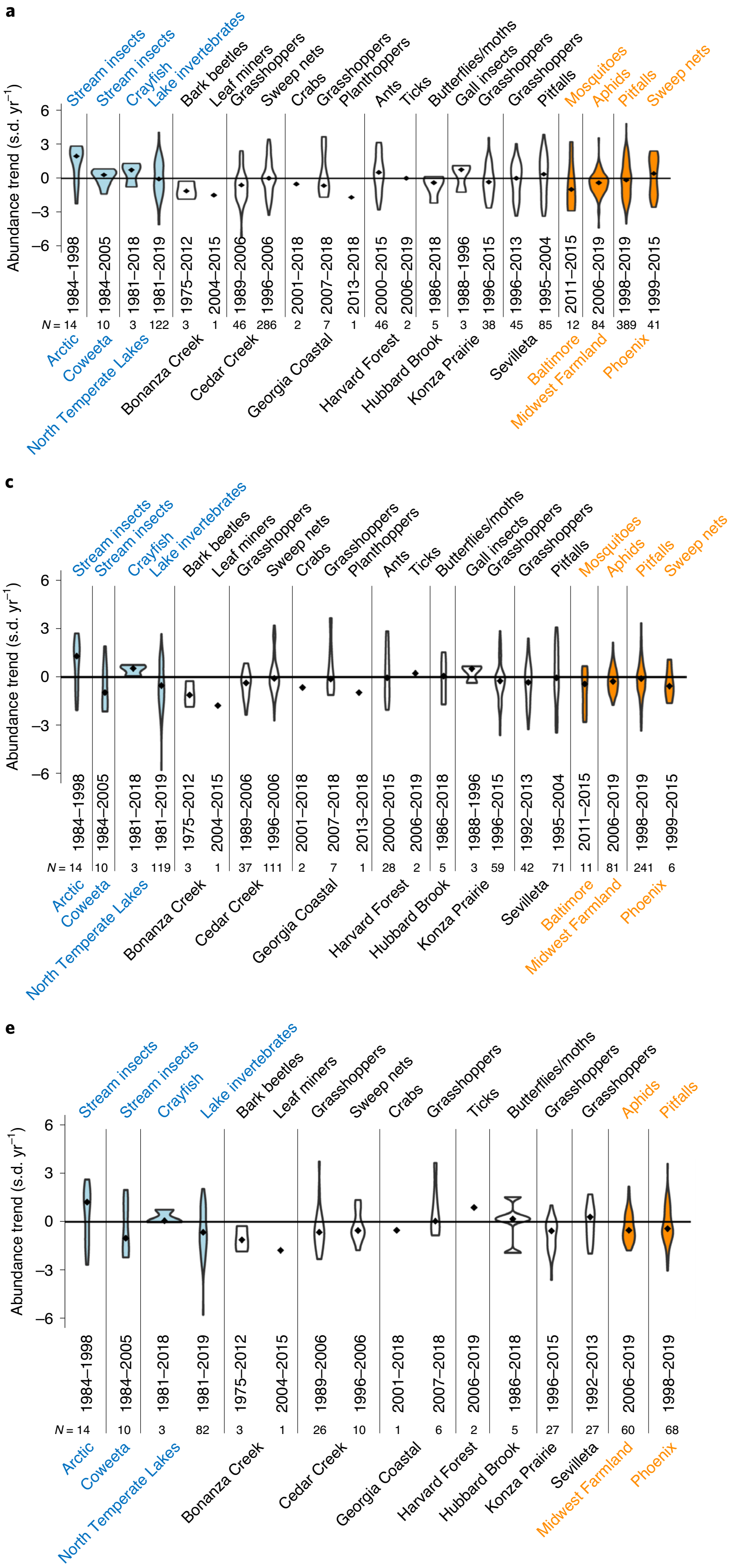
[1]Department of Entomology, University of Georgia, Athens, GA, USA. [2]Department of Biology and Health Sciences, Hendrix College, Conway, AR, USA.
✉e-mail: michael.crossley@uga.edu


a
Stream insects
Stream insects
Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Planthoppers
Ants
Ticks
Butterflies/moths
Gall insects
Grasshoppers
Grasshoppers
Pitfalls
Mosquitoes
Aphids
Pitfalls
Sweep nets
Abundance trend (s.d. yr^{-1})
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Konza Prairie
Sevilleta
Baltimore
Midwest Farmland
Phoenix
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Avg. abundance trend
1.5
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LTER
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Stream insects
Stream insects
Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Planthoppers
Ants
Ticks
Butterflies/moths
Gall insects
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Abundance trend (s.d. yr^{-1})
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Sevilleta
Baltimore
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Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Ticks
Butterflies/moths
Grasshoppers
Grasshoppers
Aphids
Pitfalls
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North Temperate Lakes
Bonanza Creek
Cedar Creek
Georgia Coastal
Harvard Forest
Hubbard Brook
Konza Prairie
Sevilleta
Midwest Farmland
Phoenix
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Avg. abundance trend
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−1.0
−1.5
LTER

Fig. 1 | Time trends in arthropod abundance among LTERs. **a**, Violin plots showing the distribution of abundance trends per taxon, where abundances were standardized by sampling effort before trend estimation. **b**, Average trend in abundance and 95% confidence intervals from **a** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($P = 0.55$). **c**, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation. **d**, Average trend in abundance and 95% confidence intervals from **c** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($P = 0.11$). **e**, Violin plots showing the distribution of abundance trends per taxon, averaged across subsites, where abundances were standardized by sampling effort before trend estimation, and time series with <10 data points were excluded. **f**, Average trend in abundance and 95% confidence intervals for **e** when trends are averaged among LTERs (d.f. = 11). Mean time trends were not significantly different from zero ($P = 0.28$). In **a**, **c** and **e**, the black diamonds within boxplots depict medians. The first and last years of LTER studies as well as the number of taxa time series are included below the violin plots. Blue shading and font indicate LTER sites reporting aquatic taxa. Orange shading and font indicate LTER sites in urban or agricultural landscapes. Unfilled violin plots and black font indicate LTER sites reporting terrestrial taxa in relatively less human-disturbed habitats.

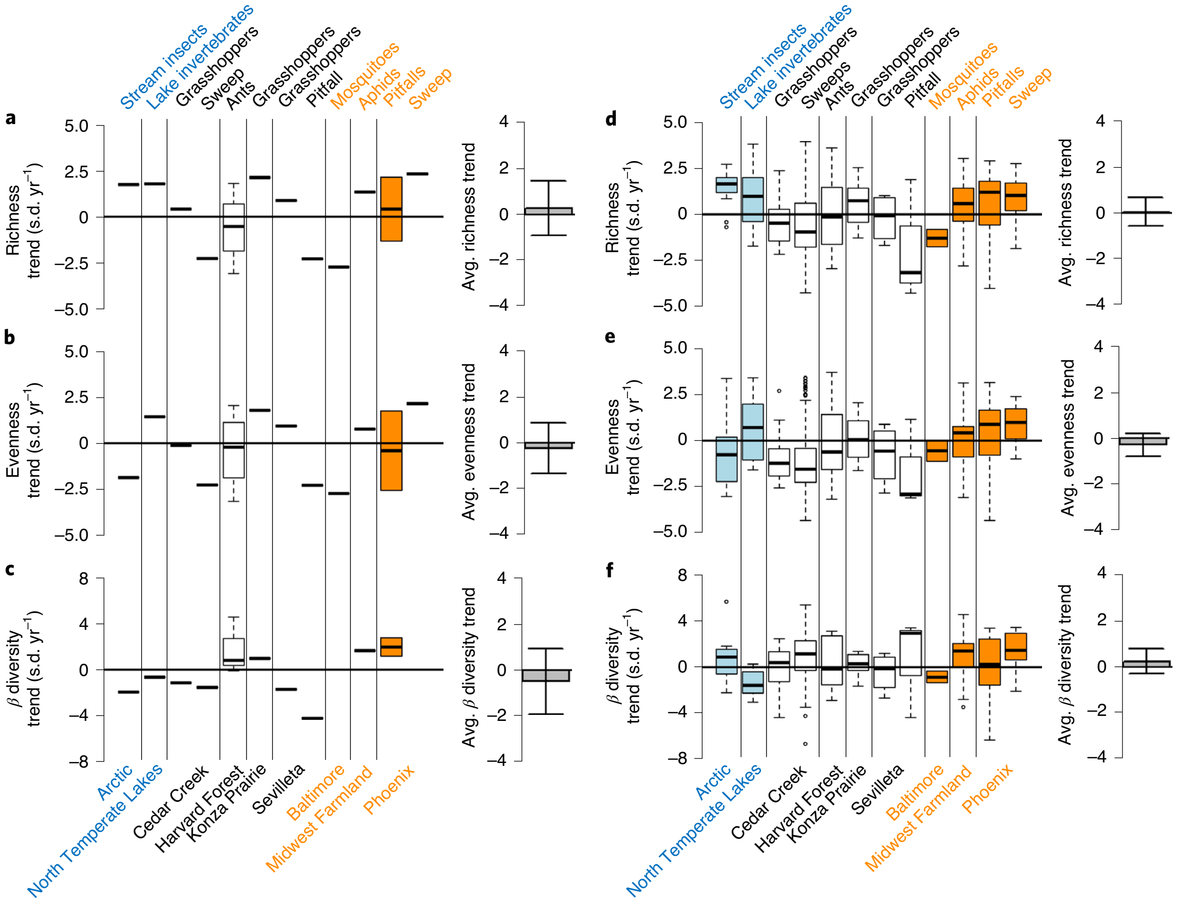

Fig. 2 | Time trends in arthropod diversity among LTERs. **a–c**, Diversity trends estimated using the dataset where arthropod abundances were standardized by sampling effort, and time series with <4 data points were excluded. **d–f**, Diversity trends estimated using the dataset where arthropod abundances were standardized by sampling effort and separated by subsite, and time series with <4 data points were excluded. **a**,**d**, Trends in taxon richness (rarefied). **b**,**e**, Trends in taxon evenness (Pielou's index). **c**,**f**, Trends in β diversity (1 − Jaccard similarity index). Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers) and outliers (circles). The right panels depict average change in diversity metrics and 95% confidence intervals among datasets (d.f. = 11). Time trends were not significantly different from zero at $\alpha = 5\%$. See the caption of Fig. 1 for a description of the coloured text.

negative (although statistically non-significant), suggesting that there may be some underlying general decline trend at LTER sites, but that the data are too few, and too variable, to clearly reveal it on their own. Interestingly, richness, evenness and β diversity patterns showed no evidence of any overall trend (positive or negative), providing little evidence of widespread biodiversity loss over time.

