Article

More losses than gains during one century of plant biodiversity change in Germany

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Long-term analyses of biodiversity data highlight a 'biodiversity conservation paradox': biological communities show substantial species turnover over the past century[1,2], but changes in species richness are marginal[1,3–5]. Most studies, however, have focused only on the incidence of species, and have not considered changes in local abundance. Here we asked whether analysing changes in the cover of plant species could reveal previously unrecognized patterns of biodiversity change and provide insights into the underlying mechanisms. We compiled and analysed a dataset of 7,738 permanent and semi-permanent vegetation plots from Germany that were surveyed between 2 and 54 times from 1927 to 2020, in total comprising 1,794 species of vascular plants. We found that decrements in cover, averaged across all species and plots, occurred more often than increments; that the number of species that decreased in cover was higher than the number of species that increased; and that decrements were more equally distributed among losers than were gains among winners. Null model simulations confirmed that these trends do not emerge by chance, but are the consequence of species-specific negative effects of environmental changes. In the long run, these trends might result in substantial losses of species at both local and regional scales. Summarizing the changes by decade shows that the inequality in the mean change in species cover of losers and winners diverged as early as the 1960s. We conclude that changes in species cover in communities represent an important but understudied dimension of biodiversity change that should more routinely be considered in time-series analyses.

Loss of biodiversity is one of the most critical environmental problems[6,7]. Globally, the extinction of many taxa has been well documented[8–10]. However, local-scale studies—that is, those at the level of communities—do not always reflect this global trend[2,3], which has sparked intense debates[11–13]. The main reason for this discrepancy between scales is that species losses and gains through time are inherently asymmetric. At any spatial scale, it only takes one individual of a new species to result in a gain, but the loss of all individuals of a species is required to lead to a loss[14]. In consequence, at a given sampled area, the loss of all individuals of one species might be compensated by single individuals of a new colonizer[3,15]. Indeed, within local communities, species turnover, rather than species loss, has been identified as the main aspect of biodiversity change[16]. For example, 28% of species were found to be replaced per decade in an analysis of global marine and terrestrial community data[2]. However, except for some studies

of forests[17,18], these analyses ignored the changes in abundance that precede species turnover.

Time series of local communities often document the abundance of each species, but this information is rarely available at larger scales. Yet the strength of these data has not been used sufficiently in assessments of global biodiversity. In the case of plant communities, the most common abundance metric is the percentage of ground covered by all individuals of a species on a particular sampling plot. This allows changes to be calculated as percentage points of cover lost or gained, which enables declines to be detected before local extinctions occur. Aggregating such cover changes across many sampling plots at a regional level allows the calculation of the rates of decrease or increase of species' mean cover; that is, identifying losers and winners. This might in turn help us to understand the discrepancies in trends in species richness that are found at different spatial scales.

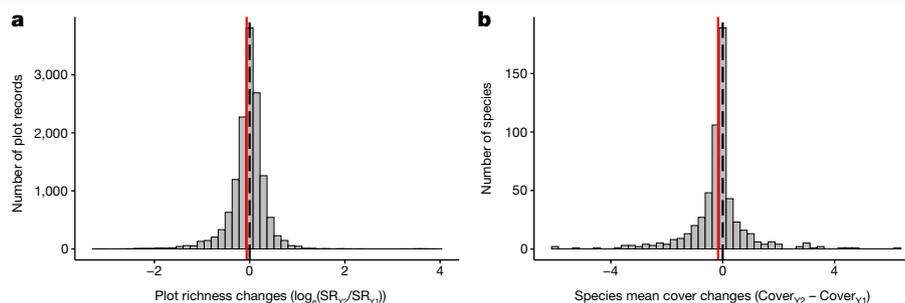A list of affiliations appears at the end of the paper.

Fig. 1 | Patterns of change in plant diversity over one century. a,b, Temporal change in plant species richness of plots (**a**) and mean cover change of species (**b**). The black dashed line shows zero change, and the red solid line shows the mean change of species richness (**a**) or the species' mean change in cover in percentage points (**b**). **a**, Comparisons of species richness (SR) in plots recorded at subsequent points in time ($n = 13,987$). An effect size of ±0.69 corresponds to double or half the initial number of species, and an effect size of ±2.3 indicates tenfold or one-tenth of the initial number of species. The estimated mean overall effect size was −0.062 according to a mixed effects model ($P = 2 \times 10^{-16}$, degrees of freedom (df) = 5,310) with a 95% confidence interval between −0.071 and −0.053. **b**, Comparisons of the species' mean changes in cover between subsequent records. Only species with at least 100 observations of change ($n = 578$) were included. The estimated overall mean of the mean cover change of species was −0.165 ± 0.089, which was significantly different from 0 according to a t-test ($P = 3.1 \times 10^{-4}$, df = 577).

For plant species, comparisons between losers and winners have only been performed with respect to occupancy at larger grain sizes. Studies based on grids of approximately 5 × 5 km reported contrasting trends in Denmark[19] and in Germany[20], with increasing and decreasing species richness, respectively. Although both studies detected an imbalance between losers and winners, it is difficult to ascertain changes in biodiversity at large grain sizes, as resurveys at that spatial resolution often differ from the initial surveys in terms of their sampling intensity. In 5-km-grid cells, species are easily overlooked, which results in pseudo-turnover with erroneous gains or losses[21]. By contrast, small-grain vegetation-plot records, ranging from a few to several hundred square metres[22], are usually thoroughly checked not only for the presence of species but also for their absence.

In vegetation science, the traditional method of analysing time series of local communities involves following a plot's trajectory through time and aggregating the changing occurrence or cover of species in the form of plot summary metrics, such as trends in species richness and diversity indices, or more sophisticated measures, such as changes in the mean ranks or abundance curves of species[23]. This type of analysis revealed both increasing[4] and decreasing[24] trends in species richness, and global syntheses consequently did not detect general trends in community-scale species richness[1,3]. However, constant community-scale richness may be combined with biodiversity loss at the regional scale. This might for example happen when few species newly colonize many communities, whereas rare species (those occurring in only a few plots) are lost completely. An early warning sign of such a development would be an asymmetry of cover trends across species; that is, with increases in cover being concentrated in a subset of species but decreases being distributed more homogeneously across many species. Indeed, a global analysis of local species turnover has suggested that it is species that are particularly widespread—often non-native species—that are increasing in abundance[25].

Here we analyse the changes in cover of individual species in 7,738 vegetation-plot time series that span almost a century and a wide range of habitat types across Germany. Apart from comparing the magnitude of cover decrements and increments, we tested for inequality in the distributions of cover losses and gains across all species. To this end, we used the Gini coefficient, a metric developed in economics to evaluate the share of incomes across the inhabitants of countries[26]. On the basis of the Gini coefficients, we also tested whether cover losses were more evenly spread among losing species than were cover gains among winning species. We here define losers and winners by their mean change in cover across all observation intervals and all plot records, which can be either negative or positive. To make sure that the observed patterns are not a result of chance alone, we also developed null models that kept species richness constant and varied the amount and direction of change and the concentration of cover losses and gains on losers and winners. We hypothesized that the divergence in the distribution between cover losses and gains is driven by (i) the proportion of species that undergo changes; (ii) the ratio of increasing to decreasing species; and (iii) the degree to which cover losses are concentrated on a specific subset of species. Then, to assess whether losers and winners (that is, those species that lost or gained cover) differed in their floristic status or habitat requirements, we analysed whether the probability of a decrease or an increase in cover depended on species being native or non-native and their habitat preference. Finally, we assessed the temporal dynamics of cover losses and gains and asked whether they occurred at the same point in time.

Changes in plant diversity from 1927 to 2020

The 7,738 vegetation-plot time series covered the period from 1927 to 2020 (Extended Data Fig. 1). Plot richness change, calculated as the log ratio of species richness (SR) at the end and the beginning of the observation time interval (Fig. 1a), varied more than tenfold in absolute numbers. Even though we observed a significant decrease in species richness over time, the estimated effect sizes were close to zero (mean $\log_e(SR_{Y2}/SR_{Y1}) = -0.062$, corresponding to a mean net loss of 0.06 species per plot). There was a tendency for shorter observation intervals to have significant increases and longer observation intervals significant decreases in species richness (Extended Data Fig. 2a–c). On average, $\log_e(SR_{Y2}/SR_{Y1})$ decreased by 0.153 per $\log_{10}$ years ($P < 0.001$ according to a mixed model), indicating that more species were lost with time. In consequence, the change in species richness was also close to zero, but was significantly positive when richness change was expressed per decade (mean $\log_e(SR_{Y2}/SR_{Y1})$ per decade = 0.062; Extended Data Fig. 3). Although decreases in species richness were greater in larger plots (mean change in $\log_e(SR_{Y2}/SR_{Y1}) = -0.064$ per $\log_{10}$ increase in plot area), species richness significantly decreased in all different categories of plot sizes (Extended Data Fig. 4a–c). Because of the overall very small effect sizes, we conclude that directional changes in mean local richness are minor at best, which is in accordance with previous studies[1,3,4]. Similarly, the effect sizes for Shannon's index of diversity, Pielou's index of evenness and the change in the species rank abundance curve (as a measure of curve change[23]) were significantly negative, but of small magnitude (Extended Data Fig. 5a–c).