Desquilbet et al.[3] begin their critique by proposing four modifications to the analysis of Crossley et al.[2]. First, Desquilbet et al.[3] suggest a minimum time series length criterion of 16 years, citing White[8]. However, White[8] prescribes no single threshold, emphasizing that "More importantly, however, there is wide distribution of estimated minimum times. Therefore, it is not wise to use a simple threshold number of years in monitoring design". In addition, White[8] states that "Approximately, 72% of the [822] populations required at least 10 years of monitoring.", which is in line with recent recommendations for analysing insect population trends[4], and

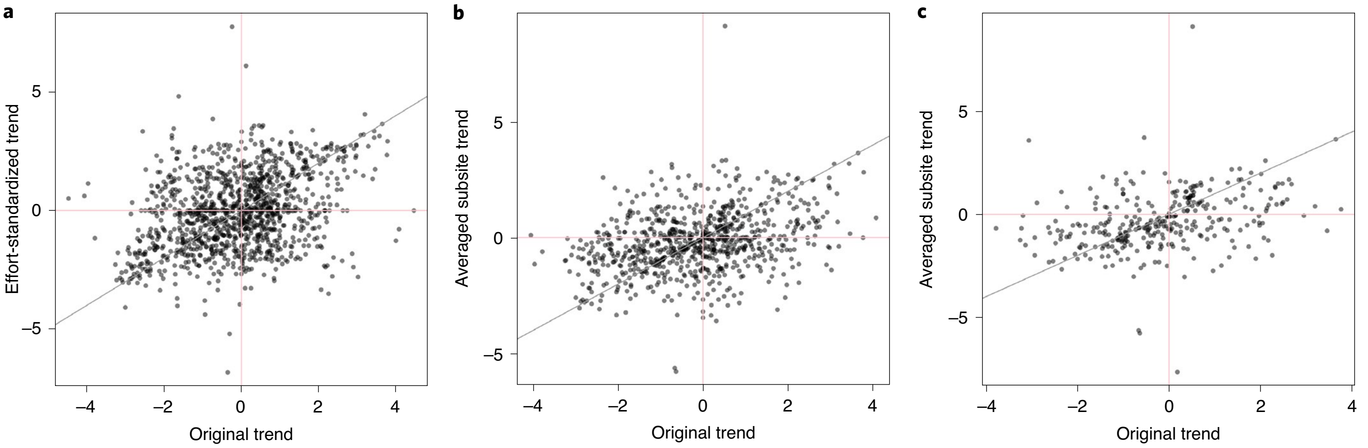

Fig. 3 | Comparison of abundance trends per taxon between original and updated datasets. a, Comparison of abundance trends per taxon, where abundances were standardized by sampling effort before trend estimation. **b**, Comparison of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation. **c**, Comparison of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation, and time series with <10 data points were excluded. Pink lines divide plots into quadrants, and grey lines depict 1:1 relationships.

which, through our reanalysis conducted in response to Welti et al.[1], we demonstrated did not alter the conclusions of Crossley et al.[2]. We also mention in the paper that restricting the analysis to only those time series that are more than 4, 8 or 15 years has relatively little effect on the results and their interpretation. Second, Desquilbet et al.[3] recommend using zero-inflated models over linear regression of log-transformed counts for estimating trends. While we appreciate this suggestion, we emphasize that there are genuine differences of opinion about how zero counts are handled in ecological data, and that log transformation is still widely accepted[9,10]. Third, Desquilbet et al.[3] recommend accounting for imperfect detection in ecological count data, illustrating their point with a large apparent increase observed for the aphid *Aphis asclepiadis* in a site where the first years of the time series reported zeros. These cases were uncommon (occurring in 195 of the 5,375 trends), and were similarly likely to contribute to large apparent declines (occurring in 109 of the 5,375 trends), among time series in Crossley et al.[2], and enforcing of stricter criteria for inclusion of time series, as done in Crossley et al.[2] and in response to Welti et al.[1], suggests that these cases did not alter the original conclusions of Crossley et al.[2]. Fourth, Desquilbet et al.[3] caution that summarizing many abundance trends drawn from different populations using violin plots, medians or means is statistically inappropriate. While we generally agree that summarizing a wealth of informative trends using a few summary statistics is not ideal, there is an understandable desire to summarize overall trends in insect abundance time series to allow some degree of comparability among studies. We stand by our original visual summary of abundance trends using violin plots, and note that this does not meaningfully diverge from other meta-analyses[11]. Last, Desquilbet et al.[3] note that analyses of diversity trends shared the same issues as analyses of abundance trends. Again, in response to Welti et al.[1] we show that reanalysis using stricter criteria does not change the original conclusions of Crossley et al.[2].

Next, Desquilbet et al.[3] raise three concerns with the inclusion of certain arthropod time series in our analysis. First, they note that a large portion of arthropod abundance trends were derived from aphid species documented by the Midwest Suction Trap Network. While this is clearly acknowledged by Crossley et al.[2], the implication that these data primarily represent pests that benefit from intensive agriculture is unfounded on two counts. First, the majority of aphid species (52 out of 96) documented by the Midwest Suction Trap Network do not feed on crops. Second, aphids do not benefit from intensive agriculture, which, to the contrary, includes in its definition the extensive use of insecticides often applied specifically to kill aphids. Rather than bias our analysis toward detection of more increasing abundance trends, inclusion of data from the Midwest Suction Trap Network introduced a substantial proportion of decreasing abundance trends, as the aphid populations monitored by the suction trap network in the Midwest appear to have been largely in decline since the early 2000s. Desquilbet et al.[3] also state that the Midwest Suction Trap Network is not an LTER. However, the data from these monitoring programmes have been included in Kellogg Biological Station's LTER datasets that are publicly available.

Desquilbet et al.[3] conclude their critique by noting that 9% of the time series were of non-insect arthropods or included both insects and other arthropods, and that they therefore should have been excluded. While we understand this sentiment, we emphasize that we clearly state throughout Crossley et al.[2] that we intentionally included all arthropods that met our sampling criteria, not just insects. This was in part to obtain data on aquatic arthropods, which often include large numbers of crustaceans.

As an aside, Desquilbet et al.[3] note that they had to re-program the R script provided in Crossley et al.[2] to make it run. In Crossley et al.[2], we state in the Code availability statement that the R code used to curate and analyse data is available at the Dryad Data Repository (https://doi.org/10.5061/dryad.cc2fqz645). We made this R code publicly available so that interested researchers could see how we handled the arthropod count data used in Crossley et al.[2]. We note that part of the issue in repeatability probably stems from unavoidable differences in how original data were compiled from online repositories, and that we have provided updated R code in response to Welti et al.[1] that we are able to run.

In conclusion, we disagree with Desquilbet et al.[3] that the issues raised about data selection and analysis invalidate the original conclusion of Crossley et al.[2] that the available data reveal no evidence of consistent, general abundance and biodiversity decline that might be expected were a dramatic "insect apocalypse" impacting the LTER sites.

Methods

Abundance trends. Using the critique provided by Welti et al.[1] as a guide, we re-curated all taxa abundance time series using four different approaches, standardizing arthropod abundances by sampling effort (definition varies among datasets) in all four. A detailed description of curation changes for each dataset and responses to critiques provided in the Supplementary Information of Welti et al.[1] is provided in Supplementary Table 1, and R code used for curation and analysis is provided on GitHub (https://github.com/mcrossley3/insectLTER). In the first approach, we summed arthropod abundances per taxon per year, standardizing abundances by associated sampling effort (for example, arthropods per sweep, aphids per day). As in the original analysis of Crossley et al.[2], we excluded time series with <4 data points. In the second approach, we further separated effort-standardized arthropod abundances by subsite (for example, watershed in Konza Prairie, experimental plot in Cedar Creek), again excluding any time series with <4 data points. In the third approach, we again separated effort-standardized arthropod abundances by subsite and excluded any time series with <4 data points, but we further removed data from subsites that were identified as inappropriate for estimation of long-term arthropod abundance trends because they involved experimental manipulation of plots (Cedar Creek arthropod sweep datasets acre153 and aage120) or physical removal of the focal taxon (North Temperate Lakes crayfish dataset knb-lter-ntl.217.9). For the Cedar Creek arthropod sweep datasets, we retained for analysis only observations from "control" plots (no exclosures, unfertilized, unburned, no experimental plant seeding). For the North Temperate Lakes crayfish dataset involving removal of rusty crayfish (*Orconectes rusticus*), we retained only data for *O. virilis*. In the fourth approach, we again separated effort-standardized arthropod abundances by subsite, but this time excluded any time series with <10 data points, following the minimum time series length recommended by Didham et al.[4] for arthropod time series analysis. For the two analyses that separated abundance time series by subsite, counts in the Konza Prairie grasshopper dataset were curated in a similar fashion to Welti et al.[5]. Specifically, counts pre-1996 were excluded, and mean abundance trends per species were separated by grazed and ungrazed treatment.

Diversity trends. Using the re-curated dataset where arthropod counts were standardized by sampling effort and/or time series were separated by subsite, we recalculated diversity metrics (rarefied richness, evenness, β diversity) and estimated time trends using the same approach as in Crossley et al.[2]. Richness was rarefied using a minimum sample that was calculated on the basis of the 0.1 quantile per dataset (or the 0.2 quantile when the 0.1 quantile was substantially smaller than the number of species reported in a dataset). As effort-standardized arthropod counts were «1 for three datasets (Midwest aphids, Central Arizona–Phoenix sweep and pitfall2), precluding richness rarefaction, standardized abundances were multiplied by a constant (20, 20 and 10, respectively). Effort-standardized abundances for these datasets could thus be interpreted as aphids per 20 days, arthropods per 20 sweeps and arthropods per 10 traps, respectively.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All curated data used for analyses in this study are available on GitHub (https://github.com/mcrossley3/insectLTER).

Code availability

R code used to curate and analyse data in this study is available on GitHub (https://github.com/mcrossley3/insectLTER).