Across all plots, there were 458,311 observations of change; that is, species × plot records × time interval combinations. There were

a

Change observations by
plot ID × species × time interval

b

Change observations
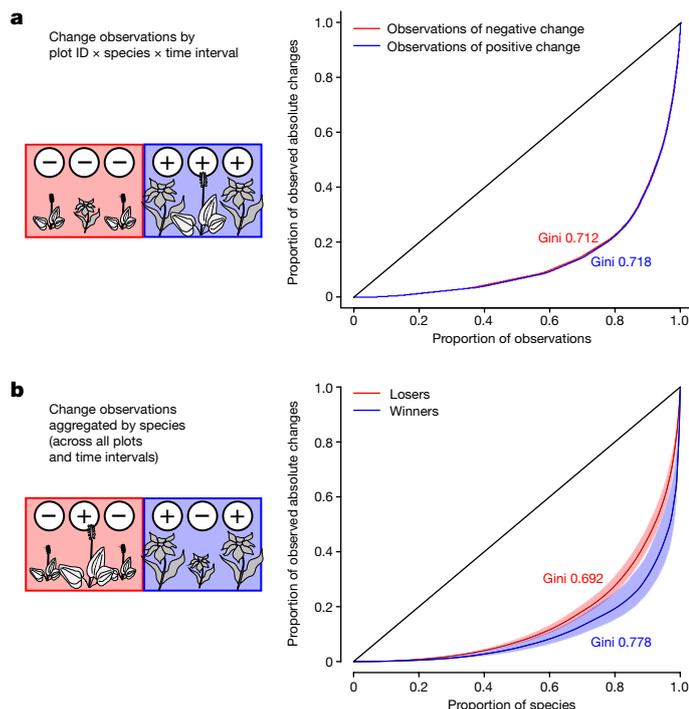aggregated by species
(across all plots
and time intervals)

Fig. 2 | Inequality of losses and gains. a,b, Lorenz curves for cover decreases (red) and increases (blue), reported in percentage points across the whole observation period from 1927 to 2020 across all change observations irrespective of species (**a**) ($n = 172{,}252$ and 166,554 observations of decrease and increase, respectively), and aggregated by species (**b**) by averaging all change observations from **a** across all plots and time intervals ($n = 1{,}011$ and 719 losers and winners; that is, species with a negative and a positive mean change in cover, respectively). The icons on the left illustrate these two types of aggregation of cover changes for six change observations, from which each three decreased (−) or increased (+). In **a**, these decreases are sorted by sign (− or +). In **b**, they are averaged by species, defining losers and winners, exemplified here as one species each with a negative or a positive mean change, respectively. The Lorenz curves show the cumulative amount of cover decrease and increase (added in order of their ascending absolute values) as a function of the cumulative number of change observations (expressed as a proportion of the total number of observations). The diagonal black line indicates the theoretical curve that would result if all observed changes were equal in size. The Gini coefficient, a measure of inequality, is the area between this diagonal line and the actual Lorenz curve divided by the entire area under the diagonal line. Thus, 0 and 1 indicate maximum equality and inequality, respectively. The differences between the Gini coefficients in both graphs were significant at $P = 0.05$, but the confidence intervals in **a** are so small that they are invisible in the graph.

more negative ($n = 172{,}252$) than positive ($n = 166{,}554$) observations, and on average, decrements were larger than increments (4.05 and 3.97 percentage points, respectively, according to a t-test ($P = 0.003$, df = 338,187). For each interval, species change was assessed as the change in per cent cover, expressed as percentage points. Across all observations, the values of both negative and positive changes in cover were not evenly distributed, which is illustrated by the Lorenz curves (Fig. 2a) and the corresponding Gini coefficients. Gini coefficients of 0.712 (95% confidence intervals (CIs): 0.710 and 0.714) and 0.718 (0.717 and 0.721 CIs) were obtained for observations of negative and positive change, respectively. Although the two Gini values were highly significantly different (non-overlapping CIs even at 99.9%), their small difference might not result in ecologically meaningful effects. Nevertheless, the finding that losses in cover were more equally distributed than gains in cover might point to an important ecological mechanism. If cover losses tend to occur in more uniform steps, whereas gains result

from both small and large increments, many small losses in cover in a plot might be offset by a few large gains. The significantly different Gini coefficients show that this was the case in a considerable number of our change observations. Moreover, cover changes also depended on interval length. Cover decreased significantly more in longer than in shorter observation intervals (by −0.042 percentage points per $\log_{10}$ interval length; $P < 0.001$ according to a mixed model), and decreased significantly more in larger than in smaller plots (by −0.14 percentage points per $\log_{10}$ area; $P < 0.001$).

Across all intervals, independent of interval length, there were more losers than winners per plot, with an average difference of 0.407 species (CIs 0.246 and 0.569; Extended Data Fig. 5d), which corresponds to the observed decrease in plot richness (Fig. 1a). Despite on average larger decrements than increments and fewer winners than losers in plots there was a significant increase of 2.5 percentage points in the mean cover of all the species in a plot across all plot records (Extended Data Fig. 5e). By contrast, we observed an insignificant decrease of 0.7 percentage points in the median cover (Extended Data Fig. 5f). These opposing directions of changes in the mean and the median cover are the direct consequence of a higher inequality of increments compared to decrements.

Although these changes in individual cover observations in plots are so subtle that they may only be detectable in large datasets, they add up when the mean changes of species are calculated. Out of the total 1,794 vascular plant species in our study, there were 41% more losers than winners, with 1,011 and 719 species, respectively. In consequence, the median across all species' mean cover changes was significantly negative (−0.063 percentage points; CIs −0.089 and −0.035; $P < 0.001$; Extended Data Fig. 6). The mean cover changes of species did not depend on their overall frequency in the dataset (regression of species' mean cover change on $\log_{10}$ frequency, $P = 0.601$). Decreases in the mean cover of species were also consistent with respect to the length of the observation interval (Extended Data Fig. 2d–f) and plot size (Extended Data Fig. 4d–f). In all analyses, not only were there more losers than winners, but the amounts of cover losses and gains were also not distributed equally within both groups. This is shown by Lorenz curves, which in Fig. 2b are based on mean cover changes per species. The Gini coefficients for species with mean negative (0.692; CIs 0.660 and 0.718) and positive (0.778; CIs 0.720 and 0816) changes differed by almost 0.1. The larger Gini coefficient for winners indicates that there were a few winners that gained disproportionately more mean cover than others, whereas the mean cover losses among losers were more equally distributed. Comparing Fig. 2a and Fig. 2b shows that two factors contributed to the inequality of biodiversity change. First, decreases occurred in smaller and more equal cover changes than gains. Second, the gains were concentrated in fewer winning species, whereas the losses were distributed among more losers.

These results also hold when rare species were excluded from the calculations. Figure 1b shows the histogram of mean cover changes for the 578 species for which at least 100 time-interval observations were available. Here, the change in mean cover was −0.165 percentage points ($P < 0.001$), which shows that species' mean losses in cover were significantly higher than species' mean gains. In other words, there was a redistribution of species: fewer species increased in dominance and frequency, whereas more species decreased in cover and sometimes disappeared locally.

Null model simulations

To understand the factors that determine the divergence in Gini coefficients between decreases and increases in cover and to disentangle those from possible species richness effects, we performed a series of null model simulations to test three different hypotheses. We hypothesized that the divergence in the distribution between cover losses and gains is driven by (i) the proportion of species that undergoes change;
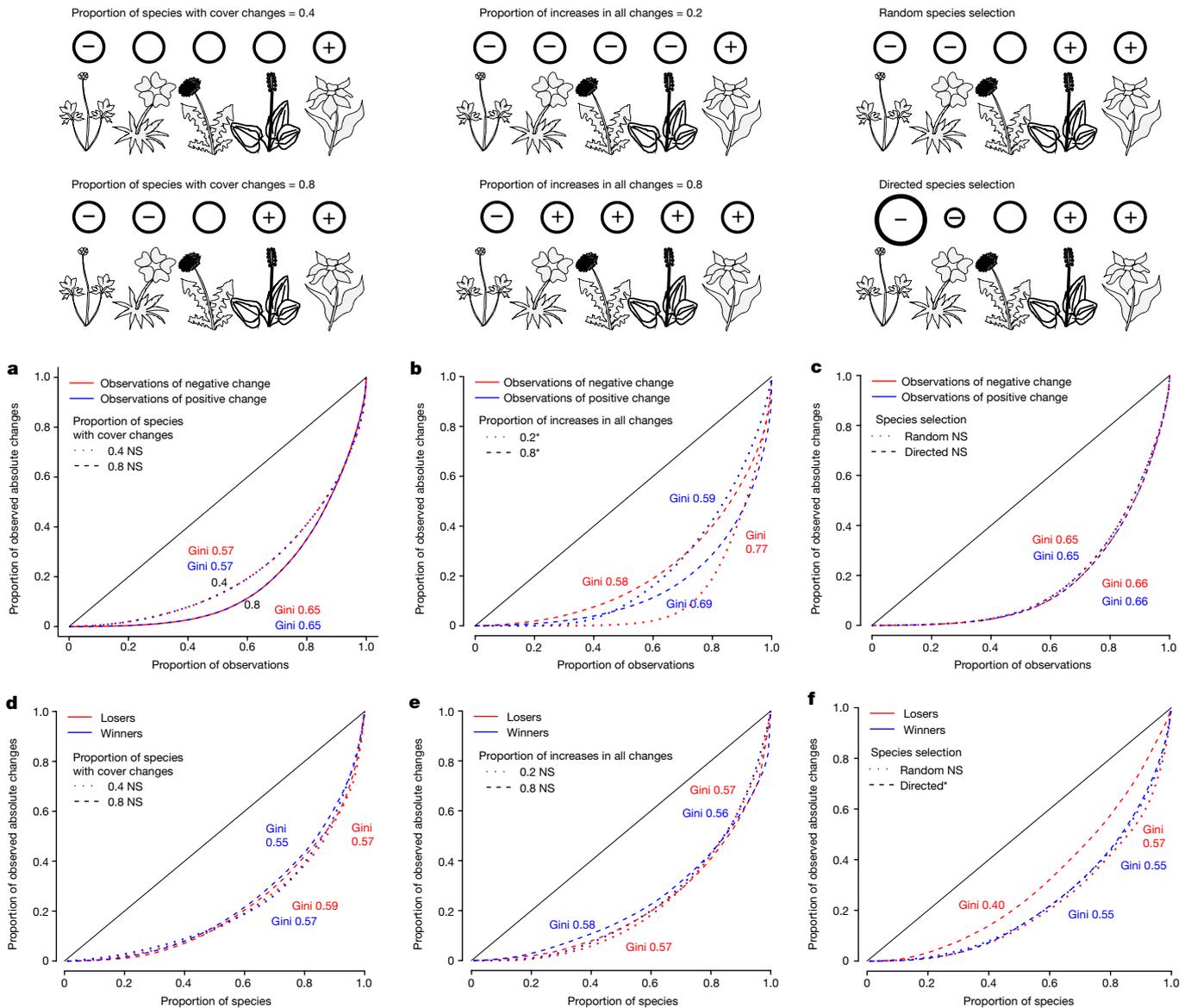
Fig. 3 | Null model simulations of changes in species cover. In all simulations, the number of species per community was kept constant, but species turnover (extinctions and colonizations) was allowed (for details see Methods and Supplementary Methods 2). The pictures at the top represent the three hypotheses being tested: the divergence in the distribution between cover losses and gains is driven by: (i) the proportion of species that undergo changes (left column, **a,d**); (ii) the ratio of increasing to decreasing species (middle column, **b,d**); or (iii) the degree to which cover losses are concentrated on a specific subset of species (right column, **c,f**). **a**–**c**, The Lorenz curves in the top row show cover changes by plot ID × species × time interval (corresponding to Fig. 2a), separated into observations of negative (red) and positive (blue) change. **d**–**f**, The graphs in the bottom row show mean cover changes per species (corresponding to Fig. 2b), separated into species with an average increase in cover and an average decrease in cover (losers in red and winners in blue). In each panel, the blue and red Gini coefficients next to each other refer to the same scenario; * indicates a statistically significant difference in the Gini coefficient between the two Gini coefficients from the same scenario; NS, not significant (all at $P = 0.05$).