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Author contributions

M.S.C., W.E.S. and M.D.M. conceived of the idea of this study. M.S.C. conducted formal analysis. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-021-01429-9.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41559-021-01429-9.

Correspondence and requests for materials should be addressed to M.S.C.

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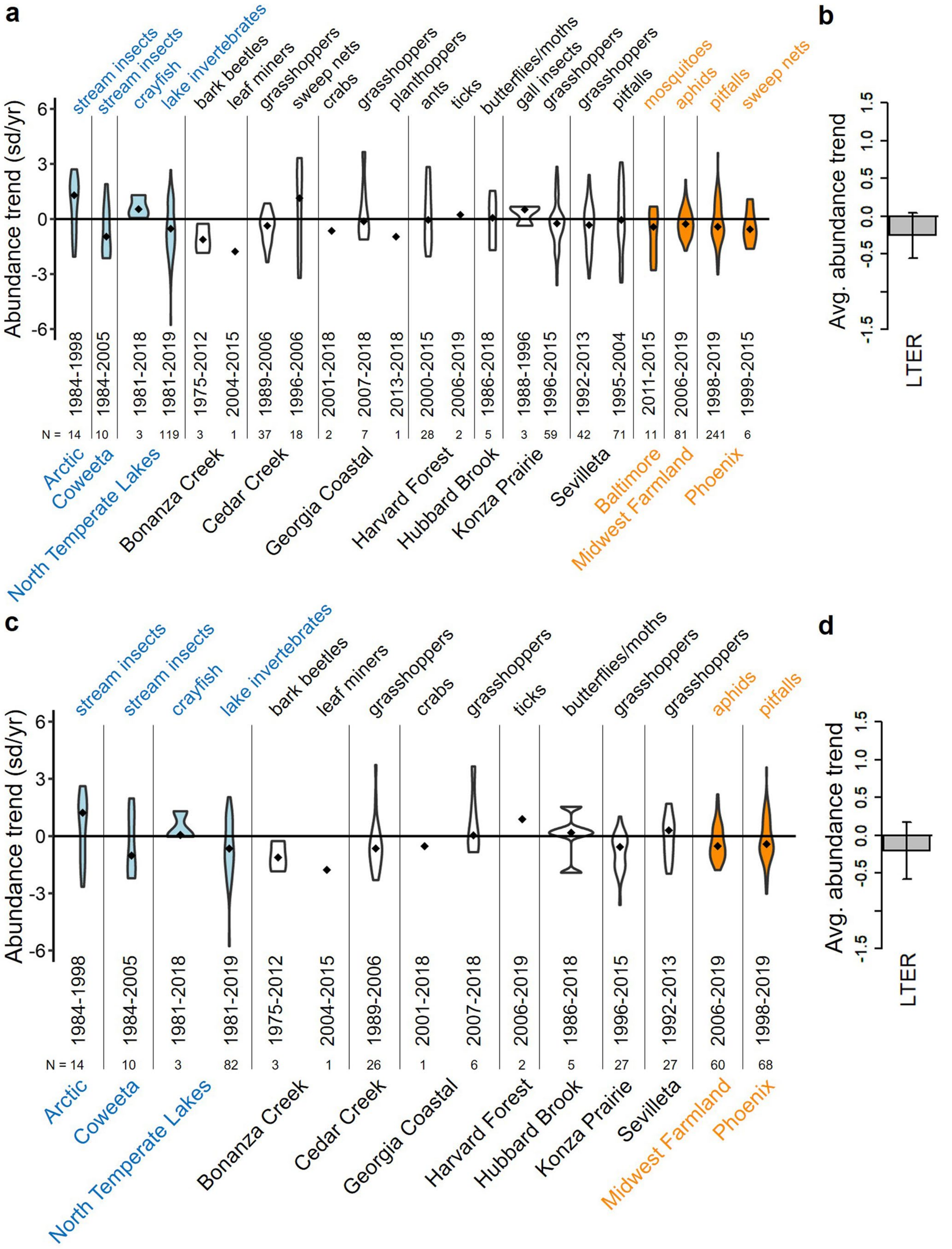
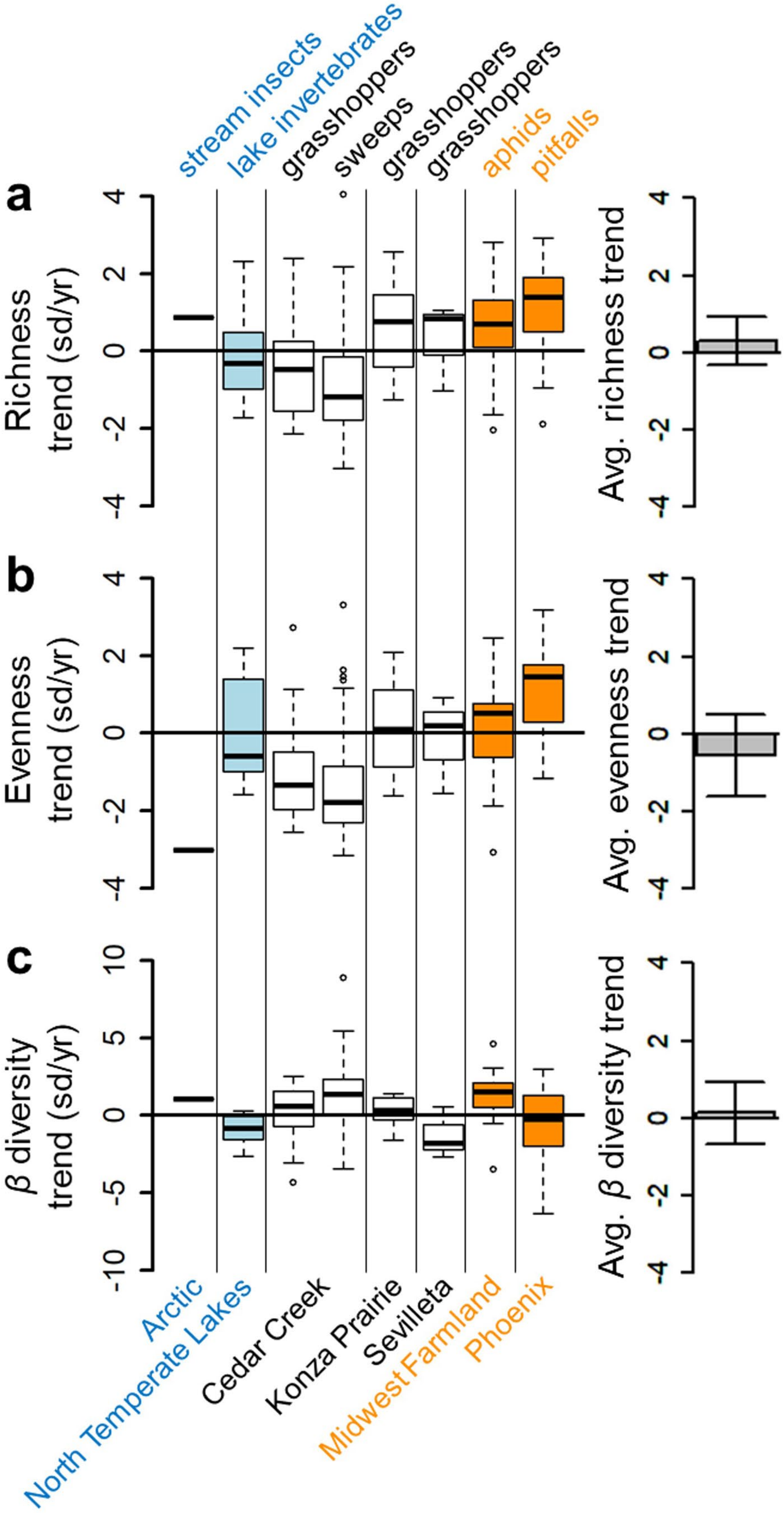


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Effort-standardized time trends in arthropod abundance among LTER subsites. a, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite prior to trend estimation, and trends with < 4 data points were excluded. This analysis differs from that depicted in Fig. 1c,d in that trends associated with experimental treatments in the Cedar Creek sweep nets and North Temperate Lakes crayfish datasets were excluded from analysis. **b**, Average trend in abundance and 95% confidence intervals from **a** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($p = 0.10$). **c**, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite prior to trend estimation, and trends with < 10 data points were excluded. This analysis differs from that depicted in Fig. 1e,f in that trends associated with experimental treatments in the Cedar Creek sweep nets and North Temperate Lakes crayfish datasets were excluded from analysis. **d**, Average trend in abundance and 95% confidence intervals from **c** when trends are averaged among LTERs (d.f. = 11). Mean time trends were not significantly different from zero ($p = 0.27$).



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Effort-standardized time trends in arthropod diversity among LTER subsites. Time trends in arthropod diversity among LTERs, using the dataset where abundances were standardized by sampling effort and separated by subsite, and time series with < 10 data points were excluded. **a**, Trends in taxon richness (rarefied). **b**, Trends in taxon evenness (Pielou's Index). **c**, Trends in β diversity (1-Jaccard Similarity Index). Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers), and outliers (circles). Right panels depict average change in diversity metrics and 95% confidence intervals among datasets (d.f.=7). Time trends were not significantly different from zero at α=5%. Please refer to Fig. 1 legend for description of colored text.

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Software and code

Policy information about availability of computer code

Data collection	All data used in this study are publicly available, and were collected as *.csv and *.txt files. Data sources are clearly defined in the manuscript methods and supplementary information files. Data curation was done in R 3.6.2. using custom code available in the supplementary information files.
Data analysis	All data analysis was done in R 3.6.2, using custom code (available in the supplementary information files) and R packages available in CRAN (as indicated in the manuscript)

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Data supporting the findings of this study (curated arthropod abundances and estimated time trends) are available at Dryad Data Repository https://doi.org/10.5061/dryad.cc2fqz645.