(ii) the ratio of increasing to decreasing species; and (iii) the degree to which cover losses are concentrated on a specific subset of species (Fig. 3; for further explanations and a graphical illustration, see Supplementary Methods 2). In all null models, the species richness of each plot was kept constant to avoid confounding effects of richness change, and only cover changes were redistributed among losers and winners (for details, see Methods). In contrast to hypothesis (i), the divergence in the distribution between cover losses and gains did not depend on the proportion of species that undergoes change. Although subjecting more species to cover changes increased the Gini coefficients for observations of both negative and positive change (Fig. 3a), this did not propagate to the mean change values of the species (Fig. 3d).

We could confirm hypothesis (ii), which posited that the ratio of increasing to decreasing species drives the divergence in the Gini coefficients of decrements and increments (Fig. 3b). Inequality is higher for the kind of change that is more frequent. However, in the empirical data, the increments were more unequal (Fig. 2a), although they are less frequent. As a corollary, the observed divergence of inequality is unlikely to be a mere consequence of the absolute number of losses and gains. There was also support for hypothesis (iii). Concentrating losses on a specific subset of species did not affect the inequality of decrements and increments across all species (Fig. 3c). However, it resulted in the mean cover losses of losers being more evenly distributed than the cover gains of winners (Fig. 3f), as in the empirical data (Fig. 2b). This
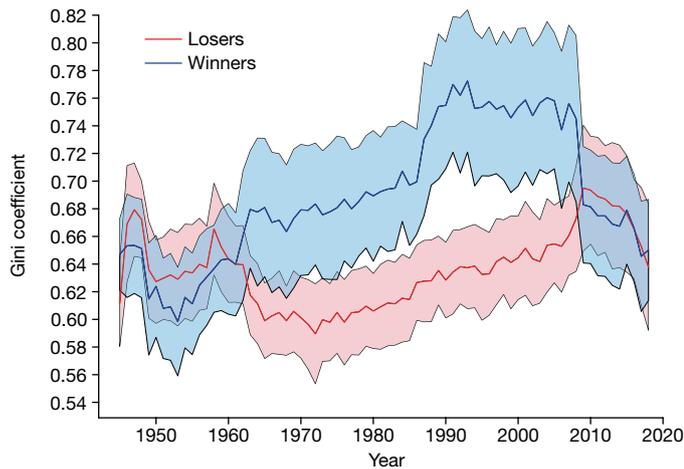
Fig. 4 | Losers and winners across one century in Germany. Probability of increase in cover for the 161 species with a significantly negative or positive change (binomial test at $P < 0.05$, with Holm correction) and at least 100 observations of change. Decreasing species are those with a probability of increasing of less than 0.5, and thus, increase less often than expected by chance, and their names are plotted below the y value of 0.5, whereas the names of increasing species are plotted above the y value of 0.5. The colours of taxon names show their floristic status, with black, purple and pink for native, archaeophyte and neophyte, respectively. The bar colour indicates the species' affinity to level 1 EUNIS habitats[66] and the error bars indicate the 95% CIs. The three most declining and increasing species are illustrated with photographs and named. Plant photographs were obtained from https://www.floraweb.de/. Copyright for *C. aureum*, *C. cyanus* and *B. racemosus*: Thomas Muer; for *I. aquifolium*: Haupt Verlag; and for *A. carinatum* and *Q. rubra*: Regensburgische Botanische Gesellschaft.

pattern was not obtained by the other two model simulations. The divergence of the Gini coefficients between losers and winners was significantly affected neither by the proportion of species that undergo cover change (Fig. 3d) nor by the proportion of increasing species (Fig. 3e). We conclude that environmental changes that threaten specific species drive the inequality of mean cover changes of losers and winners.

Losers and winners

To determine the identity of losers and winners, we focused on the 578 species with at least 100 time-interval observations, of which 161 showed significant differences in cover losses and gains across all plots (binomial test at $P < 0.05$, with Holm correction; Fig. 4). Among these 161 species with a directional change, native species decreased and neophytes increased more often than would be expected by chance (at $P < 0.05$). Comparing the habitat affinities of the species revealed that significant decreases occurred among species of mires and spring fens (level 1 EUNIS habitat Q), grasslands (R) and arable land (V), whereas forest species (T) increased more often than would be expected by chance.

The times when cover losses and gains occurred were highly species-specific, as can be inferred from the temporal course of the Gini coefficients for the 1,011 losers and 719 winners (Fig. 5). Changes started to be more unequally distributed among winners than among losers as early as in the 1960s. Since then, inequality of both gains and losses in cover increased, with cover gains always being significantly more unevenly distributed among winners than losses among losers until 2010.

Discussion

Our work reconciles some issues in the debate surrounding the 'biodiversity conservation paradox'[12]; that is, the discrepancy between observing a loss of species at a broad scale but marginal changes in species richness at the plot scale. With the support of a null model, we showed that the changes in cover may affect winners and losers differently, even if plot richness does not change. Although the observed decline in species richness might be linked to the greater number of species that lost than gained cover, our analyses show that a change of richness at the plot level is not a necessary prerequisite for this asymmetry. Overall, we found a higher number of losers than winners at the country (Germany) scale. This depends on two processes. First, cover losses were more evenly distributed than gains at the community scale. Second, cover losses and gains were concentrated in different species.

Fig. 5 | Temporal course of the inequality of species losses and gains. The Gini coefficient was calculated using a moving window approach with a window width of five years, separately for losers (species with mean cover losses; red) and winners (species with mean cover gains; blue) in this time window. The coloured lines show the mean values of 100 resampling events of 300 species each per time window; the confidence bands show the s.d. across these samples. Non-overlapping confidence bands indicate significantly different Gini coefficients between losers and winners. The increasing Gini coefficients indicate an increasingly unequal distribution of cover changes with time.

Finding 41% more losers than winners nationwide might even be considered a conservative estimate for Germany's low to mid elevations, and certainly underestimates the total change in plant biodiversity. On the one hand, our study also includes plots in the alpine region, where positive changes in richness have been described[27]. On the other hand, our work suffers from most of the shortcomings that have been noted in other studies on local time series[13,28], including the lack of spatial representativeness, varying lengths of observation intervals and a bias towards habitats that are least affected by human activities (Extended Data Figs. 7 and 8). For example, time series are usually discontinued in cases of substantial land-use change, such as when a natural (or semi-natural) habitat is converted into agricultural or urban land (with a few exceptions such as ref. [29], which is included in our analysis). In consequence, it is not surprising that the predicted 30% of local species extinctions due to land conversion[30] remain mostly unnoticed in vegetation-plot time series such as ours. We do not want to address the criticisms that have been raised with regard to calculating changes in biodiversity from local time series[13,28], which we think is mostly justified. However, we note that our time series covered about half the number of vascular plant species that occur in Germany, including rare habitats that often contain rare plant species. This means that even if the spatial representativeness is incomplete for entire Germany, the representativeness at the level of individual species is very high and gives robustness to our results.

Our results show that minor asymmetries of cover losses and gains in communities sum up when being aggregated by species, potentially hinting at population declines and extinctions at larger spatial scales. This is in agreement with trends observed across Germany[20,31–33]— including biotic homogenization[34], which has already been put forward in studies on time series[1,3], but had not yet been properly tested[13]. Homogenization occurs because, across all time series, few species consistently increase in their cover, meaning that the same species are winning in many communities. This supposedly results in a decreasing dissimilarity between communities. Other studies that analysed species changes conform to our finding of a prevalence of losers over winners, including studies from Denmark[35], the UK[36] and Germany[20]. Although neophytes were more frequently found to be increasing than decreasing, confirming global observations[37,38], most winners

were native species, as has been reported already for German forest communities[39–41]. Similarly, the habitat affinities of declining species being concentrated in mires, grasslands and arable land reflect both the trends revealed by Germany's Red List of vascular plants[42] and floristic mapping programmes[20].

Our time series also provide temporal information on species losses and gains. The strongest asymmetry between cover losses and gains occurred between the end of the 1960s and the beginning of the 21st century, indicating rapid species turnover, which is most likely to be a result of substantial changes in land use[43]. All systematic monitoring programmes on vegetation, however, only started after the year 2000, and thus cannot provide information on the second half of the 20th century. Our findings confirm the early warnings from the first Red Lists in Germany[44], as well as estimated changes in richness from floristic mapping programmes when intervals between 1997 and 2017 were compared to intervals between 1960 and 1987[20]. However, these results have to be interpreted with great caution for several reasons. First, it is probable that later time series were established at locations and habitats in the focus of nature conservation efforts, which may thus have received more favourable management than the average landscape. Second, data density on observations of species changes was highest in this intermediate period, which could give rise to a mid-domain effect[28]. In consequence, the stronger overlap of time series in the middle of the study period could have strengthened the observed trends. We note, however, that early inequalities in cover losses and gains at the plot scale will ultimately result in species extinctions at the regional scale, representing another aspect of extinction debt[45].

Overall, our analysis of local vegetation-plot time series provides a useful source of information for ongoing attempts to assess biodiversity change and understand the underlying mechanisms. We have shown that changes in species cover within communities are a neglected aspect when assessing changes in biodiversity at large spatial extents. We advocate therefore the compilation of further existing community time series worldwide, especially from vegetation plots of which few have already been mobilized in global databases, such as BioTime[46]. Compared to temporal analyses of databases[47] and meta-analyses[48], repeated observations in the same locations represent the most sensitive strategy for analysing temporal changes in vegetation[49]. However, careful quality control is a key prerequisite for this type of analysis[13]. In particular, aggregating changes across different communities by species rather than aggregating changes per plot requires much more attention, so that different taxonomies can be combined to prevent pseudo-turnover[21]. With appropriate care taken, plot time series of community data across larger regions should form a crucial backbone for future monitoring of biodiversity. Characterizing the temporal taxonomic turnover at a community scale[1,2] allows insights into the mechanisms of species losses and gains that monitoring at coarser spatial grains alone—such as floristic mapping at grid sizes of several kilometres—cannot provide.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-05320-w.

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Article

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Methods

Data compilation

We compiled as many long-term repeated vegetation-plot records from Germany as we could access, including data from published studies, as well as results from grey literature and conservation assessments. The data include 92 projects (Supplementary Table 1; for a description of the data see ref. [50]).

The different steps of data preparation and analysis are summarized in Supplementary Methods 1. Within each project, the plot resurvey ID indicates which plot observations from different times were made on the same plot or set of plots at the same site, allowing them to be compared between different points in time. Plot resurvey IDs generally refer to a single plot that was repeatedly visited (which was either permanently marked, using poles, magnets and so on, or semi-permanent; for example, provided with exact coordinates or other ways of descriptions of the exact locality). In some cases, when the exact locations were not precise, resurveys used several plots to match one previous plot, resulting in a one-to-many relationship. In this case, all plot records received the same plot resurvey ID and all plot records for the same point in time were combined. There were also resurveys with sets of plots at a site that could not be matched by single plots but only by another set of plots, resulting in a many-to-many relationship. Such resurveys were done to compare a particular community at a particular site at two points in time, each represented by a set of plots, which then all received the same plot resurvey ID. Accordingly, all plot records for the same point in time were combined by averaging the species cover values, and then treated as a single observation. Some of our studies included experimental treatments with different management of habitats (for example, abandonment or establishment of grazing, succession and disturbance). To exclude treatments that are not representative of biodiversity change in Germany, from these studies we included only the control plots[51] and plots that reflected the ambient land use at the site[52], that were unfenced[53] or that were subjected to continuous grazing[54]. At the end, 7,738 unique plot resurvey IDs remained, comprising a total of 23,641 vegetation-plot records that ranged from 1927 to 2020. We retrieved coordinates for all locations (longitude and latitude), either from the original sources or by looking up plot locations from maps. The duration and survey times of each project are shown in Extended Data Fig. 1. As different projects used different cover scales, we converted cover into per cent, following the default conversion of the Turboveg 2 program[55]. For example, for the seven-grade Braun-Blanquet scale, the transformation was r + 1 2 3 4 5 → 1% 2% 3% 13% 38% 63% 88%, respectively.

The locations of all plots of all projects are shown in Extended Data Fig. 7. We assigned the individual plot locations to the grid cells of the quadrants of German ordnance maps ('MTBQ', 0° 5′ × 0° 3′, approximately 5.6 km × 5.9 km in the centre of Germany), and tested whether the grid cells analysed differed from those without observations with respect to population density, road density, urban cover, cropland cover and protected areas. This clearly revealed that the sampled grid cells were not representative of the whole area of Germany. They showed significantly higher human population densities, road densities and urban cover, whereas the cover of cropland and the amount of protected area were lower, which indicates that many time series were made in regions with higher human pressures. Our time series were also biased with respect to habitat types. This was illustrated by assigning all plot records of the time series to EUNIS classes, using the expert system EUNIS-ESy[56] and the corresponding R code[57]. Each time series was assigned to the habitat type by using the earliest plot record that resulted in level 3 EUNIS classification (Extended Data Fig. 8). Although the time series covered 92 of the approximately 150 EUNIS habitat types encountered in Germany, most of the 23,641 plot records came from grasslands (level 1 EUNIS habitat R; n = 14,849; 62.8%), followed by forests and other wooded lands (T; n = 5,440; 23%). By contrast, arable land, which makes up more than 36% of the land cover in Germany, was only represented by 816 plot records (V; vegetated man-made habitats; 3.5%).

Taxonomic harmonization

All projects were linked to the standardized species list German SL 1.3 (ref. [58]). The nomenclature for vascular plants followed the concepts of the German taxonomic standard list[59], with additional aggregations to higher taxonomic levels according to German SL 1.3 (ref. [58]). As some authors recorded subspecies and other infraspecific taxa, species were aggregated at the species level, using vegdata[60]. Some closely related species that, from our experience, were often mistaken in the field were merged at the aggregate or genus level. Species aggregates were also used when different taxon names of the same aggregate occurred in different projects, to prevent the same taxon appearing under different taxon names. The harmonization of taxon names was a crucial step in our approach, as our aim was to assess changes in species cover across projects. We used our own R code to merge taxon names and the notation of the ESy expert system[56] to protocol all steps. The species harmonization forms the first section of the ESy system and shows which taxon names were aggregated under the name of a broader taxonomic concept (Supplementary Table 2). In addition, within single projects, we used customized aggregations when the same taxa were reported at different taxonomic levels at different points in time in the same plot resurvey IDs (Supplementary Table 3). For example, whereas *Orchis militaris* was reported in all but one year of a time series of a specific plot, only one year reported *Orchis* species at the genus level. Unaccounted for, such a leap between taxonomic levels within a time series would result in incorrect observations of species change. To avoid losing the predominant information at the species level by aggregating all records to *Orchis*, we assumed that the taxon was also *Orchis militaris* in that particular year. If more than one taxon occurred in previous years, we equally distributed the cover among those taxa. This happened, for example, when a record was taken late in spring when the two species *Anemone nemorosa* and *Anemone ranunculoides* could no longer be distinguished.

The percentage cover values of the same aggregated taxon name as well as those of taxa occurring in different layers of the same plot were merged, assuming a random overlap of their cover values and making sure that the combined cover values could not exceed 100% (ref. [56]). We removed bryophytes and lichens using the vegdata package in R[60].

Finally, the original list of 3,280 taxon names that included bryophytes and lichens was reduced to 1,794 taxon names of vascular plants. In the following, for the sake of simplicity, we refer to these taxon names as species.

Analysis of temporal change

Instead of fitting trends for individual time series, different intervals of the same time series were treated as separate observations of change. This was achieved by separating all records into 458,311 plot resurvey triplets; that is, ID × species × time interval observations, where the interval designated two subsequent observations between year 1 and year 2 for the start and the end of the interval, respectively. Separating a time series in its different intervals avoids the problem of establishing a baseline against which the changes are being compared[13,28].

Analysis of temporal change at the plot level

At the plot level, the triplets were aggregated into plot resurvey ID × time interval combinations (in total n = 13,987). With a total of 7,738 plot resurvey IDs, this corresponds to an average of 1.81 resurvey intervals per plot resurvey ID. This means that, on average, a time series had about three observation events. Although most plot resurvey IDs were only repeated once (one interval; n = 6,006), 798 had 2 intervals, 213 had 3 intervals and 721 had 4 or more intervals. The longest time series comprised 54 intervals (Uwe Wegener, montane Harz meadows[61]).

Article

For each interval and plot resurvey ID, we calculated the change in species richness (SR), Shannon's index of diversity and Pielou's index of evenness. In addition, we calculated the change in the rank abundance curves, using the formula for curve change in ref. [23]. The change in rank abundance reflects the area between the two rank abundance curves for the later observation and the earlier observation. Rank abundance curves are constructed by plotting the species' cumulative relative cover (ranging from 0 to 1) against the species' ranks in cover values, calculating ranks from highest to lowest cover and then dividing the ranks by the maximum rank (with scaled ranks ranging from 0 to 1). Furthermore, we calculated the number of species with decreases and increases in cover as well as mean and median cover across all species in a plot record.

For all change metrics that were calculated at the plot level, we calculated log response ratios of the metric at time Y2 divided by that at time Y1, except for the change in rank abundance curves and losses and gains, for which we used the difference between area and number of species, respectively. To assess the effect of plot size on the change of species richness, we tested the effect of $\log_{10}$(surface area in m^2) on $\log_e(SR_{Y2}/SR_{Y1})$. In addition, we analysed the distribution of plot records with respect to $\log_e(SR_{Y2}/SR_{Y1})$ separately for small (less than 25 m^2), medium-size (25 m^2) and large (greater than 25 m^2) plots. A similar analysis was used for testing the effect of the observation length ($\log_{10}$ interval length in years) on the change of species richness and analysing the distribution of plot records separately for short (two years or less), medium (> two years and ≤ 10 years) and long observation intervals (more than 10 years). We also expressed richness change per decade (mean $\log_e(SR_{Y2}/SR_{Y1})$ decade^{-1}). The departure of effect sizes and differences from 0 in all these analyses were assessed with mixed effects models, using the time-series ID as a random factor, thus taking into account the non-independence of intervals from the same time series. As there were 13,987 plot resurvey ID × time interval combinations, the test statistics tended to be significant, even when the mean of the test metric was close to zero. We used mixed models to calculate confidence intervals using Wald-test approximation[62].

Analysis of temporal change by species

In total, there were 458,311 plot resurvey ID × species × time interval combinations, for which the difference in cover for every species k and time interval m was calculated as $\Delta cover_{k,m} = cover_{k,m,Y2} - cover_{k,m,Y1}$ and expressed as percentage points. Here, Y2 and Y1 refer to the end and the start year of an interval, defined as the two nearest points in a time series. Similar to our analyses for the change of species richness, we also tested the effect of $\log_{10}$(surface area in m^2) and of observation length ($\log_{10}$ interval length in years) on $\Delta cover_{k,m}$, using mixed effects models with the time-series ID as random factor.

To compare the distribution of cover changes across all species, we considered observations of positive and negative cover change separately ($n = 184,678$ and 192,162 time interval observations, respectively). We then sorted the cover changes in each category (positive or negative cover changes) according to increasing absolute values and plotted the cumulative sums of cover changes against the proportion of observations in each category, thus obtaining a Lorenz curve. We calculated the unweighted Gini coefficient for each category, according to a previous report[63] and using the bias correction implemented in the DescTools package[64]:

$$G_{cover} = \frac{\sum_i^n \sum_j^n |\Delta cover_i - \Delta cover_j|}{2 \sum_i^n \sum_j^n \Delta cover_j} \frac{n}{n-1},$$

with $\Delta cover_i$ and $\Delta cover_j$ being cover changes of change observations i and j in plots, irrespective of species, and n the total number of change observations. G_{cover} is calculated separately for observations of negative and positive change, using either only all negative change observations or only all negative change observations. The Gini coefficient is

a measurement of inequality in distribution[26], given as a value between 0 and 1, with 0 indicating a perfectly equal distribution.