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Study description	We utilized a geographically and taxonomically broad suite of relatively long-term datasets available through the U.S. National Science Foundation network of Long-Term Ecological Research (LTER) sites established in 1980 to examine trends in arthropod abundance and diversity.
Research sample	Altogether, our LTER arthropod abundance meta-dataset compiled 82,777 arthropod observations from 68 datasets into 5,375 taxa time series that spans up to 36 years and is comprised of 48 arthropod orders made up of 1 to 658 taxa in a given dataset.
Sampling strategy	All data were gathered from public LTER data repositories. The only restriction was that LTER data must contain records of arthropod abundance.
Data collection	Data were downloaded from public LTER data repositories as *.csv or *.txt files.
Timing and spatial scale	Data span 1975-2019, the entire U.S. (from Alaska to Georgia, New Hampshire to Arizona).
Data exclusions	Non-arthropod LTER data were not included, except for birds and fish when available alongside arthropod data.
Reproducibility	R code used to curate and analyze data are available in the supplementary information files.
Randomization	Randomization is not relevant to this study, because we are observing trends among sites over time.
Blinding	Blinding is not relevant to this study, because human subjects were not involved.

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Methods

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Adequate statistical modelling and data selection are essential when analysing abundance and diversity trends

Marion Desquilbet [1,5], **Pierre-André Cornillon** [2,5], **Laurence Gaume** [3] and **Jean-Marc Bonmatin** [4]

ARISING FROM M. S. Crossley et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-020-1269-4 (2020).

In an analysis of a large number of time series on arthropod abundances in natural and managed areas of the United States, Crossley et al. reported no evidence of an overall decline in insect abundance and diversity[1]. We identified major concerns in the statistical analysis and inconsistencies in the selection of data, which, we argue, invalidate their conclusions. We call for a rigorous methodology in analyses of biodiversity trends because relevant information is crucial for stakeholders and policymakers.

The extent of the decline of insect populations worldwide is much debated[2–5], with major implications for public policy investment in biodiversity protection. Crossley et al. conducted a statistical analysis of 5,375 geographically and taxonomically varied time series on arthropod abundance over 4 to 36 years across the United States[1]. They concluded that there was no notable change in insect populations. However, we argue that issues in the statistical analysis and inconsistencies in data selection invalidate their conclusions.

The modelling proposed by Crossley et al.[1] relied on the following steps: (1) collecting data; (2) separating each species of each locale of each Long-Term Ecological Research (LTER) site (LLS; in the R script, a locale could be an arthropod group, a location or a collection method); (3) pre-processing data (Box 1); (4) running a different autoregressive linear model for each species of each locale of each LTER (LLS); (5) combining all estimated slopes into a 'sample'; and (6) analysing this 'sample' using violin plots, *t*-tests and confidence intervals (Fig. 1). The statistical analysis carried out in this last step relied on the assumption that the observations in the sample were independent and identically distributed. This assumption was violated for two reasons. First, the pre-processing step included a time scaling to change the minimum year of each LLS time series to 0 and its maximum year to 1. As the time length varied from 4 to 36 years depending on LLS, the scaled time x varied among LLS time series. Therefore, the estimated slopes did not represent abundance trends per year, but per time units x, varying over time series and without a clear meaning. Second, according to linear regression theory, the expectation and variance of the estimated slopes depend on the number of measures of the x variable (that is, the length of the time series) and the distances of y measures to the model (that is, the quality of the model approximation). Among LLS time series, there are different time lengths and different qualities of approximation. Therefore, the slopes cannot be independent and identically distributed, and the estimations and tests used in step 6 are not reliable. To circumvent this problem, it would be more appropriate to use a hierarchical model to analyse the whole dataset.

Other problems are as follows. First, most individual time series were too short to provide reliable estimations of the four unknown parameters specific to each LLS (Box 1). Indeed, 44% of LLS time series had only four to nine years of data. While no simple threshold exists, we do not see how to estimate four parameters reliably with fewer than ten data points, which will provide only a very imprecise estimation. Some limited sensitivity analysis was provided with a stricter data subset involving a minimum of 15 years of data, but this strict dataset included only 6% of the time series. It represented a much more limited variety of situations than the total sample and was therefore much less representative. This is another argument in favour of a global modelling approach, which would improve the precision of the trend estimate of any given LLS by using data from other LLSs.

Second, the analysis was performed at a very fine taxonomic level, implying that a high proportion of abundance counts was equal to zero (the full dataset contained 49% of zeros and the strict dataset 30.5%; moreover, 71% of the series in the full dataset, and 84% in the strict dataset, contained at least a zero). As the logarithm of zero is undefined, all zero abundance values were shifted upwards before being log-transformed by adding an arbitrary value. Such rudimentary log-transformation of count data is to be avoided because results depend on the chosen value and coefficient estimates are inaccurate[6,7]. Zero-inflated models would have dealt appropriately with the problem of high occurrence of zeros in the dataset[8].

Third, the model corrected for scale differences between abundance series without accounting for imperfect detection, which can be of particular concern for rare species. This problem may be illustrated by the case of *Aphis asclepiadis*, Northeast Purdue Agriculture Center locale, Midwest Suction Trap Network (STN) LTER site (external database S1[9]). In the ten years of records, its abundance was 0 for the nine first years and 1 for the last year. This time series (like the others in the dataset) was not composed of abundance levels, but estimations of abundance. Owing to imperfect detection, a shift of an estimated abundance from 0 to 1 provides poor information on the real abundance trend. After scaling log abundances (Box 1), this uninformative *A. asclepiadis* data

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[1] Toulouse School of Economics, INRAE, University of Toulouse Capitole, Toulouse, France. [2] Université de Rennes, CNRS, IRMAR - UMR 6625, Rennes, France. [3] AMAP, University of Montpellier, CNRS, CIRAD, INRAE, IRD, Montpellier, France. [4] Centre de Biophysique Moléculaire, CNRS, Orléans, France. [5] These authors contributed equally: Marion Desquilbet, Pierre-André Cornillon. ✉e-mail: marion.desquilbet@inrae.fr

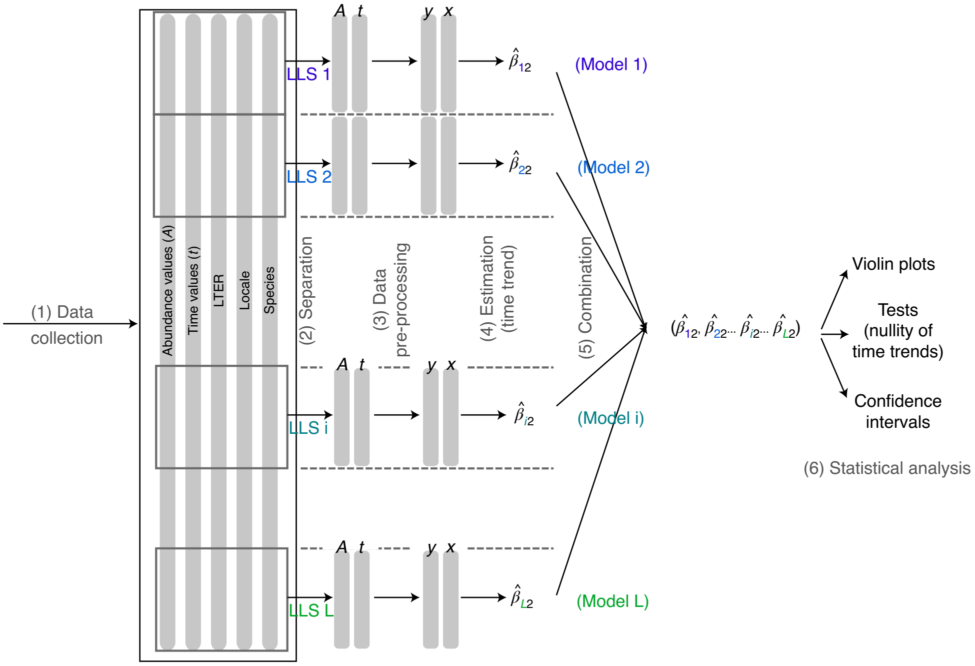

Fig. 1 | Modelling steps in Crossley et al.[1] and arising problems. Time trends were estimated separately for each species of each LLS. The time scaling was performed on LLSs of different time lengths and the quality of approximation varied among LLSs. Therefore, the abundance time trends were not independent and identically distributed, as assumed when calculating the average abundance trends, confidence intervals and significance tests associated with the violin plots of Fig. 2 in Crossley et al.[1]. A global hierarchical modelling would have circumvented this problem.

Box 1 | Model used by Crossley et al.[1]

Each time series i was composed of abundance levels A_{it} for LLS i and for years t_{i1} to t_{iT_i}, where T_i is the length of time series i.

The first step of data pre-processing consisted in log-transforming abundances. For LLS i in year t, the abundance value A_{it} was replaced either by its logarithm, log A_{it}, or, if $A_{it} = 0$, by the logarithm of a constant, log c_i, where c_i was half the minimum non-zero abundance in time series i, to obtain a series of log-transformed abundances a_{it}.

In a second step, log abundances were scaled: the empirical mean $\bar{a}_i$ of log-transformed abundances of the series was subtracted from each a_{it} value and this difference was divided by the empirical standard deviation, s_i, of log-transformed abundances. This yielded the scaled logarithm of abundance of LLS i in year t, y_{it}, defined as $y_{it} = (a_{it} - \bar{a}_i)/s_i$.

In a third step, the authors transformed all time units using a common scale varying between 0 (the first year of the LLS abundance time series) and 1 (its last year). The scaled year x_{it} was obtained by transforming the first year of time series t_{i1} to 0 and its last year t_{iT_i} to 1, and scaling all years accordingly as $x_{it} = (t_{it} - t_{i1})/(t_{iT_i} - t_{i1})$.

The proposed modelling was a linear model with a Gaussian autoregressive error of order 1:

$$y_{it} = \beta_{i1} + \beta_{i2}x_{it} + \varepsilon_{it}, \text{ where } \varepsilon_{it} = \rho_i \varepsilon_{i,t-1} + \eta_{it},$$

and where the error term, η_{it}, follows a normal law of mean 0 and variance σ_i^2.

For each individual LLS time series, this model implied the estimation of four parameters, β_{i1}, β_{i2}, ρ_i and σ_i, the slope β_{i2} representing the abundance trend and therefore being the parameter of interest.

series was erroneously modelled as having the highest abundance increase of all the time series (external database S2[9]), while it could just reflect the rarity of the species or its poor detection. The same slope could have been obtained with a time series reflecting an important abundance change with, for example, 100 insects in all years except the last year with 1,000 insects. In total, 16% of the time series included abundance values of only 0 and 1, and 27% of the time series included abundance values of only 0, 1 and 2. Simple models of occurrence and abundance have already been developed to cope with the problem of imperfect detection[10].