Across all plot resurvey IDs, there were 458,311 species × time interval combinations with a value for cover change. For species comparisons, we aggregated cover changes by species across all plot resurvey IDs and intervals. We counted the number of positive, zero or negative cover changes per species and subjected them to an exact binomial test, using the stats package. We adjusted the significance levels for multiple testing using Holm correction. When showing changes by species in graphs (Fig. 4), we confined the list to those species with $P < 0.05$ after Holm correction and with 100 or more time interval observations ($n = 161$). To compare the distribution of cover changes among all species, we calculated the mean cover change per species, expressed as percentage points in cover. As the cover changes were highly dependent on species and many species occurred only rarely in the time series, we tested the probability of increase with a non-parametric exact binomial test. We assigned the floristic status native, archaeophyte and neophyte (the latter two being exotic species arriving in Germany before or after 1492, respectively) to these 161 species, using the BIOLFLOR database[65]. We assigned species to their preferred habitat using the level 1 habitats of the EUNIS habitat classification[66]. This was achieved by assigning all 225,606 vegetation plots in the German Reference Vegetation Database[67] to EUNIS classes, using the expert system EUNIS-ESy[56] and the corresponding R code[57]. We then calculated the affinity of the 161 species with a significant change to each of the 150 EUNIS classes that occurred in Germany, using the Φ coefficient of association[68,69]. Then, the habitat preference of a species was defined as the EUNIS class to which the species had the highest Φ coefficient. For further analysis, we used the highest hierarchy of the EUNIS system (level 1). To assess which categories of floristic status and EUNIS habitat level 1 preference departed from the expected probability to increase, which is 0.5, we scaled the probability response to −1 to 1 and calculated linear models without intercept. In addition, we tested whether mean cover changes of species depended on their overall frequency in the dataset and analysed subsets of species on the basis of different interval lengths and plot sizes in which the species occurred, using the same categories of interval lengths and plots sizes as used for analysing species richness.

We calculated the Gini coefficient for inequality of changes, separately for species with negative and positive mean cover changes (that is, losers and winners), respectively. The Gini coefficient based on species means was also calculated using the DescTools package[64], and is defined as:

$$G_{\overline{cover}} = \frac{\sum_i^N \sum_j^N |\overline{\Delta cover}_i - \overline{\Delta cover}_j|}{2 \sum_i^N \sum_j^N \overline{\Delta cover}_j} \frac{N}{N-1}$$

with $\overline{\Delta cover}_i$ and $\overline{\Delta cover}_j$ being the mean cover changes of species i and j and N the total number of species. $G_{\overline{cover}}$ is calculated separately for losers or winners, using either only all negative or positive species mean cover changes. Applied in this way, the Gini coefficient $G_{\bar{x}}$ indicates that either the losses or the gains in cover were not distributed equally among species. To assess the significance in the difference between the Gini coefficients of losers and winners, we calculated 95% confidence intervals from bootstrapping, using percentiles, bias correction and 1,000 replicates. For analysing the temporal course of the distribution of cover changes in the groups of winners and losers, we calculated the Gini coefficient $G_{\bar{x}}$ as described above using a moving window of five years, using only records from 1945 onwards because of data scarcity before this date. Cover changes of all resurvey ID × species × time interval combinations were aggregated by species and year for all years that fell into a window of five years. In every window, 300 species were resampled by chance, which was 100 times, and Gini coefficients were calculated separately for all decrements and increments of the means of these 300 resampled species. Temporal trends with confidence intervals were calculated from the Gini coefficients from these 100 runs.

Null model scenarios

To assess the mechanisms that might drive the inequality of cover changes among losers versus winners, we set up a simple model, serving as a theoretical null expectation (see illustration in Supplementary Methods 2). Corresponding to our data analysis, the null model was not spatially explicit. In contrast to previously developed null models[1], our aim was also not to model stochastic colonization or extinction, but stochastic changes in cover, which to our knowledge had not been attempted before. Extinction only happened when cover decreased below zero, and was exactly counterbalanced by colonization. In this way, we kept species richness constant, in contrast to previous null models[1]. Our null model also differs from traditional null models in community ecology, which reshuffle cover values across communities and/or species[70–72] but do not allow for random decreases and increases in cover.

Simulating random communities. We simulated random communities and subjected them to different scenarios. First, we created a pool of 200 species with frequencies randomly drawn from a log-normal distribution, using the rlnorm function in R (mean log = 1.5, sdlog = 1.2). Summing up all frequencies resulted in a total of 1,810 occurrences. We then drew random species richness values for 100 communities from a normal distribution, varying the mean and standard deviation to obtain the same total number of occurrences (1,810), which was achieved by using a Gaussian distribution with mean = 19.13 and s.d. = 9 species). We chose these parameters in a way to be similar to the richness distribution of our empirical dataset (mean = 23.4, s.d. = 13.7). Finally, cover values were randomly assigned to the species in each community according to a broken-stick distribution[73], using the drbs function of the sads package[74], which resulted in a sum of a total cover of 100% in each community.

Imposing cover change with three different scenarios. We then introduced different types of change to this random community, using three different scenarios. In all scenarios, the species richness was kept constant, which reflected our own findings and those of previous studies[1,3–5]. However, we allowed species turnover by replacing species that—owing to randomly introduced decreases—had cover values of less than 0. Newly colonizing species were randomly selected from the pool of 200 species, with the drawing probability weighted by the species' frequency. In scenarios 1 and 2, this made sure that the species frequency distribution in the species pool remained constant (except for random noise). Species decreases in cover were introduced by varying three parameters, which corresponded to the three scenarios in which these parameters were varied: (1) the proportion of species affected by cover change in a community (to simulate different rates of turnover in community composition); (2) the proportion of species with an increase in cover among those species affected by change (to simulate differences in the distribution of cover losses and gains, irrespective of species); and (3) the identity of the species to decrease in cover (to simulate that cover losses and gains might be concentrated in certain species). Decrements were either assigned randomly or according to the descending ID of the species, which resulted in species with higher ID values being more frequently selected for losing cover than other species.

In each community, according to these parameters, species were randomly chosen that underwent a decrease. The cover of all decreasing species in each community was summed up and redistributed according to a geometric distribution. For example, in a community of 24 species in which 50% of all species were selected to change in cover and 50% of those were subjected to decrease in cover, the summed cover of these 6 species was redistributed (but randomly assigned) to the same 6 species as 0.125, 0.0625, 0.03125, 0.015625, 0.0078125 and 0.0078125. Note that the smallest change occurred twice to result in a sum of 0.25. If the decrease in cover assigned to a species was larger

than the current cover of that species, its cover became 0 and the species was replaced as described above. The actually applied decrements were then assigned to the species that—according to the given parameters—were selected for increase. The number of increasing species also comprised the newly colonizing species in a community. If the number of decreasing and increasing species was the same, the exact same cover changes of decreasing species were randomly assigned as increments to the increasing species, taking the decrements and changing their sign. In this case, the absolute values of all increments and decrements across all communities were exactly the same, and, thus could not result in differences in the equality of their distribution. If the number of decreasing species was higher than that of increasing species, each two randomly chosen decrements were combined until the number of required increments was reached. Conversely, if the number of decreasing species was lower than that of increasing species, randomly selected decrements were divided by 2 until the number of required increments was reached. In the latter two cases, the equality of the distribution of decrements was no longer the same as that of the increments.

For all scenarios, we measured the inequality of increments and decrements by the Gini coefficient as described above. As in the empirical data, we calculated the Gini coefficient (i) across all cover changes, separately for increments and decrements, but irrespective of species; and (ii) on species-aggregated mean values of increments and decrements.

The analyses were calculated in R v.4.0.3 using the packages stats, foreign, reshape2, data.table, tidyverse, Hmisc, sads and Desctools. Graphs were produced with the packages ggplot2, egg and vcd.

Reporting summary

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

Data availability

All data are available as a data paper[50] and available at https://doi.org/10.25829/idiv.3514-0qsq70 under the terms specified by CC BY 4.0.

Code availability

The R code for retrieving resurvey ID × species × time interval combinations and that was used to calculate the results presented in this paper is provided in Supplementary Code 1 and is available at https://github.com/idiv-biodiversity/ReSurveyGermany_Analysis. The R code that was used to produce the null models in Supplementary Code 2 is available at https://github.com/idiv-biodiversity/ReSurveyGermany_null_models.

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Article

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Author contributions U.J. and H.B. conceived the idea for the project. All authors were involved in collecting datasets, developing the conceptual framework and interpreting the results. H.B. performed the statistical analyses and developed the null model. U.J. and H.B. wrote the first draft of the manuscript. All authors commented on and agreed with the final version 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 Helge Bruelheide.
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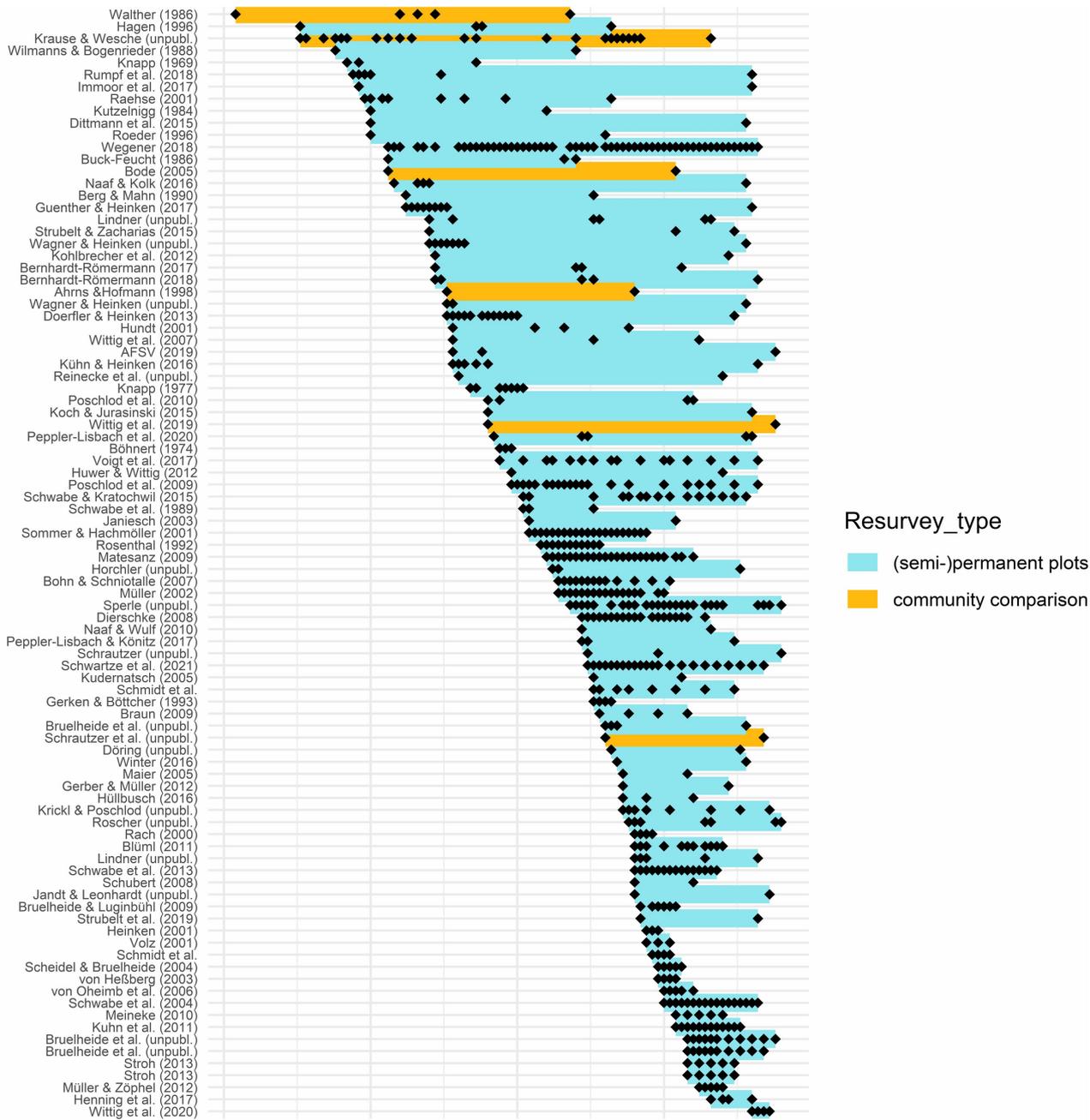
Extended Data Fig. 1 | Temporal coverage of the 92 projects included in the study. The coloured lines indicate the start and the end of a project, black diamonds show in which years surveys were made. Resurvey type refers to either studies that were repeated within a particular community across a site without attempts to match plots (community comparison), or were carried out on matched plots, which were either permanently marked or relocated from exact descriptions (semi-permanent). The lower graph shows the number of times a particular year was included in any of the time series.
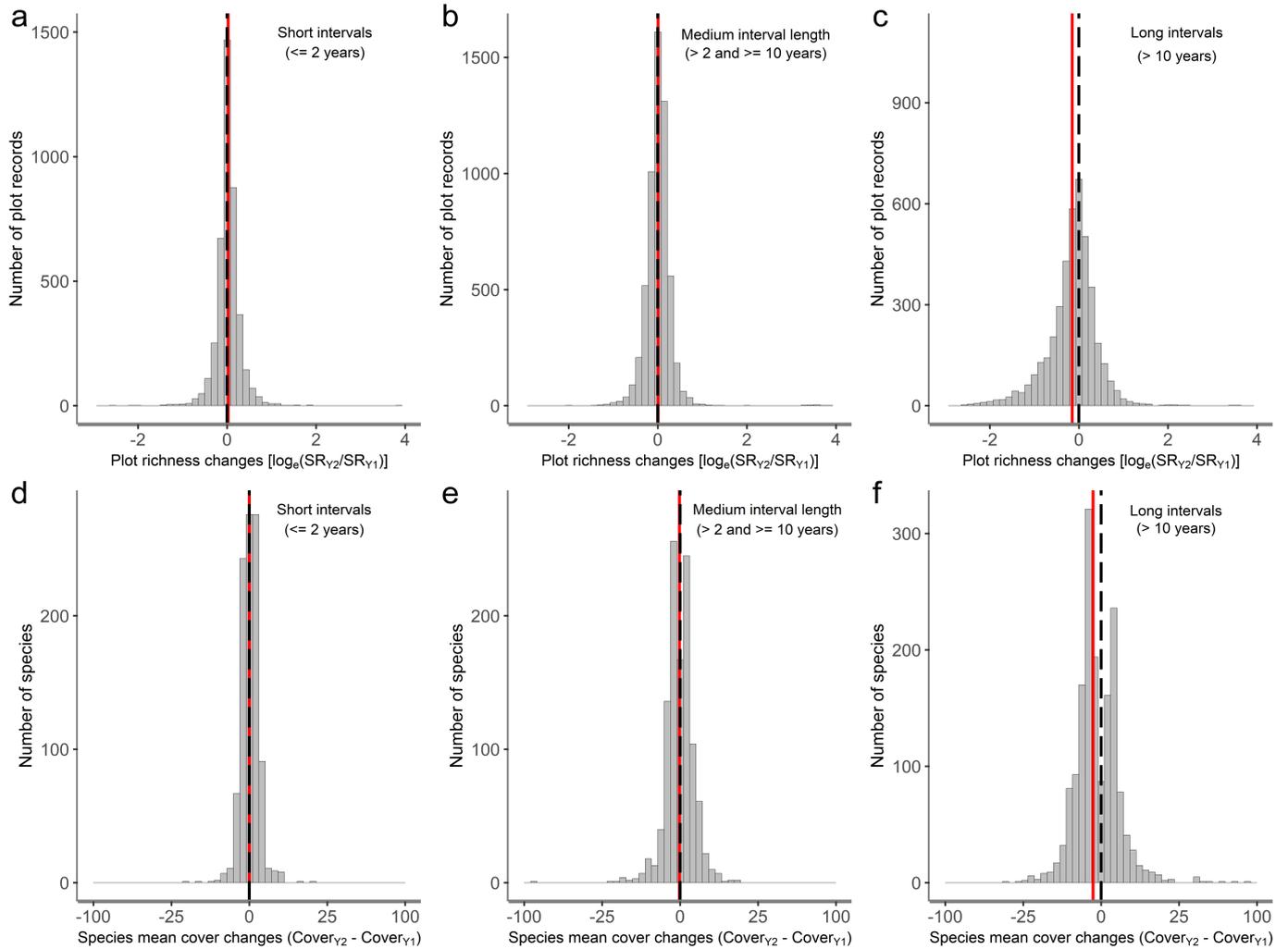
**Extended Data Fig. 2 | Effect of the length of the observation interval on plant diversity change.** The temporal change of species richness (SR) in plot records (a–c) and mean cover change of species (d–f) is shown separately for short (≤ 2 years), medium (> 2 and ≤ 10 years) and long observation intervals (> 10 years). The black dashed line shows zero change, while the red solid line in a)–c) shows the mean change of richness and in d)–f) the species' median change in cover in percentage points. According to a mixed effects model estimated mean overall effect size was in a) +0.025 (p = 3.9 x 10⁻⁹, df = 4,142), b) +0.007 (p = 0.093, df = 3,903) and c) −0.150 (p < 2 x 10⁻¹⁶, df = 8,612). In d)–f) plot Interval comparisons of the mean of all cover changes per species between time points Y1 and Y2 of the start and end year, respectively, are shown on an axis with a sign*square root-transformation. According to an exact binomial test estimated overall median of cover change was in d) 0 (95 per cent confidence interval 0 and 0.007), e) −0.02 (CI −0.02 and 0) and f) −0.26 (CI −0.53 and 0.002).
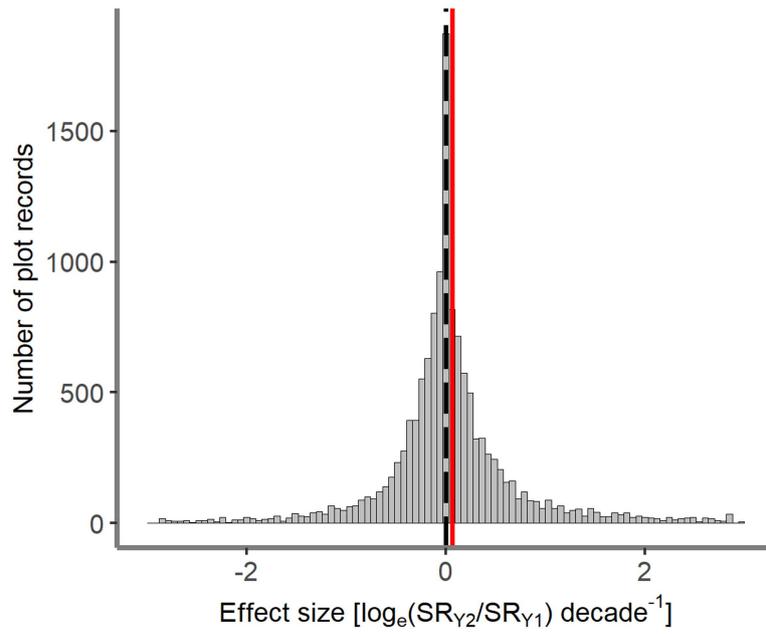
**Extended Data Fig. 3 | Temporal change of plant richness expressed per decade.** Interval comparisons of species richness (SR) in plot records between time points Y1 and Y2 of the start and end year, respectively, and divided by the length of the interval in decades ((Y2-Y1)*10) (n = 13,987). Estimated overall effect size was +0.062 according to a mixed effects model (p = 1.8 x 10^{-7}) with a 95% confidence interval between +0.039 and +0.086.
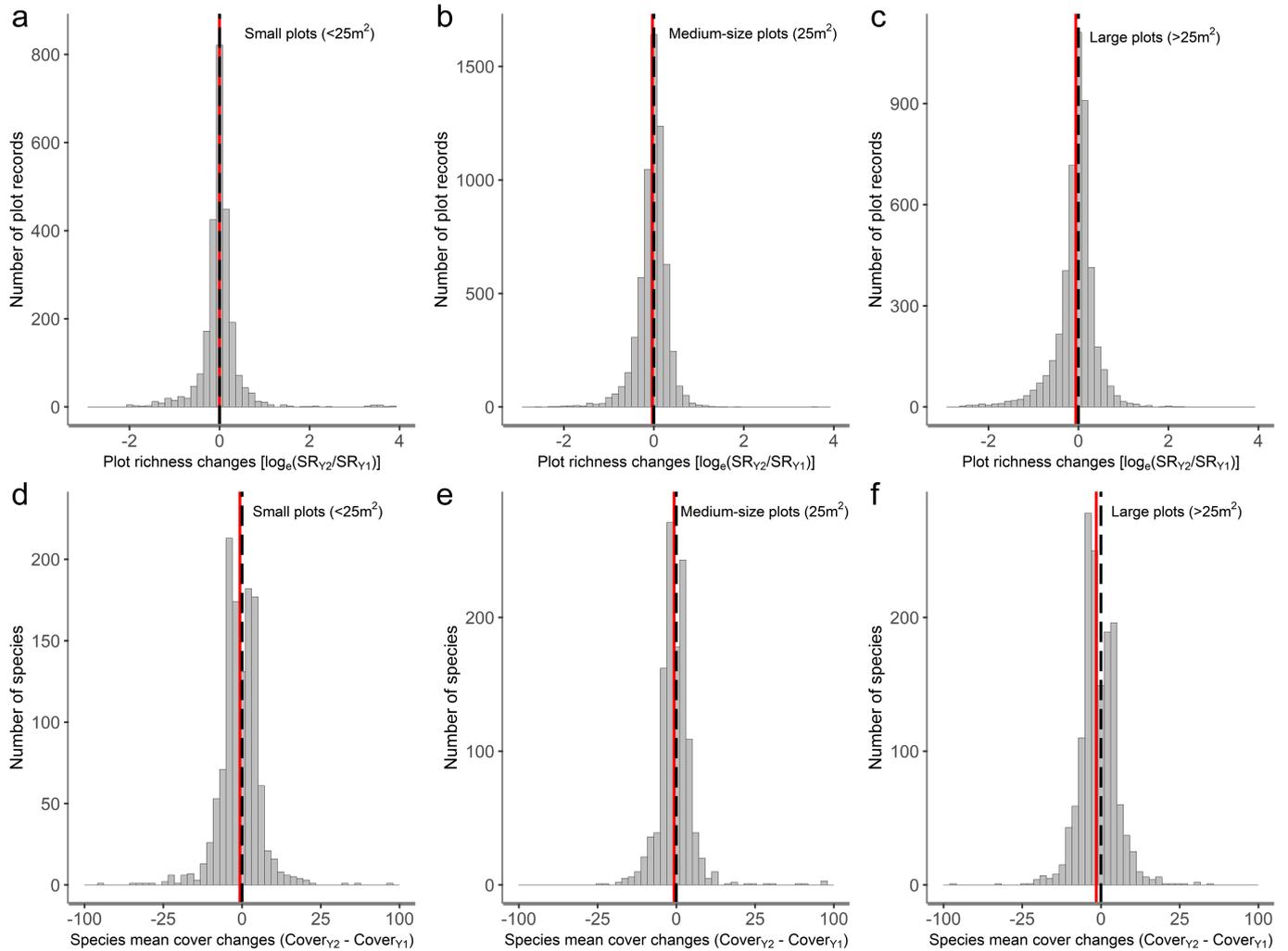