As all analyses of diversity (richness, evenness and β diversity) in the article relied on these estimations of abundance and on the same modelling, they shared similar methodological problems.

We also point out that we had to reprogram the R script provided by the authors using their external databases S1 and S2[9] to make it run.

Regarding data selection, the article is intended to analyse insect abundance trends in US LTER sites, but it departs from this description in two ways. First, 39.5% of the time series are from the STN. One suction trap of the STN is located in the Kellogg LTER site and all STN data, encompassing ten US states, were incorporated into the Kellogg LTER site dataset up to 2014[11]. But the dataset used in the analysis (https://suctiontrapnetwork.org), spanning up to 2019, is not linked to a LTER site. Its inclusion may bias results by minimizing the damages of intensive farming, because the STN exclusively provides data on aphids and primarily aims to document pest aphids[11], which benefit from intensive agriculture[12,13], unlike most insects (for example, aphid natural enemies[13] or bees[14]).

Second, the reference to insects in the title of the article is confusing as almost 10% of the time series were of non-insect arthropods or included insects and other arthropods. In Fig. 2 of Crossley et al.[1], 3 of the 22 violin plots concerned or involved crustaceans. Unlike the rest of the dataset, the violin plot from the Coweeta LTER site related to aquatic invertebrate communities in terms of functional feeding groups, and not species. These inconsistencies add to other criticisms of this article[15] regarding unaccounted-for changes in sampling location and sampling effort at LTER sites and the unaccounted-for impact of experimental conditions on insect populations.

In conclusion, the methodology chosen in this article is very approximate with several identified problems likely to substantially bias the results. The analysis would have required an adequate global model for all data, considering all our criticisms and those of Welti et al.[15]. We call for the application of rigorous standards for analyses on global change, especially because results can have a substantial impact on policy decision-making and the fate of biodiversity.

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Author contributions

M.D. and P.-A.C. performed both the detailed and overall analysis of the article and wrote the original draft. P.-A.C. examined and reprogrammed the R code. L.G. contributed to the argumentation and extensively edited the manuscript. J.-M.B. contributed to the analysis of data selection in the article. All authors contributed to the general comment and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.D.

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MATTERS ARISING
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M. S. Crossley et al. reply

Michael S. Crossley[1]✉, William E. Snyder[1] and Matthew D. Moran[2]

REPLYING TO E. A. R. Welti et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-021-01424-0 (2021)
REPLYING TO M. Desquilbet et al. *Nature Ecology & Evolution* https://doi.org/10.1038/s41559-021-01427-x (2021)

Our recent study showing no broad-scale declines in arthropods at Long Term Ecological Research (LTER) sites across the USA has garnered critiques from peers. Welti et al.[1] note that the LTER sites have complex management histories, and describe instances where we failed to correct for changes in sampling intensity through time. Using their critique as a guide, we re-curated the LTER metadata to maintain per-sample (for example, per sweep, pitfall trap and so on) arthropod numbers. We then repeated the analyses of abundance and biodiversity trends for the arthropod taxa and sites described in Crossley et al.[2], using several different approaches to generate trends for each taxon and site. Results were generally similar to our original findings, with broad variation in abundance and biodiversity trends among taxa and sites, but no clear overall pattern of abundance or biodiversity changes, supporting our original conclusion that LTER data do not show evidence of an "insect apocalypse". Desquilbet et al.[3] raise additional concerns that pertain to two aspects of our original study: selection criteria for studies included; and analysis. We find that the criticism of time series included is unwarranted, because the data from the Midwest Suction Trap Network are curated by an LTER and our inclusion of non-insect arthropods was intentional and clearly stated in the manuscript. The criticisms of our analysis are more substantial, but mostly represent ongoing debate on how to analyse time series data and what criteria should be utilized to include a time series. We note that within our publication, we address this uncertainty in several places and point out that changing criteria for time series inclusion has little effect on our results. We find that the criticisms of Desquilbet et al.[3] raise some important questions, but mostly reflect differences in opinion and not substantial flaws in our analysis and interpretation.

Welti et al.[1] begin their critique by noting that sampling intensity varied at several LTERs through time, and that Crossley et al.[2] failed to account for these changes when summing to generate taxon abundance trends. They also noted one instance (crayfish in North Temperate Lakes) where a coding error removed several time trends from analysis. We re-curated the metadata to correct these errors. Then, we repeated abundance and biodiversity trend analyses after collapsing abundances into a single trend per taxon (Methods). We found broad variability among taxa and sites in whether arthropods were decreasing or increasing through time (Fig. 1a), yielding a net trend whose distribution overlapped with zero (Fig. 1b). Likewise, sites varied in whether the various biodiversity metrics were showing gains or declines, yielding no net directional trend across sites (Fig. 2a). These results are generally consistent with the findings of Crossley et al.[2].

Second, Welti et al.[1] note that a simple summing across subplots at each LTER site may have masked important plot-specific differences in abundance trends that, had they been considered, would have revealed general arthropod declines. To address this possibility, we calculated separate abundance trends for each species, at each subsite, before averaging these values to arrive at the single species-specific abundance trend per site. The revised dataset contained many more subsites within each main site, such that the overall number of abundance trends considered in the meta-analysis increased ~80%. We also separately calculated biodiversity trends for each subsite. Here again, results were broadly consistent with those in Crossley et al.[2], with broad variability in trends by species and site but no clear overall directional trend (Figs. 1c,d and 2b).

Third, Welti et al.[1] suggest that three datasets are not appropriate for estimation of long-term arthropod trends because they involve observations made in experimentally manipulated plots (Cedar Creek arthropod sweep datasets acre153 and aage120) or physical removal of the focal taxon (North Temperate Lakes crayfish dataset knb-lter-ntl.217.9). While we consider these instances to be a special case where drivers of arthropod abundance change are potentially well known and not grounds to exclude trend data from our meta-analysis, a reanalysis of abundance trends after excluding these data did not change overall results (Extended Data Fig. 1).

Finally, since our paper was published, Didham et al.[4] have suggested that time series that include at least 10 points may provide the most reliable measure of arthropod abundance change through time. Thus, we again repeated our analyses using only those subsites that included 10 or more data points. This process removed one LTER site (Baltimore) altogether, and several subdatasets at particular sites (Fig. 1e). However, the general patterns were similar to those generated with other data treatments, with variability in abundance (Fig. 1f) and biodiversity (Extended Data Fig. 2) trends but no clear overall directional change across sites. Despite a consistent finding of broad variability in trends, we note that a proportion of abundance trends changed sign after standardizing taxa counts by sampling intensity and accounting for subsite structure (Fig. 3). However, a roughly equal proportion of changing trends switched from positive to negative and vice versa, suggesting no upward bias in abundance trend estimates in our original analysis (for example, 19% and 17% changed to decreasing or increasing, respectively, after accounting for sampling effort).

A key challenge when searching for evidence of recent declines in many animal and plant groups is that it often is necessary to rely on data collected for other purposes. We acknowledge that there may be longer-term periodicity in arthropod trends, as elegantly described at several of the LTER sites[5,6], that make it difficult to isolate any recent arthropod declines. However, autoregressive models are capable of detecting general declines embedded within data that show periodicity[7]. Last, we found that net abundance trends across our many different analysis approaches were consistently weakly

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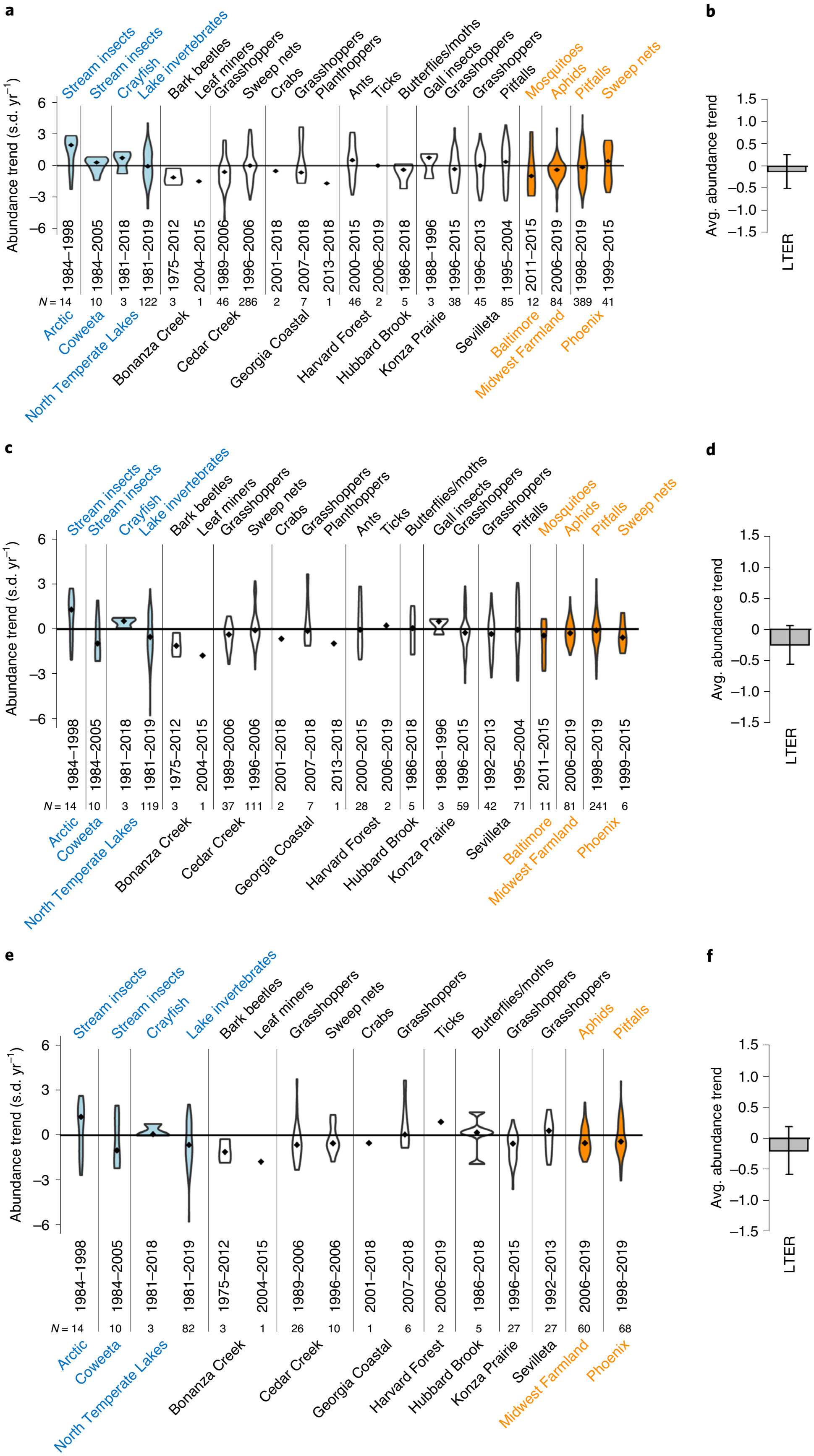
[1]Department of Entomology, University of Georgia, Athens, GA, USA. [2]Department of Biology and Health Sciences, Hendrix College, Conway, AR, USA.
✉e-mail: michael.crossley@uga.edu