Extended Data Fig. 4 | Effect of plot surface area on plant diversity change. The temporal change of species richness (SR) in plot records (a–c) and mean cover change of species (d–f) is shown shown separately for small (> 25 m²), medium-size (25 m²) and large plots (>25 m²). The black dashed line shows zero change, while the red solid line in a)–c) shows the mean change of richness and in d)–f) the species' median change in cover in percentage points. According to a mixed effects model estimated mean overall effect size was in a) −0.03 (p = 0.064, df = 487), b) −0.031 (p = 1.55 x 10⁻¹³, df = 4,204) and c) −0.095 (p < 2 x 10⁻¹⁶, df = 9,124). In d)–f) plot Interval comparisons of the mean of all cover changes per species between time points Y1 and Y2 of the start and end year, respectively, are shown on an axis with a sign*square root-transformation. According to an exact binomial test estimated overall median of cover change was in d) −0.017 (95 per cent confidence interval −0.065 and −0.001), e) −0.019 (CI −0.043 and −0.006) and f) −0.26 (CI −0.134 and −0.050).
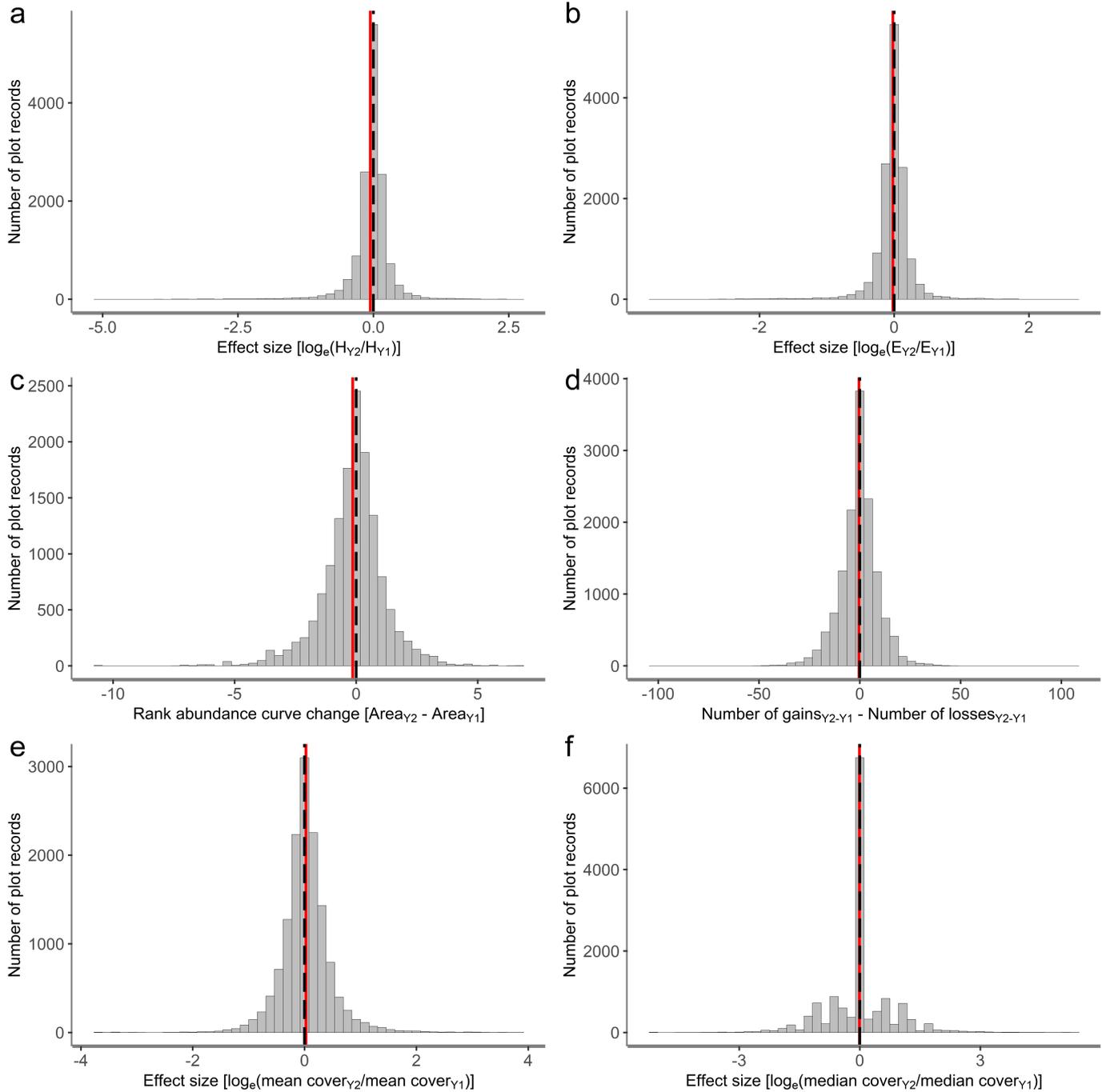
Extended Data Fig. 5 | Different measures of temporal change of plant diversity. The histograms show the interval comparisons of plot records between time points Y1 and Y2 of the start and end year, respectively. The black dashed line shows the zero change, while the red solid line shows the mean change as predicted from a mixed effects model. a) Change in Shannon's index of diversity (H). Estimated mean effect size for H −0.055 (p = 2.2 x 10⁻¹⁶, df = 5,462, 95% confidence interval −0.064 and −0.047). b) Change in Pielou's index of evenness (E). Estimated mean effect size for E −0.019 (p = 2.6 x 10⁻¹⁶, 95% confidence interval −0.024 and −0.015). c) Difference in the area under the rank abundance curves. Estimated mean difference −0.143 (p = 0.00211, 95% confidence interval −0.194 and −0.091). d) Difference in the number of cover gains and losses. Estimated mean difference −0.407 (p = 7.9 x 10⁻⁷, 95% confidence interval −0.569 and −0.246). e) Change in mean cover of all the species in a plot (in per cent covered ground). Estimated mean effect size for mean cover +0.025 (p = 1.0 x 10⁻¹⁰, 95% confidence interval +0.018 and +0.033). f) Change in median cover of all the species in a plot (per cent of covered ground). Estimated mean effect size for median cover −0.007 (p = 0.2984, 95% confidence interval −0.021 and +0.007).
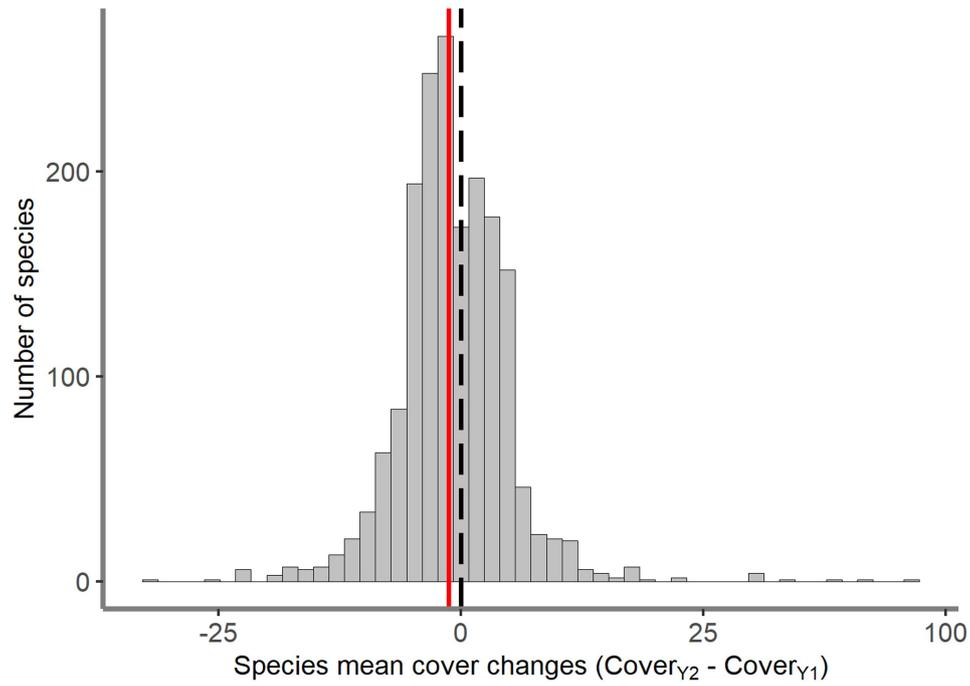
Extended Data Fig. 6 | Temporal change in mean cover change of all species. Plot Interval comparisons of the mean of all cover changes per species in percentage points between time points Y1 and Y2 of the start and end year, respectively, shown on an axis with a sign*square root-transformation. The black dashed line shows the zero change, while the red solid line shows the median change in cover across all species. All species in the dataset were included (n = 1,794). Estimated overall median of cover change was −0.0625 (95 per cent confidence interval −0.089 and −0.035) and significantly different from zero according to an exact binomial test (p < 0.001).
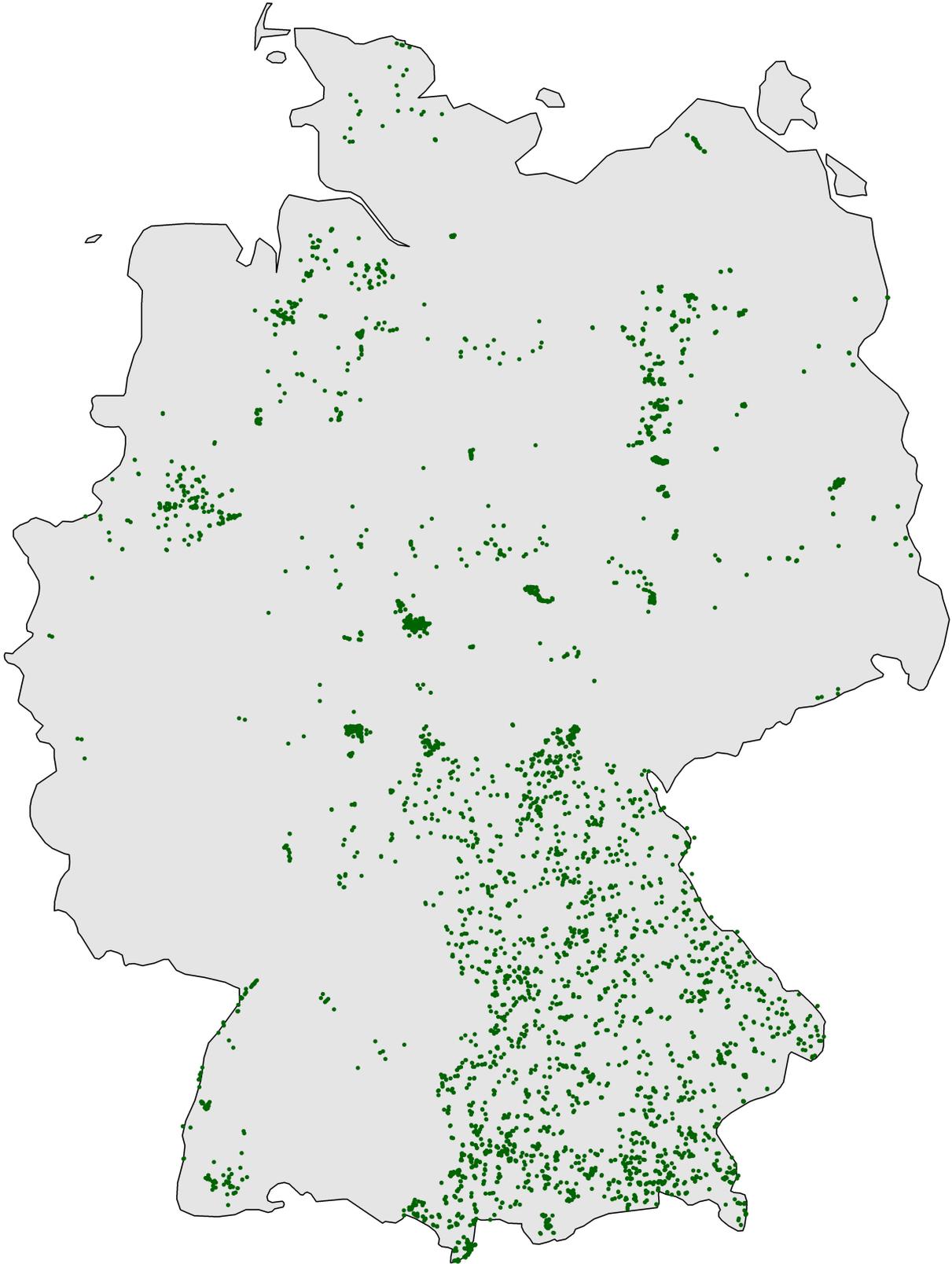
Extended Data Fig. 7 | Map of plot locations of all plots of all projects. One or several of the total of n = 23,641 plot records are summarized under the same plot resurvey ID (n = 7,738). Note that the more complete coverage of Bavaria resulted from including the grassland monitoring Bavaria which started in 2002[75]. The map was produced using rnaturalearthdata (free vector and raster map data at naturalearthdata.com).
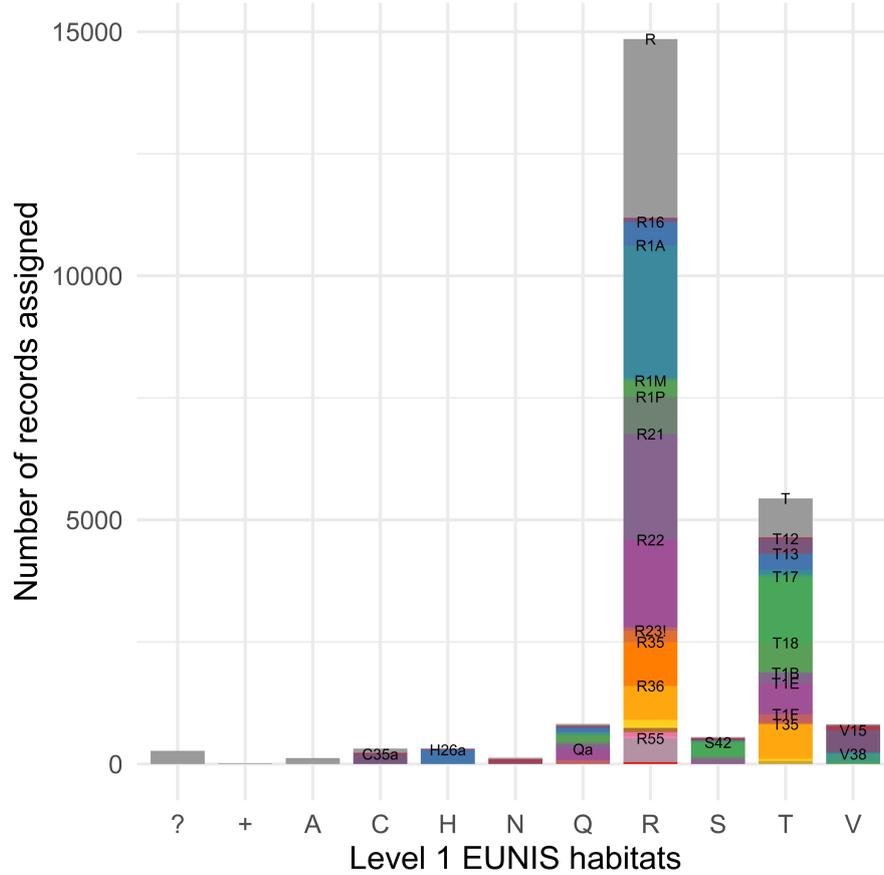
Extended Data Fig. 8 | Assignment of time-series plot records to EUNIS habitat types. Each time series was assigned to the habitat type by using the earliest plot record that matched with the level 3 EUNIS classification. The classification was based on the EUNIS-ESy expert system[56] using the R code implementation[57]. ?: plots not assigned to any level 3 EUNIS habitat type, +: assigned to more than one level 3 EUNIS habitat type, A: Marine habitats, C: Inland surface waters, H: Inland sparsely vegetated habitats or devoid of vegetation, N: Coastal habitats, Q: Wetlands, R: Grasslands and lands dominated by forbs, mosses or lichens, S: Heathlands, scrub and tundra, T: Forests and other wooded land, V: Vegetated man-made habitats, including arable land. Labels for EUNIS habitats were only printed at the top of the corresponding bar section when the number of assigned records was ≥150.

nature portfolio

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Sampling strategy	Compile all available data

Data collection	Compile all available data
Timing and spatial scale	Compile all available data
Data exclusions	Experimental treatments were excluded
Reproducibility	n/a
Randomization	n/a
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Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

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Flow Cytometry

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☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

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Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI   ☐ Used   ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

| Volume censoring | *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.* |

Statistical modeling & inference

| Model type and settings | *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).* |

| Effect(s) tested | *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.* |

Specify type of analysis: ☐ Whole brain   ☐ ROI-based   ☐ Both

| Statistic type for inference
(See Eklund et al. 2016) | *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.* |

| Correction | *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).* |

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

| Functional and/or effective connectivity | *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).* |

| Graph analysis | *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).* |

| Multivariate modeling and predictive analysis | *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.* |