a
Stream insects
Stream insects
Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Planthoppers
Ants
Ticks
Butterflies/moths
Gall insects
Grasshoppers
Grasshoppers
Pitfalls
Mosquitoes
Aphids
Pitfalls
Sweep nets
Abundance trend (s.d. yr^{-1})
6
3
0
−3
−6
1984–1998
1984–2005
1981–2018
1981–2019
1975–2012
2004–2015
1989–2006
1996–2006
2001–2018
2007–2018
2013–2018
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1986–2018
1988–1996
1996–2015
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1999–2015
N = 14
10
3
122
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1
46
286
2
7
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46
2
5
3
38
45
85
12
84
389
41
Arctic
Coweeta
North Temperate Lakes
Bonanza Creek
Cedar Creek
Georgia Coastal
Harvard Forest
Hubbard Brook
Konza Prairie
Sevilleta
Baltimore
Midwest Farmland
Phoenix
b
Avg. abundance trend
1.5
1.0
0.5
0
−0.5
−1.0
−1.5
LTER
c
Stream insects
Stream insects
Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Planthoppers
Ants
Ticks
Butterflies/moths
Gall insects
Grasshoppers
Grasshoppers
Pitfalls
Mosquitoes
Aphids
Pitfalls
Sweep nets
Abundance trend (s.d. yr^{-1})
6
3
0
−3
−6
1984–1998
1984–2005
1981–2018
1981–2019
1975–2012
2004–2015
1989–2006
1996–2006
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1998–2019
1999–2015
N = 14
10
3
119
3
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37
111
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59
42
71
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81
241
6
Arctic
Coweeta
North Temperate Lakes
Bonanza Creek
Cedar Creek
Georgia Coastal
Harvard Forest
Hubbard Brook
Konza Prairie
Sevilleta
Baltimore
Midwest Farmland
Phoenix
d
Avg. abundance trend
1.5
1.0
0.5
0
−0.5
−1.0
−1.5
LTER
e
Stream insects
Stream insects
Crayfish
Lake invertebrates
Bark beetles
Leaf miners
Grasshoppers
Sweep nets
Crabs
Grasshoppers
Ticks
Butterflies/moths
Grasshoppers
Grasshoppers
Aphids
Pitfalls
Abundance trend (s.d. yr^{-1})
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1984–1998
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1975–2012
2004–2015
1989–2006
1996–2006
2001–2018
2007–2018
2006–2019
1986–2018
1996–2015
1992–2013
2006–2019
1998–2019
N = 14
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3
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3
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26
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6
2
5
27
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60
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Arctic
Coweeta
North Temperate Lakes
Bonanza Creek
Cedar Creek
Georgia Coastal
Harvard Forest
Hubbard Brook
Konza Prairie
Sevilleta
Midwest Farmland
Phoenix
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Avg. abundance trend
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1.0
0.5
0
−0.5
−1.0
−1.5
LTER

Fig. 1 | Time trends in arthropod abundance among LTERs. a, Violin plots showing the distribution of abundance trends per taxon, where abundances were standardized by sampling effort before trend estimation. **b**, Average trend in abundance and 95% confidence intervals from **a** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($P = 0.55$). **c**, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation. **d**, Average trend in abundance and 95% confidence intervals from **c** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($P = 0.11$). **e**, Violin plots showing the distribution of abundance trends per taxon, averaged across subsites, where abundances were standardized by sampling effort before trend estimation, and time series with <10 data points were excluded. **f**, Average trend in abundance and 95% confidence intervals for **e** when trends are averaged among LTERs (d.f. = 11). Mean time trends were not significantly different from zero ($P = 0.28$). In **a**, **c** and **e**, the black diamonds within boxplots depict medians. The first and last years of LTER studies as well as the number of taxa time series are included below the violin plots. Blue shading and font indicate LTER sites reporting aquatic taxa. Orange shading and font indicate LTER sites in urban or agricultural landscapes. Unfilled violin plots and black font indicate LTER sites reporting terrestrial taxa in relatively less human-disturbed habitats.

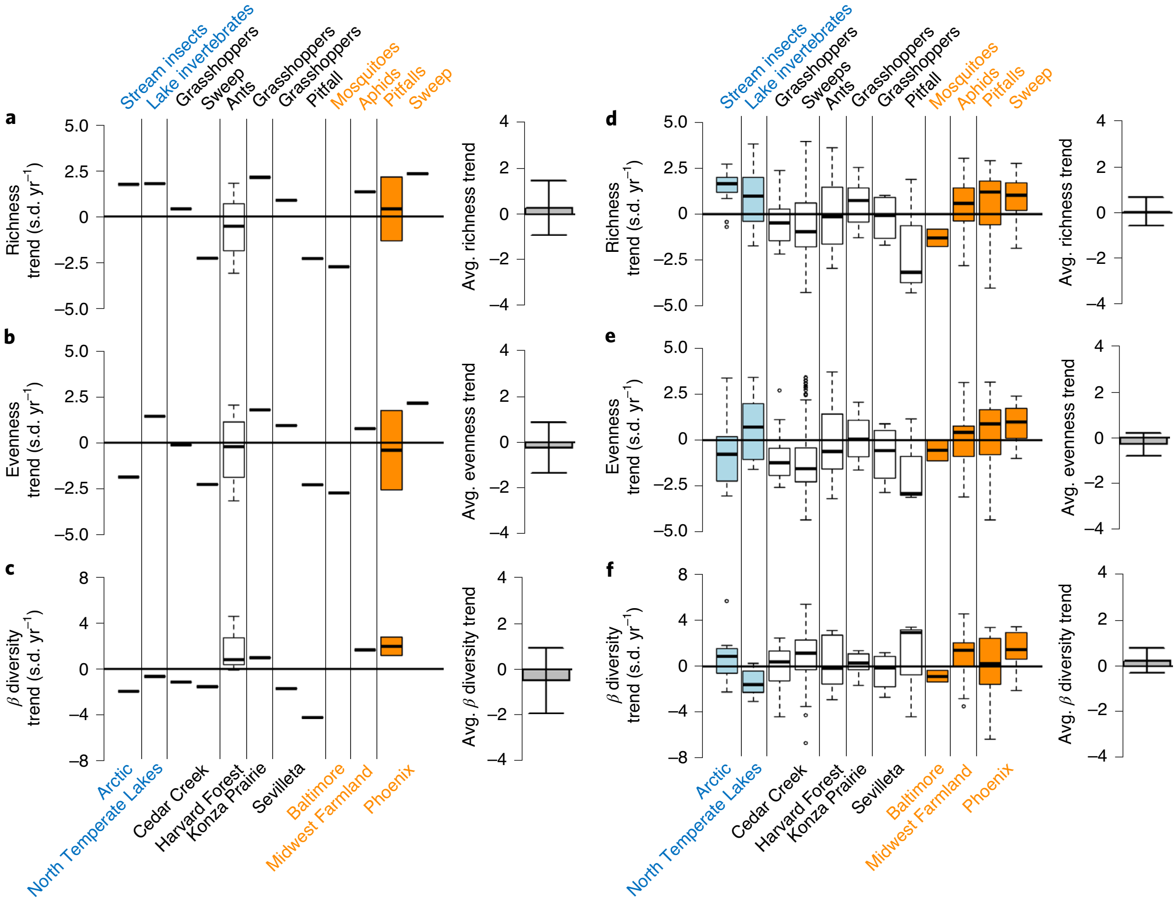

Fig. 2 | Time trends in arthropod diversity among LTERs. a–c, Diversity trends estimated using the dataset where arthropod abundances were standardized by sampling effort, and time series with <4 data points were excluded. **d–f**, Diversity trends estimated using the dataset where arthropod abundances were standardized by sampling effort and separated by subsite, and time series with <4 data points were excluded. **a**,**d**, Trends in taxon richness (rarefied). **b**,**e**, Trends in taxon evenness (Pielou's index). **c**,**f**, Trends in β diversity (1 – Jaccard similarity index). Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers) and outliers (circles). The right panels depict average change in diversity metrics and 95% confidence intervals among datasets (d.f. = 11). Time trends were not significantly different from zero at $\alpha = 5\%$. See the caption of Fig. 1 for a description of the coloured text.

negative (although statistically non-significant), suggesting that there may be some underlying general decline trend at LTER sites, but that the data are too few, and too variable, to clearly reveal it on their own. Interestingly, richness, evenness and β diversity patterns showed no evidence of any overall trend (positive or negative), providing little evidence of widespread biodiversity loss over time.

Desquilbet et al.[3] begin their critique by proposing four modifications to the analysis of Crossley et al.[2]. First, Desquilbet et al.[3] suggest a minimum time series length criterion of 16 years, citing White[8]. However, White[8] prescribes no single threshold, emphasizing that "More importantly, however, there is wide distribution of estimated minimum times. Therefore, it is not wise to use a simple threshold number of years in monitoring design". In addition, White[8] states that "Approximately, 72% of the [822] populations required at least 10 years of monitoring.", which is in line with recent recommendations for analysing insect population trends[4], and

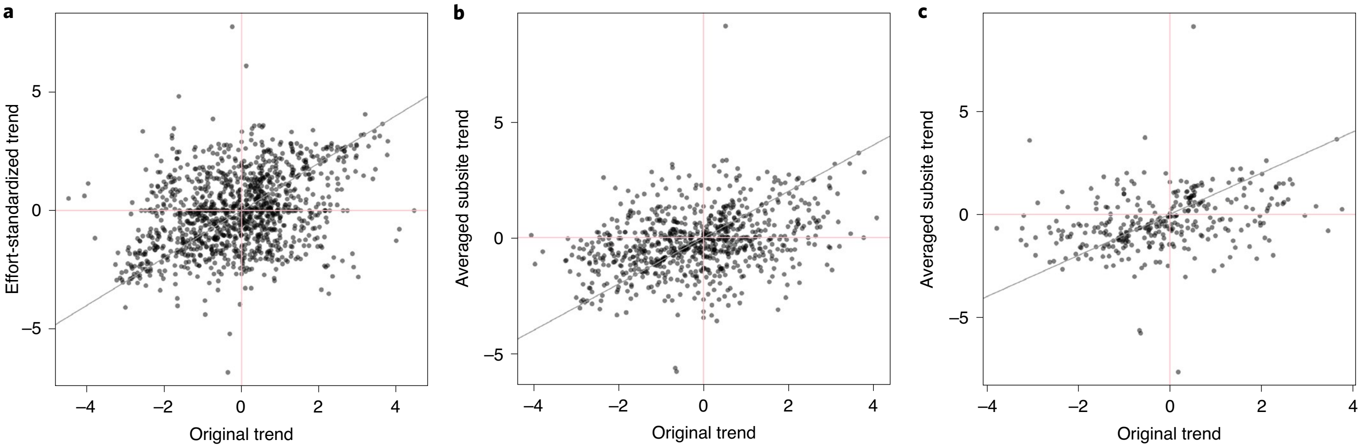

Fig. 3 | Comparison of abundance trends per taxon between original and updated datasets. a, Comparison of abundance trends per taxon, where abundances were standardized by sampling effort before trend estimation. **b**, Comparison of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation. **c**, Comparison of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite before trend estimation, and time series with <10 data points were excluded. Pink lines divide plots into quadrants, and grey lines depict 1:1 relationships.

which, through our reanalysis conducted in response to Welti et al.[1], we demonstrated did not alter the conclusions of Crossley et al.[2]. We also mention in the paper that restricting the analysis to only those time series that are more than 4, 8 or 15 years has relatively little effect on the results and their interpretation. Second, Desquilbet et al.[3] recommend using zero-inflated models over linear regression of log-transformed counts for estimating trends. While we appreciate this suggestion, we emphasize that there are genuine differences of opinion about how zero counts are handled in ecological data, and that log transformation is still widely accepted[9,10]. Third, Desquilbet et al.[3] recommend accounting for imperfect detection in ecological count data, illustrating their point with a large apparent increase observed for the aphid *Aphis asclepiadis* in a site where the first years of the time series reported zeros. These cases were uncommon (occurring in 195 of the 5,375 trends), and were similarly likely to contribute to large apparent declines (occurring in 109 of the 5,375 trends), among time series in Crossley et al.[2], and enforcing of stricter criteria for inclusion of time series, as done in Crossley et al.[2] and in response to Welti et al.[1], suggests that these cases did not alter the original conclusions of Crossley et al.[2]. Fourth, Desquilbet et al.[3] caution that summarizing many abundance trends drawn from different populations using violin plots, medians or means is statistically inappropriate. While we generally agree that summarizing a wealth of informative trends using a few summary statistics is not ideal, there is an understandable desire to summarize overall trends in insect abundance time series to allow some degree of comparability among studies. We stand by our original visual summary of abundance trends using violin plots, and note that this does not meaningfully diverge from other meta-analyses[11]. Last, Desquilbet et al.[3] note that analyses of diversity trends shared the same issues as analyses of abundance trends. Again, in response to Welti et al.[1] we show that reanalysis using stricter criteria does not change the original conclusions of Crossley et al.[2].

Next, Desquilbet et al.[3] raise three concerns with the inclusion of certain arthropod time series in our analysis. First, they note that a large portion of arthropod abundance trends were derived from aphid species documented by the Midwest Suction Trap Network. While this is clearly acknowledged by Crossley et al.[2], the implication that these data primarily represent pests that benefit from intensive agriculture is unfounded on two counts. First, the majority of aphid species (52 out of 96) documented by the Midwest Suction Trap Network do not feed on crops. Second, aphids do not benefit from intensive agriculture, which, to the contrary, includes in its definition the extensive use of insecticides often applied specifically to kill aphids. Rather than bias our analysis toward detection of more increasing abundance trends, inclusion of data from the Midwest Suction Trap Network introduced a substantial proportion of decreasing abundance trends, as the aphid populations monitored by the suction trap network in the Midwest appear to have been largely in decline since the early 2000s. Desquilbet et al.[3] also state that the Midwest Suction Trap Network is not an LTER. However, the data from these monitoring programmes have been included in Kellogg Biological Station's LTER datasets that are publicly available.

Desquilbet et al.[3] conclude their critique by noting that 9% of the time series were of non-insect arthropods or included both insects and other arthropods, and that they therefore should have been excluded. While we understand this sentiment, we emphasize that we clearly state throughout Crossley et al.[2] that we intentionally included all arthropods that met our sampling criteria, not just insects. This was in part to obtain data on aquatic arthropods, which often include large numbers of crustaceans.

As an aside, Desquilbet et al.[3] note that they had to re-program the R script provided in Crossley et al.[2] to make it run. In Crossley et al.[2], we state in the Code availability statement that the R code used to curate and analyse data is available at the Dryad Data Repository (https://doi.org/10.5061/dryad.cc2fqz645). We made this R code publicly available so that interested researchers could see how we handled the arthropod count data used in Crossley et al.[2]. We note that part of the issue in repeatability probably stems from unavoidable differences in how original data were compiled from online repositories, and that we have provided updated R code in response to Welti et al.[1] that we are able to run.

In conclusion, we disagree with Desquilbet et al.[3] that the issues raised about data selection and analysis invalidate the original conclusion of Crossley et al.[2] that the available data reveal no evidence of consistent, general abundance and biodiversity decline that might be expected were a dramatic "insect apocalypse" impacting the LTER sites.

Methods

Abundance trends. Using the critique provided by Welti et al.[1] as a guide, we re-curated all taxa abundance time series using four different approaches, standardizing arthropod abundances by sampling effort (definition varies among datasets) in all four. A detailed description of curation changes for each dataset and responses to critiques provided in the Supplementary Information of Welti et al.[1] is provided in Supplementary Table 1, and R code used for curation and analysis is provided on GitHub (https://github.com/mcrossley3/insectLTER). In the first approach, we summed arthropod abundances per taxon per year, standardizing abundances by associated sampling effort (for example, arthropods per sweep, aphids per day). As in the original analysis of Crossley et al.[2], we excluded time series with <4 data points. In the second approach, we further separated effort-standardized arthropod abundances by subsite (for example, watershed in Konza Prairie, experimental plot in Cedar Creek), again excluding any time series with <4 data points. In the third approach, we again separated effort-standardized arthropod abundances by subsite and excluded any time series with <4 data points, but we further removed data from subsites that were identified as inappropriate for estimation of long-term arthropod abundance trends because they involved experimental manipulation of plots (Cedar Creek arthropod sweep datasets acre153 and aage120) or physical removal of the focal taxon (North Temperate Lakes crayfish dataset knb-lter-ntl.217.9). For the Cedar Creek arthropod sweep datasets, we retained for analysis only observations from "control" plots (no exclosures, unfertilized, unburned, no experimental plant seeding). For the North Temperate Lakes crayfish dataset involving removal of rusty crayfish (*Orconectes rusticus*), we retained only data for *O. virilis*. In the fourth approach, we again separated effort-standardized arthropod abundances by subsite, but this time excluded any time series with <10 data points, following the minimum time series length recommended by Didham et al.[4] for arthropod time series analysis. For the two analyses that separated abundance time series by subsite, counts in the Konza Prairie grasshopper dataset were curated in a similar fashion to Welti et al.[5]. Specifically, counts pre-1996 were excluded, and mean abundance trends per species were separated by grazed and ungrazed treatment.

Diversity trends. Using the re-curated dataset where arthropod counts were standardized by sampling effort and/or time series were separated by subsite, we recalculated diversity metrics (rarefied richness, evenness, β diversity) and estimated time trends using the same approach as in Crossley et al.[2]. Richness was rarefied using a minimum sample that was calculated on the basis of the 0.1 quantile per dataset (or the 0.2 quantile when the 0.1 quantile was substantially smaller than the number of species reported in a dataset). As effort-standardized arthropod counts were «1 for three datasets (Midwest aphids, Central Arizona–Phoenix sweep and pitfall2), precluding richness rarefaction, standardized abundances were multiplied by a constant (20, 20 and 10, respectively). Effort-standardized abundances for these datasets could thus be interpreted as aphids per 20 days, arthropods per 20 sweeps and arthropods per 10 traps, respectively.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All curated data used for analyses in this study are available on GitHub (https://github.com/mcrossley3/insectLTER).

Code availability

R code used to curate and analyse data in this study is available on GitHub (https://github.com/mcrossley3/insectLTER).

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Author contributions

M.S.C., W.E.S. and M.D.M. conceived of the idea of this study. M.S.C. conducted formal analysis. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to M.S.C.

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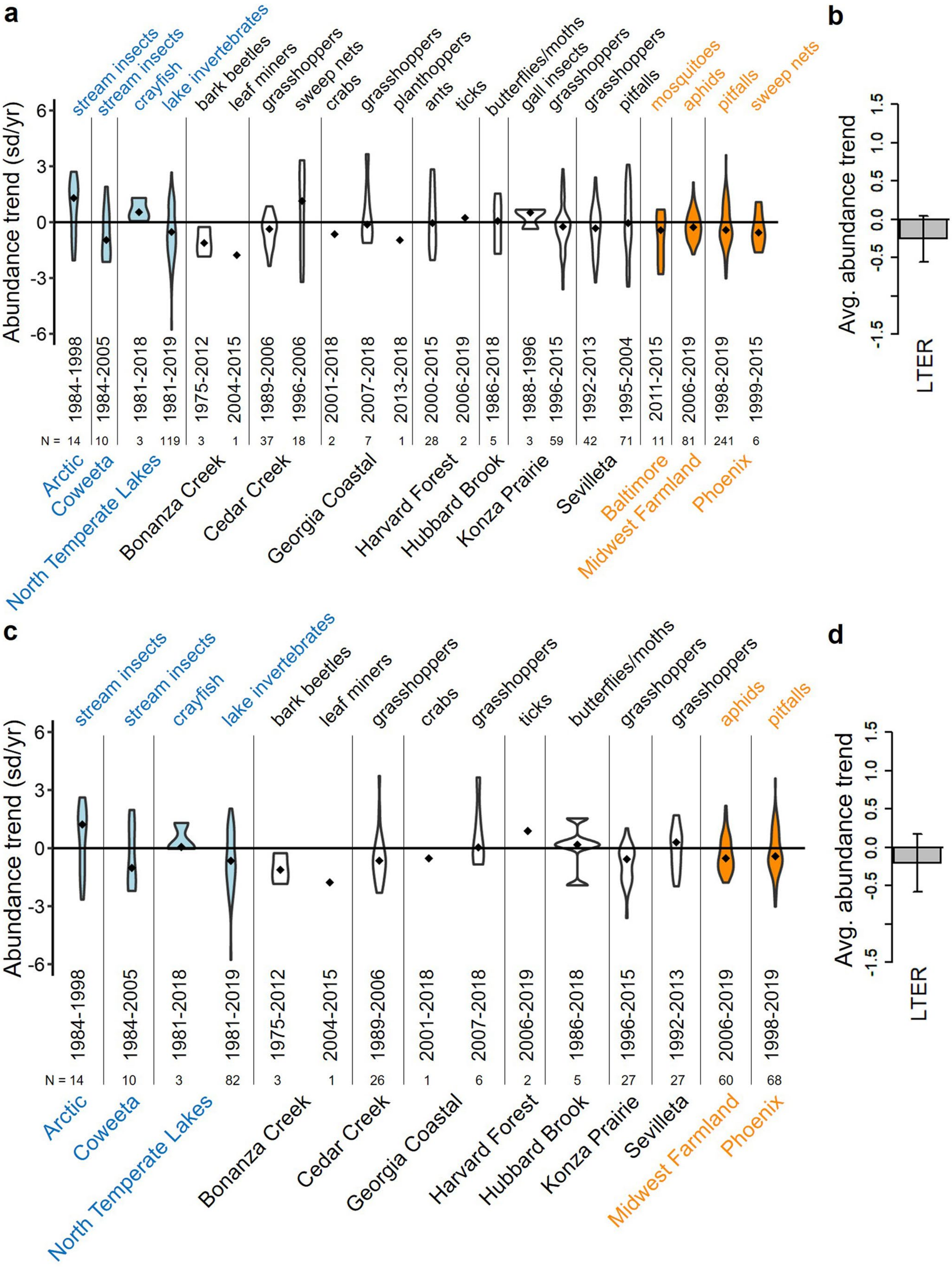
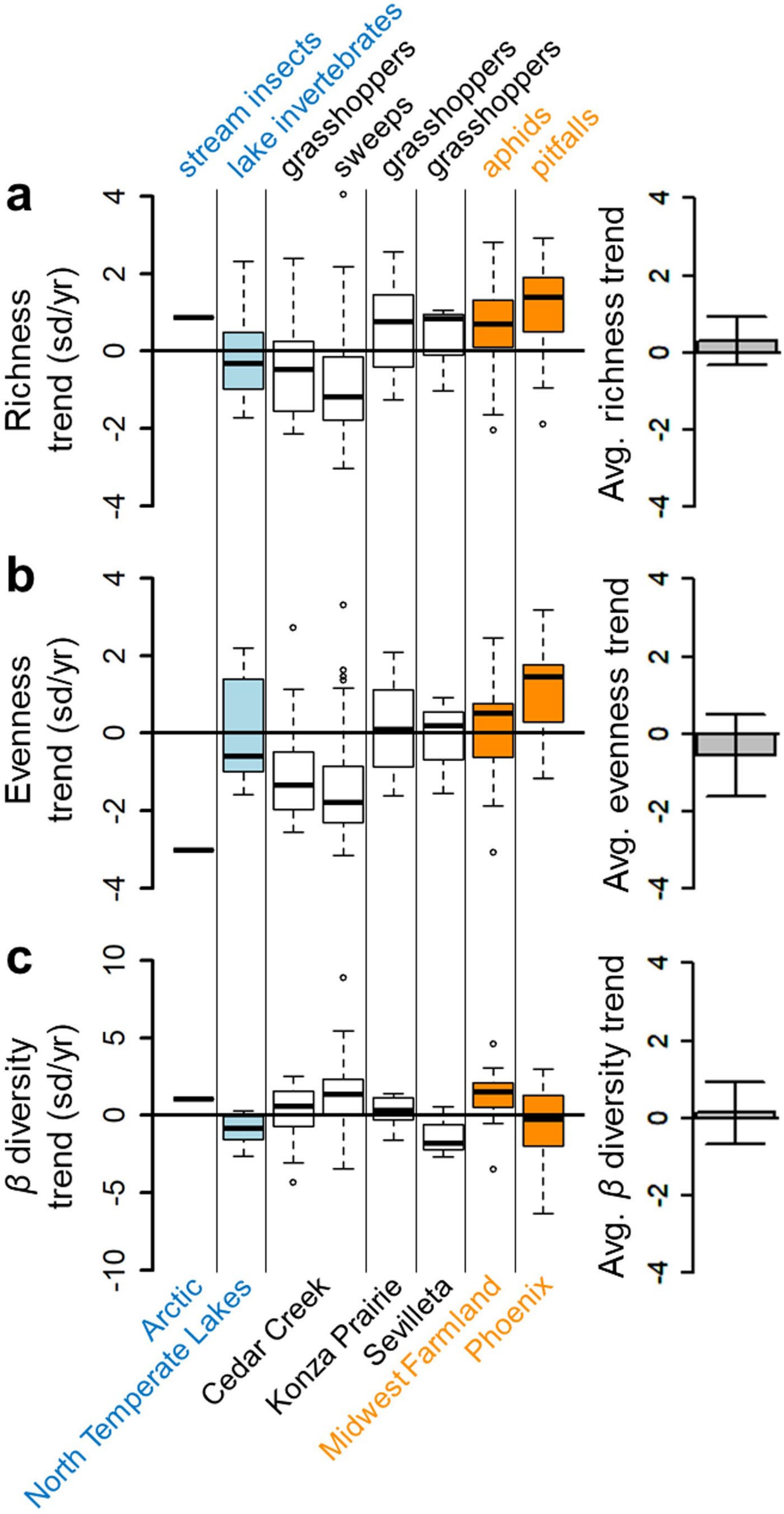


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Effort-standardized time trends in arthropod abundance among LTER subsites. a, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite prior to trend estimation, and trends with < 4 data points were excluded. This analysis differs from that depicted in Fig. 1c,d in that trends associated with experimental treatments in the Cedar Creek sweep nets and North Temperate Lakes crayfish datasets were excluded from analysis. **b**, Average trend in abundance and 95% confidence intervals from **a** when trends are averaged among LTERs (d.f. = 12). Mean time trends were not significantly different from zero ($p = 0.10$). **c**, Violin plots showing the distribution of abundance trends per taxon averaged among subsites, where abundances were standardized by sampling effort and separated by subsite prior to trend estimation, and trends with < 10 data points were excluded. This analysis differs from that depicted in Fig. 1e,f in that trends associated with experimental treatments in the Cedar Creek sweep nets and North Temperate Lakes crayfish datasets were excluded from analysis. **d**, Average trend in abundance and 95% confidence intervals from **c** when trends are averaged among LTERs (d.f. = 11). Mean time trends were not significantly different from zero ($p = 0.27$).



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Effort-standardized time trends in arthropod diversity among LTER subsites. Time trends in arthropod diversity among LTERs, using the dataset where abundances were standardized by sampling effort and separated by subsite, and time series with < 10 data points were excluded. **a**, Trends in taxon richness (rarefied). **b**, Trends in taxon evenness (Pielou's Index). **c**, Trends in β diversity (1-Jaccard Similarity Index). Boxplots depict trends among insects as medians (thick line), 25th and 75th percentiles (box edges), 95th percentiles (whiskers), and outliers (circles). Right panels depict average change in diversity metrics and 95% confidence intervals among datasets (d.f.=7). Time trends were not significantly different from zero at α=5%. Please refer to Fig. 1 legend for description of colored text.

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Corresponding author(s): Michael Crossley

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All data used in this study are publicly available, and were collected as *.csv and *.txt files. Data sources are clearly defined in the manuscript methods and supplementary information files. Data curation was done in R 3.6.2. using custom code available in the supplementary information files.
Data analysis	All data analysis was done in R 3.6.2, using custom code (available in the supplementary information files) and R packages available in CRAN (as indicated in the manuscript)

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Data supporting the findings of this study (curated arthropod abundances and estimated time trends) are available at Dryad Data Repository https://doi.org/10.5061/dryad.cc2fqz645.

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We utilized a geographically and taxonomically broad suite of relatively long-term datasets available through the U.S. National Science Foundation network of Long-Term Ecological Research (LTER) sites established in 1980 to examine trends in arthropod abundance and diversity.
Research sample	Altogether, our LTER arthropod abundance meta-dataset compiled 82,777 arthropod observations from 68 datasets into 5,375 taxa time series that spans up to 36 years and is comprised of 48 arthropod orders made up of 1 to 658 taxa in a given dataset.
Sampling strategy	All data were gathered from public LTER data repositories. The only restriction was that LTER data must contain records of arthropod abundance.
Data collection	Data were downloaded from public LTER data repositories as *.csv or *.txt files.
Timing and spatial scale	Data span 1975-2019, the entire U.S. (from Alaska to Georgia, New Hampshire to Arizona).
Data exclusions	Non-arthropod LTER data were not included, except for birds and fish when available alongside arthropod data.
Reproducibility	R code used to curate and analyze data are available in the supplementary information files.
Randomization	Randomization is not relevant to this study, because we are observing trends among sites over time.
Blinding	Blinding is not relevant to this study, because human subjects were not involved.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

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☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging