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High plant diversity and slow assembly of old-growth grasslands

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A.N.N and J.W.V. conceived the study, conducted the literature review, and wrote the manuscript. A.N.N. performed the statistical analyses.

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Main Text

Abstract

Earth's ancient grasslands and savannas—hereafter *old-growth grasslands*—have long been viewed by scientists and environmental policymakers as early successional plant communities of low conservation value. Challenging this view, emerging research suggests that old-growth grasslands support substantial biodiversity and are slow to recover if destroyed by human land uses (e.g., tillage agriculture, plantation forestry). But despite growing interest in grassland conservation, there has been no global test of whether old-growth grasslands support greater plant species diversity than *secondary grasslands* (i.e., herbaceous communities that assemble after destruction of old-growth grasslands). Our synthesis of 31 studies, including 92 timepoints on six continents, found that secondary grasslands supported 37% fewer plant species than old-growth grasslands (log response ratio = -0.46), and that secondary grasslands typically require at least a century, and more often millennia (projected mean 1400 yr), to recover their former richness. Young (< 29 yr) secondary grasslands were composed of weedy species, and even as their richness increased over decades to centuries, secondary grasslands were still missing characteristic old-growth grassland species (e.g., long-lived perennials). In light of these results, the view that all grasslands are weedy communities, trapped by fire and large herbivores in a state of arrested succession, is untenable. Moving forward, we suggest that ecologists should explicitly consider grassland assembly time and endogenous disturbance regimes in studies of plant community structure and function. We encourage environmental policymakers to prioritize old-growth grassland

conservation and work to elevate the status of old-growth grasslands, alongside old-growth forests, in the public consciousness.

Significance Statement

The idea that grasslands can be ancient, particularly in climates that also support forests, is not widely recognized. Consequently, scientists and conservation planners often misinterpret old-growth grasslands to be low-diversity, successional vegetation, from which little is lost through conversion to tillage agriculture or tree plantations. We used a global analysis of herbaceous plant communities to show that after old-growth grasslands are destroyed, the recovery of plant diversity requires hundreds to thousands of years. Such slow rates of recovery underscore the need to replace outdated models of forest succession with models that emphasize the importance of fire, herbivory, and long periods of time to grassland biodiversity. This study offers evidence that old-growth grasslands, like old-growth forests, should be prioritized for conservation.

Main Text: Introduction

Grasslands (broadly defined, including savannas and open-canopy grassy woodlands) occupy 28% of the terrestrial biosphere (1), house a significant proportion of global biodiversity (2), and support the livelihoods of at least a billion people via multitude of ecosystem services (e.g. provisioning of water and carbon storage; 3). Given the global importance of grasslands, it is critical that we accurately conceptualize grassland ecological dynamics to advance our understanding of plant community responses to environmental change. Hindering such advances, the idea of climate-determined succession (4), one of the dominant ecological paradigms of the past century, underemphasizes two ubiquitous aspects of grassland ecology and evolution: fires and large herbivores (5).

Fire and herbivores shaped the ecology and evolution of Earth's grasslands for millions of years before the existence of humans (6). Through consumption of aboveground plant biomass, these two agents of endogenous disturbance (7, 8) maintain grasslands in places where the climate and soils are suitable for the development of forests (9). When interpreted through the lens of climatic determinism, with a focus on trees rather than herbaceous plant community dynamics, frequent fire and herbivory can appear to reset, or arrest, ecological succession (10, 11). This successional narrative has contributed to a crisis in grassland conservation: around the world disturbance-dependent grasslands are widely misclassified as degraded forests (12, 13), overlooked for their conservation value (14), targeted for agricultural conversion (15), and viewed as opportunities for carbon sequestration through tree planting and fire exclusion (i.e., afforestation and woody encroachment) (16, 17).

The old-growth grassland concept (18)—modelled on parallel ideas in forest ecology and conservation (19)—is a direct challenge to the narrative that most of earth's grasslands are successional communities that ought to become forests (11; 20). Of primary importance, the concept posits that old-growth grasslands are ecologically distinct from recently formed (secondary) grasslands (18). It is also important to note that fire and megafaunal herbivory—the endogenous disturbances that maintain old-growth grassland diversity (8)—are detrimental to many old-growth forests (e.g., 21). Further, readily visible indicators of old-growth forests, such as trees with large girths, are inapplicable to old-growth grasslands, where signs of antiquity are often underground (18). Indeed, old-growth grasslands are characterized by slow-growing, long-lived herbaceous plants, with a suite of traits, including underground storage organs, bud banks, and rhizomes, which enable resprouting and clonal growth after fire and herbivory (22, 23).

Although old-growth grasslands include some of the most biodiverse terrestrial ecosystems, there has been no global-scale test of whether old-growth grasslands are, in fact, more species-rich than secondary grasslands. Several examples suggest that high species richness is characteristic of old-growth grasslands. The savannas of the South American Cerrado support 4800 endemic plant and vertebrate species (24). The Shola grasslands of India, home to endangered Asian elephants and Bengal tigers, are rich with herbaceous plants (278 species) (25). The world record for local-scale plant species richness (89 vascular species/ m^2) is held by a montane grassland in Argentina (26). At the 100 to 1000-m^2 scales, fire-dependent grasslands of the North American Coastal Plain can be as rich in vascular plant species as tropical forests (14). While illustrative of the potential diversity of grassland plant communities, these examples do not tell us how quickly old-growth grassland plant diversity recovers after intensive land-use change, such as agriculture or afforestation.

To test the relevance of the old-growth grassland concept to our understanding of global patterns of herbaceous plant diversity, we conducted a meta-analysis of 31 pairs of old-growth grasslands and secondary grasslands on six continents (Fig. 1). Because the application of the term ‘old growth’ to grasslands is recent, we included studies that used a variety of synonymous adjectives, including: ancient, intact, native, natural, pristine, reference, remnant, semi-natural, and undisturbed (*SI Appendix,* Fig. S1). For our analysis, we compared species richness between pairs of old-growth and secondary grasslands using a random effects model of the log response ratio [lnRR, calculated as $\log_e$(secondary grassland richness/old-growth grassland richness)] (27). To determine the rate at which secondary grasslands recover the species richness of old-growth grasslands, we conducted a mixed-effects linear meta-regression (27) of 92 secondary grassland ages (range 1 to 251 years) extracted from the 31 studies. As is standard in meta-analyses (27), we weighted each study by the inverse of the associated variance and evaluated the robustness of our findings through assessments for publication bias and sensitivity (see Methods).

Following the core meta-analysis, we used data from a subset of studies to better understand how variation in species richness and grassland age relate to plant community composition. We first assessed the relationship between total species richness in secondary grasslands and the recovery of old-growth grassland community composition (n = 10 studies). We then assessed the relationship between grassland age and the number of weedy species (including ruderals and exotics; n =11 studies with 29 timepoints). In combination, we expect these analyses of grassland species richness, assembly time, and community composition to validate patterns that many grassland ecologists have recognized in specific ecosystems around the world (e.g., 28, 29). Through global meta-analysis, we hope to expand recognition of the high species diversity and slow assembly of old-growth grasslands more broadly among ecologists, environmental policymakers, and the public.

Results

Our results showed that secondary grasslands support 63% of the species richness of old-growth grasslands (95% CI: 53%, 76%; global weighted mean lnRR: -0.46, 95% CI: -0.64, -0.28; Fig. 2). For individual studies, lnRR ranged from -1.8 to 0.4, with only 2 of 31 studies reporting secondary grasslands to be richer than old-growth grasslands (Fig. 2). The weighted mean lnRR was associated with a high level of between-study heterogeneity (Q = 230, I^2 = 90%, P < 0.0001), which is typical of ecological meta-analyses (30). Post-hoc assessments suggested that our estimate of the global weighted mean (i.e., lnRR = -0.46) is robust (*SI Appendix,* Table S1, *SI Appendix,* Fig. S2-S5). Tests for publication bias (26) were negative (*SI Appendix,* Table S2, *SI Appendix,* Fig. S6-S7).

Secondary grassland age was weakly, but positively, related to the recovery of plant species richness (Fig. 3, P = 0.0001, R^2 = 0.041). The upper bound of the 95% confidence interval for the meta-regression model yielded a minimum global recovery time (to lnRR = 0) for plant species richness of ~160 years. Extrapolation of the regression equation, which should be interpreted cautiously, projected a mean time of ~1400 years for richness to recover. At the last time point for which we have data (i.e., 251 years, which is the oldest value permitting interpolation), the regression equation predicted secondary grasslands to recover 84% of the richness of old-growth grasslands (lnRR = -0.17).

Even as richness increased with time, the communities of plants recolonizing secondary grasslands remained distinct from those of old-growth grasslands. Based on regression of the subset of ten studies that reported community similarity indices (Fig. 4*A*), we projected that recovery of species richness (i.e., to lnRR = 0) would equate to just 43% (95% CI: 31%, 56%) compositional similarity between old-growth and secondary grasslands. These persistent differences in community composition can be explained in part by a preponderance of weedy species in secondary grasslands. Regression of 29 timepoints from 11 sites indicated that in the initial 29 to 130 yr of recovery (range based on lower 95% CI and mean regression equation for lnRR=0; Fig. 4*B*) secondary grasslands supported more weedy species than did old-growth grasslands.

Discussion

By demonstrating that secondary grasslands support just 63%, and are missing 37%, of the herbaceous plant species richness of old-growth grasslands (Fig. 2), this meta-analysis provides support for the applicability of the old-growth grassland concept at the global scale (18). Evidence of the slow assembly of old-growth grasslands (Fig. 3) underscores recent calls to move away from the view of most grasslands as a successional stage (11), toward recognition that endogenous disturbances can sustain species-diverse grasslands for very long periods of time in climatic zones that can also support forests (5, 9, 10, 17, 22). Compared to old-growth forests, which are widely recognized and intensively studied (31, 32), we still know relatively little about old-growth grasslands. We hope that these results will motivate future studies, analogous to research on secondary forests (31), to better understand the recovery rates of secondary grasslands with different land-use histories (*SI Appendix*, Fig. S8), and to compare grasslands of the tropics to those of temperate latitudes (*SI Appendix*, Fig. S9) (22).

In our analysis, we focused on one aspect of plant diversity—species richness—a community metric available from the modest number of 31 grassland studies that included both old-growth and secondary grasslands. Because species richness figures prominently in the application of community ecology to questions of global change [e.g., (33)], we suggest that recognizing old-growth grasslands as distinct from secondary grasslands will improve our understanding of the relationships among plant diversity, community assembly time, and ecosystem functioning (18, 34). Doing so would pull together disparate, but clearly related, lines of research, such as those framed around agricultural legacies (29), fire exclusion (17), declines of native megafauna (35), woody encroachment (36), and nutrient pollution (37), to help clarify the distinct ecological consequences of human activities for old-growth versus secondary grasslands.

Our results show that old-growth grasslands, once destroyed, require at least a century, and more typically millennia, to recover their plant species richness (Fig. 3); full recovery of plant community composition will take even longer (Fig. 4*A*). To be clear, the recovery of species richness is not the same as the recovery of community composition; two communities can have the same number of species, while the identity of those species can be quite different (38). For the subset of sites that provided compositional data, recovery of species richness equated to just 43% similarity in community composition between secondary and old-growth grasslands (Fig. 4*A*). Thus, our estimate of the time required for secondary grasslands to attain the richness of old-growth grasslands (160 to 1400 yr; Fig. 3) is certainly less than the time required for secondary grasslands to recover the community composition (i.e., the full suite of species and abundances) of old-growth grasslands. This echoes research on secondary tropical forests, where tree species richness typically rebounds within 50 yr, but recovery of the composition of old-growth forests requires many centuries or longer (39).

One reason that richness is thought to recover more rapidly than community composition is because weedy species are quick to colonize secondary grasslands (e.g., 40). Consistent with this idea, and based on the 11 studies that reported data on weedy plants (including, ruderal and non-native species), we found that compared to old-growth grasslands, young secondary grasslands supported more weedy species. Evidence that elevated numbers of weedy species persisted for 29 to 130 yr in secondary grasslands (Fig. 4*B*) underscores recent calls for grassland experiments to consider compositional changes over longer periods of time [i.e., >10 yr (41)]. From our analysis, it appears that while weedy species partially compensated for reduced species richness in secondary grasslands, certain species characteristic of old-growth grasslands remained missing even after many decades to centuries (Fig. 3, Fig. 4*A, B*).

Why are certain old-growth grassland species missing in secondary grasslands? A plausible explanation is that above-ground disturbances (i.e., fire and herbivory), which select for persistence in old-growth species, are fundamentally different from the anthropogenic disturbances, like tillage agriculture, that destroy underground organs and select for secondary grassland species with high colonization ability (18). To explore this possibility, we revisited the results and discussions of the 31 studies included in the meta-analysis and found that the missing species most frequently described by authors (*SI Appendix*, Dataset 1) were native perennial grasses (typically with C_4 photosynthesis) and native perennial forbs (often species with underground storage organs). Missing species were also described as fire-promoting and shade-intolerant, with high capacity to resprout. Authors further described missing species as being stress-tolerant with poor colonization ability, producing seeds dispersed by gravity or ants, forming limited seed banks, and relying on clonal growth or asexual reproduction. In addition, authors noted missing species that were of conservation concern in specific regions. These included: medicinally important species in Africa; annual hemiparasites in Asia; composites (Asteraceae) and legumes (Fabaceae) in North and South America; perennial sedges and orchids, and threatened IUCN Red-list species in Europe; woody sub-shrubs (underground trees) in South America, and endemic grasses in Australia (*SI Appendix*, Dataset 1). These descriptions match the key functional types of old-growth grassland species that are thought to be most vulnerable to anthropogenic environmental change (22).

In addition to functional traits (e.g., persistence-colonization trade-off; 42, 43), a multitude of ecological mechanisms likely contribute to the lower richness and slow recovery of secondary grasslands relative to old-growth grasslands. Indeed, a central focus of grassland restoration ecology is to identify these mechanisms and overcome the limitations to old-growth grassland community assembly (44). In some ecosystems, landscape effects, such as spatial isolation (45) or limited habitat connectivity (46), restrict the arrival of plant propagules. In others, site-level conditions, such as severely altered soil conditions (47) and species interactions (e.g., priority effects and plant-soil feedbacks; 48) limit the establishment of old-growth grassland species. Also important, but often overlooked in grassland restoration studies (49), is the role of vegetation-disturbance feedbacks (e.g., 50; 51). Plant communities determine the quantity and quality of biomass available for fire and herbivores to consume, and in turn fire and herbivores influence grassland community composition via selection on plant traits (52). Given their differences in species composition (Fig.4*A, B*), we should expect pairs of old-growth and secondary grasslands to also differ in aspects of their disturbance regime, such as frequency, seasonality, and intensity, even if they experience the same disturbance type (i.e., fire, grazing, or haying). In sum, altered disturbance regimes, reinforced by vegetation-disturbance feedbacks (53), should be considered among the probable mechanisms for reduced species richness and slow compositional recovery of secondary grassland communities (8).

Conclusion

In light of the high diversity of old-growth grasslands (Fig. 2) and the many documented challenges to their restoration (8), we encourage environmental policymakers to give old-growth grasslands equal consideration as old-growth forests (32) in efforts to conserve earth's biodiversity. We are particularly concerned that recent research and emerging land-use policies, meant promote tree-planting for carbon sequestration, are a threat to undervalued grassland biodiversity and ecosystem services (16, 17). Fundamental to these afforestation efforts has been the assumption that old-growth grasslands that occur where climate-vegetation models suggest forest as the potential vegetation must be degraded. Our analysis shows that the reality on the ground is much more complicated. Indeed, most of the species-rich old-growth grasslands in this analysis occur in climates that can support forests [Fig. 1; (5)]. We urge conservation initiatives to safeguard against the conversion of old-growth grasslands for tree planting or tillage agriculture, to maintain biodiverse grasslands with frequent fires and megafaunal herbivores, and to emphasize the recovery of grassland plant communities in efforts to restore Earth's biodiversity.

Materials and Methods

Literature search and screening

To identify studies that compared species richness in old-growth grasslands and secondary grasslands, we conducted a literature search of peer-reviewed journal articles in the Web of Science database (27, 54). This initial Web of Science search yielded a total of 8336 articles. We examined the titles of these 8336 articles for relevance and retained 745 articles. We then screened the abstract (and methods in some cases) of these 745 articles to arrive at a shortlist of 99 articles for detailed examination. We then examined in detail the full texts of these 99 articles, which resulted in a final set of 31 articles that met our eligibility criteria (described below) for inclusion in the analyses (*SI Appendix,* Fig. S1) (55).

For the initial search, we used the advanced search function in Web of Science to identify articles published from 1 January 1900 to 14 November 2018 that fit the following topic search (TS) criteria (i.e., terms found in titles, abstracts, and key words):

TS = (savanna* OR grassland* OR woodland* OR pine OR pinus OR eucalypt* OR cerrado OR prairie OR veld* OR steppe) AND TS=(herb* OR grass* OR forb* OR understor*) AND TS=(richness OR diversity) AND TS=("old growth" OR secondary OR succession* OR remnant* OR "old field" OR restor* OR reference OR abandon* OR "post agric*" OR "woody encroach*" OR mine OR mining OR degrad* OR pasture OR plantation OR afforest*)

We subsequently scrutinized the articles to ensure that they met the following criteria. 1) Study sites were grasslands, broadly defined to include savannas and open-canopy grassy woodlands (22). As such, the studies in our analysis encompass herbaceous-dominated ecosystems with scattered trees that are often called ‘forests’ or ‘woodlands’ (12). 2) Studies included old-growth grasslands (18) that were either clearly described in the article or that we were able to verify through correspondence with the authors. Because there is a wide range of synonymous terminology for old-growth grasslands in the literature, we screened studies to ensure that there were no major human-induced structural or functional alterations to the historical herbaceous plant communities, and that current ecosystem management closely resembled historical, endogenous disturbance regimes (8). As such, we included study sites that supported large herbivores (domestic livestock and/or native megafauna), were burned with prescribed fire or wildfire, or where other regular aboveground disturbance (i.e., mowing or haying) served as a surrogate for fire and herbivory (18). 3) Studies included secondary grasslands (11) on sites previously occupied by old-growth grasslands that had been destroyed by tillage agriculture, tree plantations, or other intensive land uses. 4) Studies reported data for herbaceous plant species richness for both old-growth and secondary grasslands. 5) Study plots were not treated with nutrient additions (e.g., nitrogen or phosphorous fertilizers). 6) Studies were conducted in a unique location; where multiple papers provided data for the same study location, we excluded all but the most complete (i.e., best replicated, longest duration) paper for that location. 7) We only included studies on 'actively restored' grasslands (i.e., restoration treatments such as sowing seed mixtures, soil or hay transfer) if the study included a control treatment of 'passive restoration' (i.e., secondary grassland communities assembling without propagule additions). For such studies, we only extracted data from the passively restored secondary grasslands and the paired old-growth grasslands.

Data extraction

Response variables

Total species richness: We extracted the mean total species richness per unit area for the old-growth grasslands and secondary grasslands in each study. For studies that only presented richness in figures (n = 20), we calculated the mean richness using the image analysis software ImageJ (56). For studies (n = 2) that reported median richness but not mean, we used the median, since these studies had either non-normal data (as verified from figures) or a large (>25) sample size (57). In cases where studies appeared to have measured richness, but did not report richness, we contacted the authors (n = 4).

Weedy species richness: For each study, we determined whether the authors presented data on weedy species. We included any group of species that the authors identified using one, or a combination of, the following terms: ruderals (including annuals, perennials or both), weedy species, arable weeds, alien species, exotic species, or invasive species.

Using the same approaches as for total species richness (described above), we were able to extract weedy species richness from 11 studies, yielding 29 time points.

Compositional similarity: 10 out of the 31 studies reported the similarity of old-growth and secondary grassland plant communities as Jaccard's (n = 4), Bray-Curtis (n = 3) and Sorensen's (n = 3) indices. For studies that reported dissimilarity we converted the index to similarity (i.e., 1-dissimilarity). Because these indices range from 0 to 1, we analysed them without further transformation (58).

<u>Predictor and moderator variables</u>

Location: We used the latitude and longitude of the sites reported in the methods of each study to map study locations (Fig. 1*A*) using Q-GIS v 2.10.1 (59).

Precipitation and temperature: We obtained mean annual precipitation and temperature for each location from the WorldClim2 database (60). In cases where the author-reported precipitation deviated more by more than 100 mm (n = 4 cases) from the WorldClim2 data, we used the author-reported values. We plotted mean annual precipitation versus mean annual temperature (Fig. 1*B*) with Minitab (Minitab I.N.C., Pennsylvania State University, USA).

Type of secondary grassland: We used authors' descriptions of land-use history to classify secondary grasslands into one or more of the following categories for supplemental analyses: tree-plantations/woody encroachment; tillage agriculture; soil excavation; planted pasture; or other (*SI Appendix,* Table S3, *SI Appendix,* Fig. S8).

Age of secondary grassland: We determined the assembly time (in years) for each secondary grassland based on author-reported time since the last grassland-damaging [i.e., exogenous (8)] disturbance or time since land abandonment. If secondary grasslands were classified by a range of ages, we used the mean of the range. For open-ended classes, we approximated the value to get a conservative estimate of age [e.g. for Öster *et al.* (61), we considered the class “<10 years” to be 5 years and “>50 years” to be 55 years]. In the one study with multiple sites of different ages (i.e., Brudvig *et al.* (62), with sites of age 90 years, 69 years, 58 years) we calculated a weighted mean age (weighted by sample size). For studies (n = 3) that provided a large number (>30) of data points across a range of secondary grassland ages, we extracted a subset of discrete time points to represented the range. For the two (of the 92 secondary grassland timepoints) that were sampled after one growing season, we coded their age as one year rather than as a fraction of a year, to avoid giving them undue weight in our log(time) regression (Fig. 2).

Statistical analyses

<u>Effect size</u>

To compare the differences between old-growth and secondary grassland species richness, we calculated the log response ratio (lnRR) as the effect size (63). The lnRR has been widely used in ecology (64) and has several desirable properties. A major advantage of lnRR over other effect-size metrics is that it does not require variance data for computation (63). For our calculations, $\mathrm{lnRR} = \log_e (\bar{Y}_s / \bar{Y}_o)$ where $\bar{Y}_s$ = species richness for secondary grasslands, $\bar{Y}_o$ = species richness for old-growth grasslands. Thus, values of

$\ln RR < 0$ indicated old-growth grasslands have greater richness than secondary grasslands, whereas $\ln RR > 0$ indicated secondary grasslands are richer than old-growth grasslands, and $\ln RR = 0$ indicated both grassland classes are equally rich in species. For studies that provided more than one data point by time, we calculated the composite effect size per study (65).

Variance and weights

We calculated the variance of lnRR (V_{lnRR}) based upon reported sample sizes (66) using $V_{lnRR} = (N_s + N_o)/(N_s \times N_o)$, where N_s is the sample size for secondary grasslands and N_o is the sample size for old-growth grasslands. By calculating V_{lnRR} in this manner, we were able to standardize the variance estimates across studies and obtain estimates for those studies that did not report variance, or that had pseudo-replicated or otherwise poorly described study designs (67, 68) (*SI Appendix,* Table S1). We weighted the lnRR for each study by the inverse variance, such that studies with higher variance were given lower weights (27). We used OpenMEE (69) to perform the meta-analysis (Fig. 2), including tests for heterogeneity, publication bias, and sensitivity. We considered meta-analysis results to be statistically significant (at $\alpha = 0.05$) if the 95% confidence intervals (CI) of the overall mean lnRR did not include zero.

Handling non-independent observations

Several studies reported multiple (non-independent) data points ($n = 18$), unbalanced study designs, or both. We calculated the variance of the composite lnRR for studies with unequal sample sizes (and thus unequal variances) as $(1/n)^2 (\sum V_{i,\, i = 1 \text{ to } n} + 2\sum_{i,j} (r_{ij} \sqrt{V_i} \sqrt{V_j}))$, where n=number of observations in the study and r_{ij} = correlation coefficient (covariance) between the pair of effect sizes under consideration (27, 65). If a study had equal sample sizes (and thus equal variances), the above formula was simplified to: (V/n) × Variance Inflation Factor (VIF) (27), where VIF = 1 + (n-1) × r. This formula thus takes into account the non-independence of multiple observations reported by a single study through the covariance factor. The possible values of 'r' range from 0 to 1 (assuming the correlation is positive), but we cannot know the true value of r in the selected studies. To apply $r = 0$ would be to assume that observations are independent, and result in an underestimation of the variance. Conversely, to apply $r = 1$ would assume perfect (100%) correlation and certainly overestimate the variance (65). We chose to apply $r = 0.5$ as a plausible value for our analysis (65), and verified that other plausible values (i.e., $r = 0.25$ and 0.75), yielded similar results (*SI Appendix,* Table S1, *SI Appendix,* Fig. S2, S3).

Between-study heterogeneity

We calculated the weighted overall mean effect size (lnRR) for $n = 31$ studies using a random effects model, with between-study variance estimated using the restriction maximum likelihood (REML) approach (70). We also used REML to calculate effects by sub-groups using moderator variables (as in *SI Appendix,* Fig. S8). To test if there was significant heterogeneity associated with the effect sizes, we computed the Q statistic (a measure of between-study variance), and tested this against a X^2 distribution [with n-1 degrees of freedom (dof), n = number of studies] (27). Because the Q statistic has low power and is not intuitive by itself (27, 65), we also report heterogeneity with the I^2

statistic [approximately equal to 100 × (Q-dof/Q)], which yields a more intuitive measure of heterogeneity, from 0 to 100% (65).

Sensitivity analyses

We performed several sensitivity analyses to verify that the meta-analysis results were robust. 1) To determine if using the pseudo-replicated (within-study) sample sizes affected our conclusions, we performed a post-hoc sensitivity analysis by repeating our meta-analysis without the two studies with highest sample sizes (and consequently highest weights) (*SI Appendix,* Table S1, *SI Appendix,* Fig. S5). Given the overall result did not change with exclusion of these highly weighted studies, this analysis supported our use of study sample sizes to estimate variances (71, 72). 2) To assess the sensitively of our results to our assumption of covariance of $r = 0.5$, we repeated the analysis using other plausible covariance values ($r = 0.25$, $r = 0.75$) and verified that results did not deviate substantially with changes in r [as in (73); *SI Appendix,* Table S1, *SI Appendix,* Fig. S2, S3]. 3) Given the high between-study heterogeneity obtained from the weighted model, and to further assess the sensitivity of the calculated overall mean lnRR to the weighting of studies, we calculated an unweighted mean lnRR (27) (*SI Appendix,* Fig. S4).

Meta-regression of species richness and time

We tested for the effect of secondary grassland age on lnRR using a meta-regression mixed-effect model that treated each study as a random effect [i.e., REML method; (27)]. To understand the proportion of variance explained by the regression model (27), we calculated R^2 as $Q_M/(Q_M + Q_E)$. We performed the meta-regression using the *metafor* package (70) in R (v 3.6.3) (74). We created all the regression analyses figures using the *ggplot2* package (75) in R (v 3.6.3) (74).

Regression analyses of compositional similarity and weedy species

To understand the relationship between the lnRR of total species richness and compositional similarity of secondary grasslands to old-growth grasslands, we performed fixed-effect linear regression in the R base package *stats* (v 3.6.3) (74) for the $n = 10$ studies that reported compositional similarity data. To analyse how lnRR of weedy species richness changes with secondary grassland age, we constructed a linear mixed-effect model to predict weedy species lnRR (with secondary grassland age as the fixed effect and study as the random effect) using the *nlme* package (76) in R (v 3.6.3) (74) for $n = 11$ studies (and 29 timepoints). For both these analyses, we chose not to weight the data points (as would be done in meta-regression) given that these studies represent a relatively small subset of the full meta-analysis dataset.

Exploratory models of unexplained variance in lnRR

Given the high between-study heterogeneity ($I^2 = 90\%$, Fig. 2), we explored whether unexplained variance in lnRR was attributable to variables that were not part of our core hypothesis [as in (77)]. We constructed linear mixed-effect models, with study as a random effect, to predict lnRR based on: continent, latitude, secondary grassland age, MAP, MAT, type of secondary grassland, and sample area (*SI Appendix,* Table S3). We generated a starting model using the *nlme* package (76) in R (v 3.6.3) (74). We then used a step backward selection method, based on Akaike Information Criteria (AIC), to identify the best model [*MASS* package (78)]. The marginal R^2 value associated with each model was calculated separately by using the *PIECEWISESEM* package (79). To visualize the relationships between lnRR and the continuous and categorical predictor variables retained in the top models (ΔAIC < 2 compared to the best model; *SI Appendix,* Table S3), we presented results by secondary grassland type (*SI Appendix,* Fig. S8) and conducted a meta-regression for latitude (*SI Appendix,* Fig. S9).

Publication bias

To assess publication bias, we first performed non-parametric correlation tests (Spearman's rho and Kendall's tau) between the standardized effect sizes and the composite variance as a substitute (27) (*SI Appendix,* Table S2). A significant positive or negative correlation would indicate publication bias (27). Second, we performed a Cumulative Meta-Analysis (CMA) to assess publication bias (*SI Appendix,* Fig. S6) with publications sorted by year and using a random-effects model (80). The CMA re-calculates the cumulative effect size after adding studies, one by one (80). In the end, if the effect sizes do not converge with the calculated effect size, this would suggest bias (27). Lastly, to assess publication bias, we calculated the Rosenberg's fail-safe number — i.e., the number of studies with the same weight as the average of the current set of studies that would be needed to render the results non-significant at α=0.05 (81); results are considered unbiased if the number is high (> 5× n + 10) (65). We chose this metric because it uses a weighted approach, whereas alternative metrics (e.g., Rosenthal's and Orwin's) use an unweighted approach (27). To test if variation in sampling area affected lnRR, we conducted a regression between plot size in each study and lnRR (*SI Appendix,* Fig. S7).

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Data availability statement: All data used for the analyses is provided in *SI Appendix* Dataset 1

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Figure Captions:

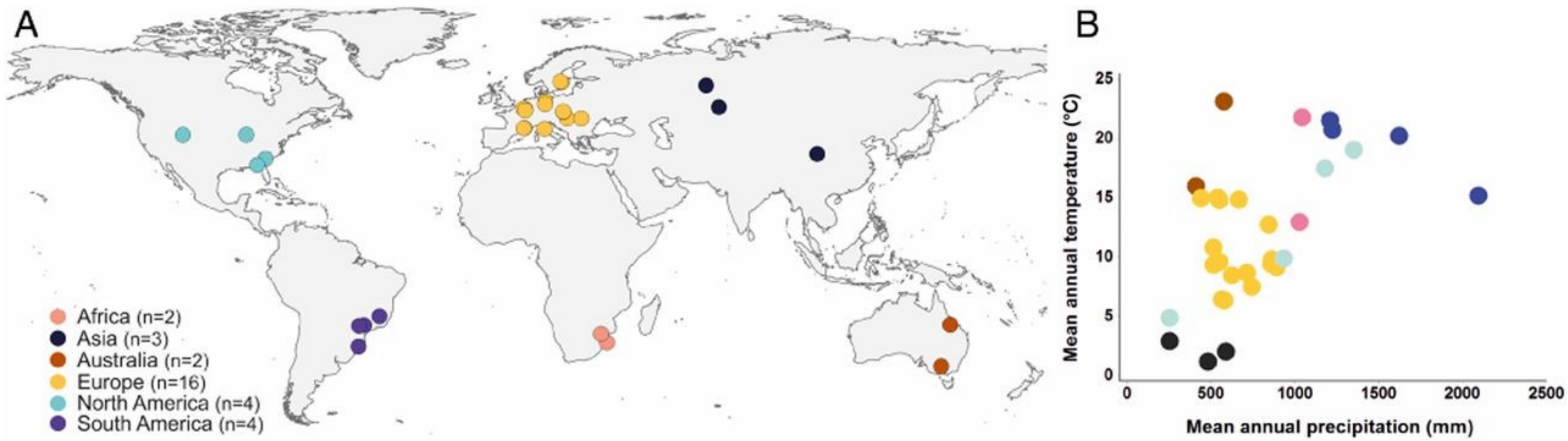
Figure 1. Geographic and climatic distribution of paired old-growth grassland and secondary grassland study sites. (*A*) Locations of the 31 studies included in the meta-analysis. (*B*) Bi-variate plot of mean annual precipitation and mean annual temperature for each study location.

Figure 2. Global comparison of species richness in old-growth grasslands and secondary grasslands. The 31 plant community studies (left column) are listed alphabetically by continent and author, and are marked by the type of aboveground disturbance that currently maintains old-growth grasslands at each site. For each study, boxes and solid lines display the natural logarithm of the response ratio [$\log_e$ (secondary grassland richness/old-growth grassland richness)] and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study (see methods). Response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean response ratio (-0.46, I^2 = 90%, P = 0.0001) equates to secondary grasslands supporting 63% of the richness of (or 37% fewer species than) old-growth grasslands (ends of the diamond indicate the 95% CI: -0.64 to -0.28, equivalent to 53% and 76%). See *SI Appendix*, Dataset 1 for full study citations.

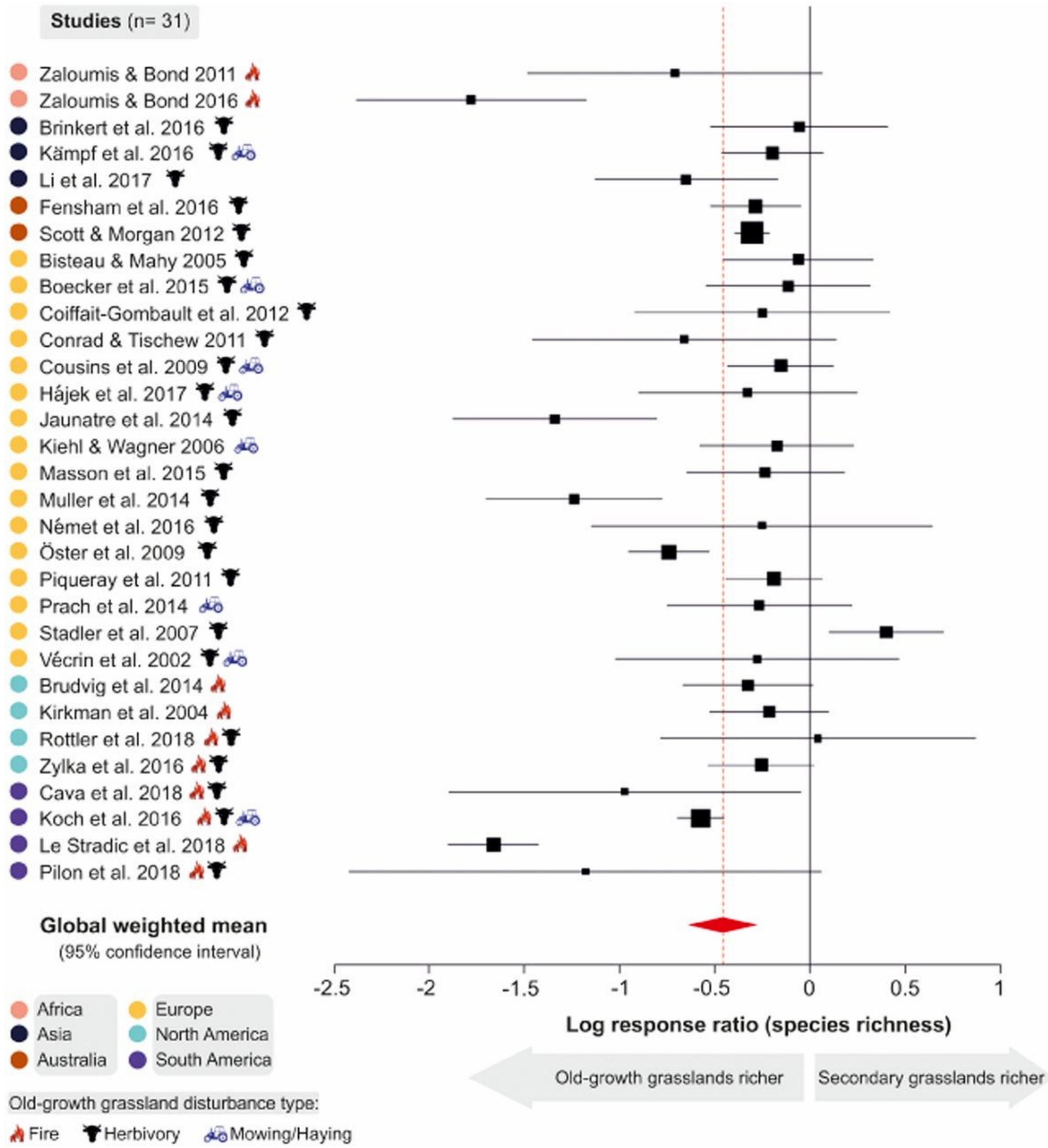
Figure 3. Relationship between secondary grassland age and the recovery of old-growth grassland species richness. Black circles represent secondary grassland age (n = 92, range: 1 to 251 years, extracted from n = 31 studies) and are scaled in proportion to their weight (see Methods; note age is represented on a $\log_{10}$ scale). The meta-regression model accounts for this weight and the random effect of each study location. The regression equation, lnRR = 0.2279 [$\log_{10}$(secondary grassland age)] - 0.7201 (R^2 = 0.041, P = 0.0001), is displayed as a solid black line; grey shading indicates the 95% confidence interval. The horizontal dashed line indicates the response ratio at which secondary and old-growth grassland species richness is equal (lnRR = 0). Response ratios less than zero indicate secondary grasslands that have fewer species compared to old-growth grasslands.

Figure 4. Indicators of plant community composition in relation to secondary grassland species richness and age. (*A*) Relationship between the log response ratio (lnRR) of total species richness and the compositional similarity between secondary and old-growth grassland communities. Data are from n = 10 studies that reported similarity indices. The regression equation, similarity = 0.2681(lnRR) + 0.4332 (R^2 = 0.536, P = 0.016), is displayed as a solid black line; grey shading indicates the 95% confidence interval. The horizontal dashed line (similarity = 0.5) indicates the level at which secondary grasslands are 50% similar to old-growth grasslands in species composition. At lnRR = 0, secondary and old-growth grasslands are equal in total species richness. (*B*) Relationship between the lnRR of weedy species richness and age of secondary grasslands. The mixed-effect

regression model is based on the $n = 11$ studies (random effect) that reported weedy species richness for $n = 29$ timepoints (age as fixed effect). The regression equation, $\ln RR = -0.8597\,[\log_{10}(\text{secondary grassland age})] + 1.8164$ ($R^2 = 0.274$, $P < 0.0001$), is displayed as a solid black line; grey shading indicates the 95% confidence interval. At $\ln RR = 0$, secondary and old-growth grasslands are equal in weedy species richness.


A
B
Africa (n=2)
Asia (n=3)
Australia (n=2)
Europe (n=16)
North America (n=4)
South America (n=4)
Mean annual temperature (°C)
Mean annual precipitation (mm)
0
5
10
15
20
25
0
500
1000
1500
2000
2500

699 Fig. 1


Studies (n= 31)
Zaloumis & Bond 2011
Zaloumis & Bond 2016
Brinkert et al. 2016
Kämpf et al. 2016
Li et al. 2017
Fensham et al. 2016
Scott & Morgan 2012
Bisteau & Mahy 2005
Boecker et al. 2015
Coiffait-Gombault et al. 2012
Conrad & Tischew 2011
Cousins et al. 2009
Hájek et al. 2017
Jaunatre et al. 2014
Kiehl & Wagner 2006
Masson et al. 2015
Muller et al. 2014
Német et al. 2016
Öster et al. 2009
Piqueray et al. 2011
Prach et al. 2014
Stadler et al. 2007
Vécrin et al. 2002
Brudvig et al. 2014
Kirkman et al. 2004
Rottler et al. 2018
Zylka et al. 2016
Cava et al. 2018
Koch et al. 2016
Le Stradic et al. 2018
Pilon et al. 2018
Global weighted mean
(95% confidence interval)
-2.5 -2 -1.5 -1 -0.5 0 0.5 1
Log response ratio (species richness)
Africa
Asia
Australia
Europe
North America
South America
Old-growth grasslands richer
Secondary grasslands richer
Old-growth grassland disturbance type:
Fire
Herbivory
Mowing/Haying
700 Fig. 2

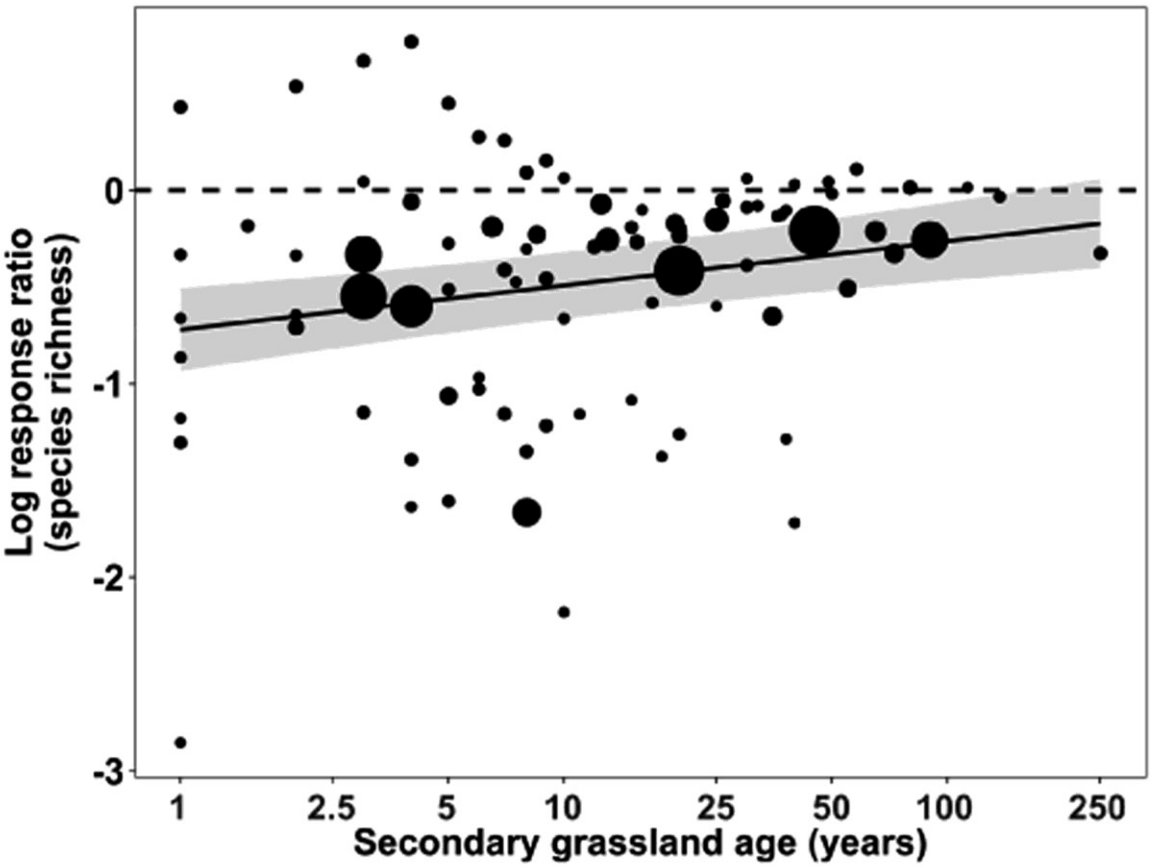
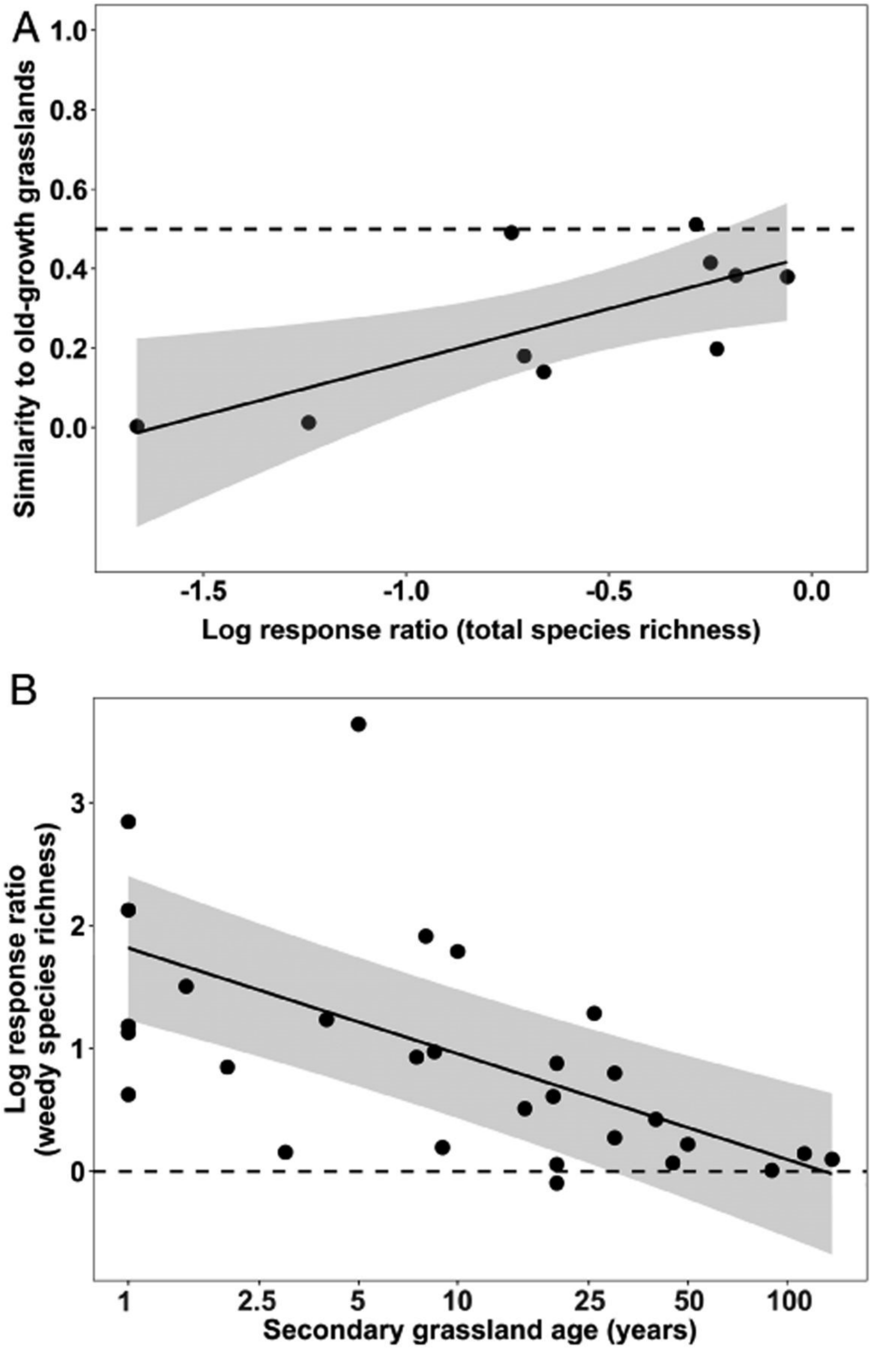

Log response ratio
(species richness)
0
-1
-2
-3
1
2.5
5
10
25
50
100
250
Secondary grassland age (years)

Fig. 3


A
Similarity to old-growth grasslands
1.0
0.8
0.6
0.4
0.2
0.0
-1.5
-1.0
-0.5
0.0
Log response ratio (total species richness)
B
Log response ratio
(weedy species richness)
3
2
1
0
1
2.5
5
10
25
50
100
Secondary grassland age (years)
Fig. 4

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Supplementary Information for

High plant diversity and slow assembly of old-growth grasslands.

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This PDF file includes:

Tables S1 to S3
Figures S1 to S9
Legend for Dataset S1

Other supplementary materials for this manuscript include the following:

Dataset S1

SUPPLEMENTARY INFORMATION (TABLES)

Table S1. Sensitivity analyses for the global meta-analysis comparing species richness of old-growth and secondary grasslands. Compared to the global meta-analysis (r = 0.5, lnRR = -0.46, Fig. 2), models with plausible covariances that were lower (r = 0.25) and higher (r = 0.75) yielded very similar results (lnRR = -0.46 and -0.45, respectively). An unweighted model (lnRR = -0.48) and a weighted model that excluded the two highest-weighted studies (lnRR = -0.46) confirmed that the global meta-analysis results were not driven by weighting. Columns in the table report: lnRR, the associated confidence intervals (CI), p-values for the heterogeneity test (P), the between-study heterogeneity (I^2), and the number of studies included in the model (n).

Model	Purpose of Sensitivity Test	LnRR	95% CI	P	I^2	n
Weighted mean lnRR, random effects model (r = 0.5)	Global meta-analysis (Fig. 2)	-0.458	-0.637, -0.278	< 0.0001	90%	31
Weighted mean lnRR, random effects model (r = 0.25)	To determine the effect of low plausible covariance estimate on results (*SI Appendix,* Fig. S2)	-0.463	-0.643, -0.284	< 0.0001	91%	31
Weighted mean lnRR, random effects model (r = 0.75)	To determine the effect of high plausible covariance estimate (*SI Appendix,* Fig. S3)	-0.450	-0.629, -0.272	< 0.0001	89%	31
Unweighted mean lnRR	To determine the effect of weighting on the global meta-analysis results (*SI Appendix,* Fig. S4)	-0.484	-0.662, -0.305	< 0.0001	61%	31
Weighted mean lnRR, random effects model (r = 0.5), two highest-weighted studies excluded	To determine whether results were heavily influenced by the two highest-weighted studies (*SI Appendix,* Fig. S5)	-0.461	-0.656, -0.266	< 0.0001	85%	29

Table S2. Tests for bias. We performed three tests to assess publication bias (27, 80, 81), and one test for the influence of sample plot size on lnRR, all of which were negative. The rows describe the tests, associated statistics, test interpretations, and the result of the tests.

TEST DESCRIPTION	TEST STATISTICS	INTERPRETATION	RESULT
Correlation between standardized effect sizes and standard errors (27)	Spearman's rho = -0.176, P= 0.343; Kendall's tau = -0.1185, P= 0.349	Correlations were not significant	Negative
Cumulative meta-analysis (80) (*SI Appendix*, Fig. S6)	NA	Over time (publication year), effect size became more negative, and converged with global mean lnRR	Negative
Rosenberg's fail safe number (81)	Fail-safe number: 2165	Fail-safe number was greater than the minimum cut-off of 165 (i.e., 5× n+10; where n = number of studies)	Negative
Relationship between plot size and lnRR (*SI Appendix,* Fig. S7)	Slope: -0.00076, R^2 = 0.0021, P = 0.806	Variation in sample area among studies did not influence global mean lnRR	Negative

Table S3. Models to identify potential sources of unexplained variation in Log response ratio. We began by defining a linear mixed effect model of lnRR values, from $n = 92$ time points, with seven predictor variables as fixed effects, and study sites ($n = 31$) as random effects. We then used a step-backward selection method based on Akaike Information Criteria (AIC) to identify the best model. We calculated the ΔAIC for each model in relation to the best model. For predictor variables that appeared in models with ΔAIC < 2 (in bold), and were not part of the core hypotheses (i.e., secondary grassland type and latitude, as opposed to secondary grassland age, Fig. 3), we produced supplemental figures to visualize their relationships with lnRR (*SI Appendix,* Fig. S8, S9). Abbreviations are as follows: MAP, Mean annual precipitation; MAT, Mean annual temperature; SG_type, type of secondary grassland; plot_area , size of the sampling unit in each study; Latitude, site location in degrees north or south of the equator; and log_time, base 10 logarithm of secondary grassland age.

Model	Parameters	AIC	ΔAIC	R^2
LnRR~ Continent + Latitude + MAP + MAT + log_time + SG_type+ plot_area	7	85.61	7.38	0.399
LnRR ~ Continent + Latitude + MAP + log_time + SG_type + plot_area	6	83.65	5.42	0.399
LnRR ~ Continent + Latitude + MAP + log_time + SG_type	5	81.71	3.48	0.398
LnRR ~ Continent + Latitude + log_time + SG_type	4	80.34	2.11	0.379
LnRR ~ Latitude + log_time + SG_type	**3**	**78.85**	**0.62**	**0.356**
LnRR ~ Latitude + log_time	**2**	**78.23**	**0**	**0.322**
LnRR ~ log_time	1	85.48	7.25	0.14

SUPPLEMENTARY INFORMATION (FIGURES)

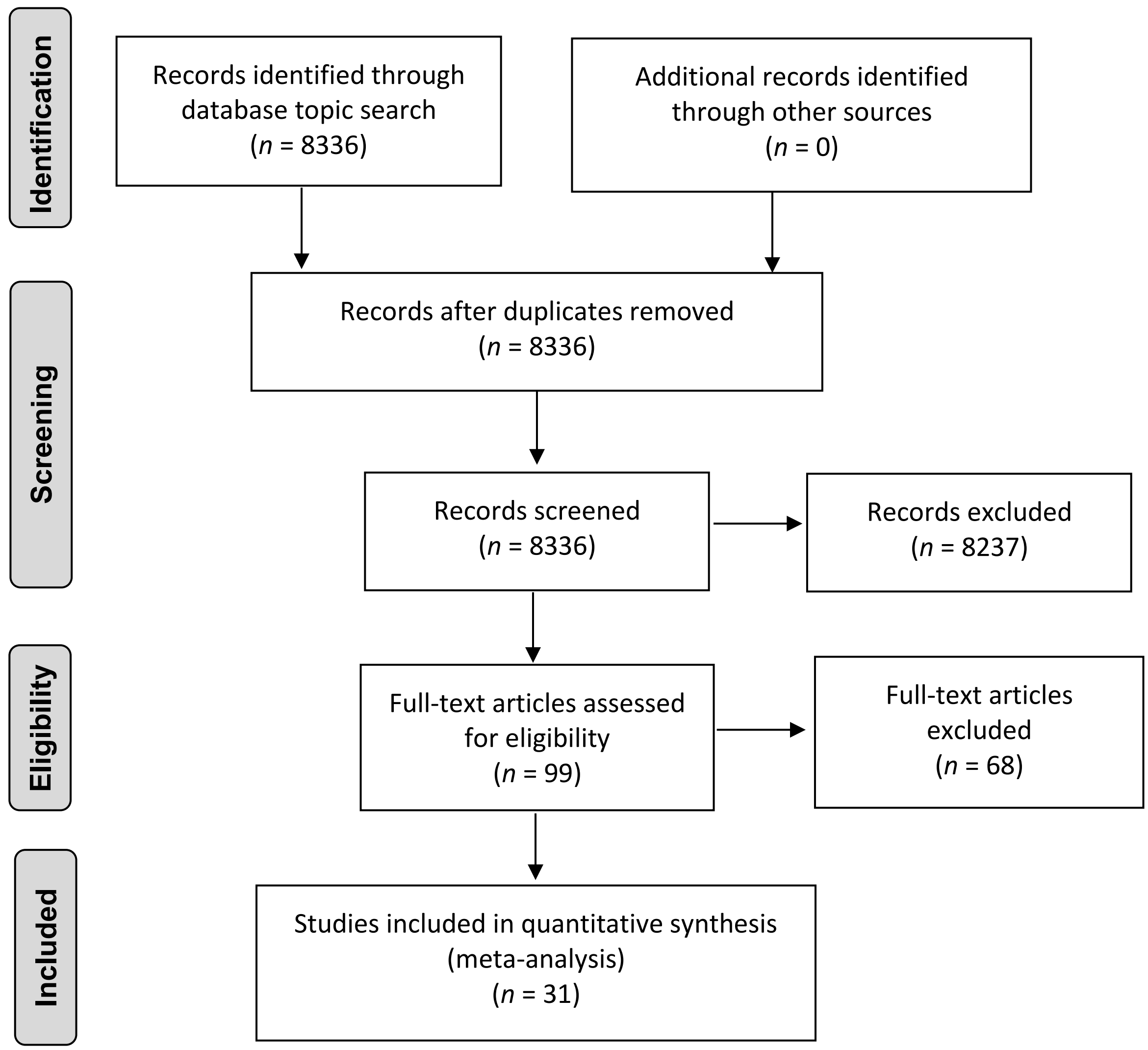

Figure S1. Flowchart of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for step-wise selection of studies (55). Identification: The Web of Science topic search yielded a total of 8336 articles (we were unable to identify additional records in recent review articles). Screening: We examined the titles of the 8336 articles to eliminate those that were obviously irrelevant (for ambigous titles, we further screened the abstract and methods), which resulted in 99 articles for the final screening: Lastly, we read the full texts of the 99 articles and determined that 31 articles met the eligibility criteria (see Methods) to be included in the analysis.

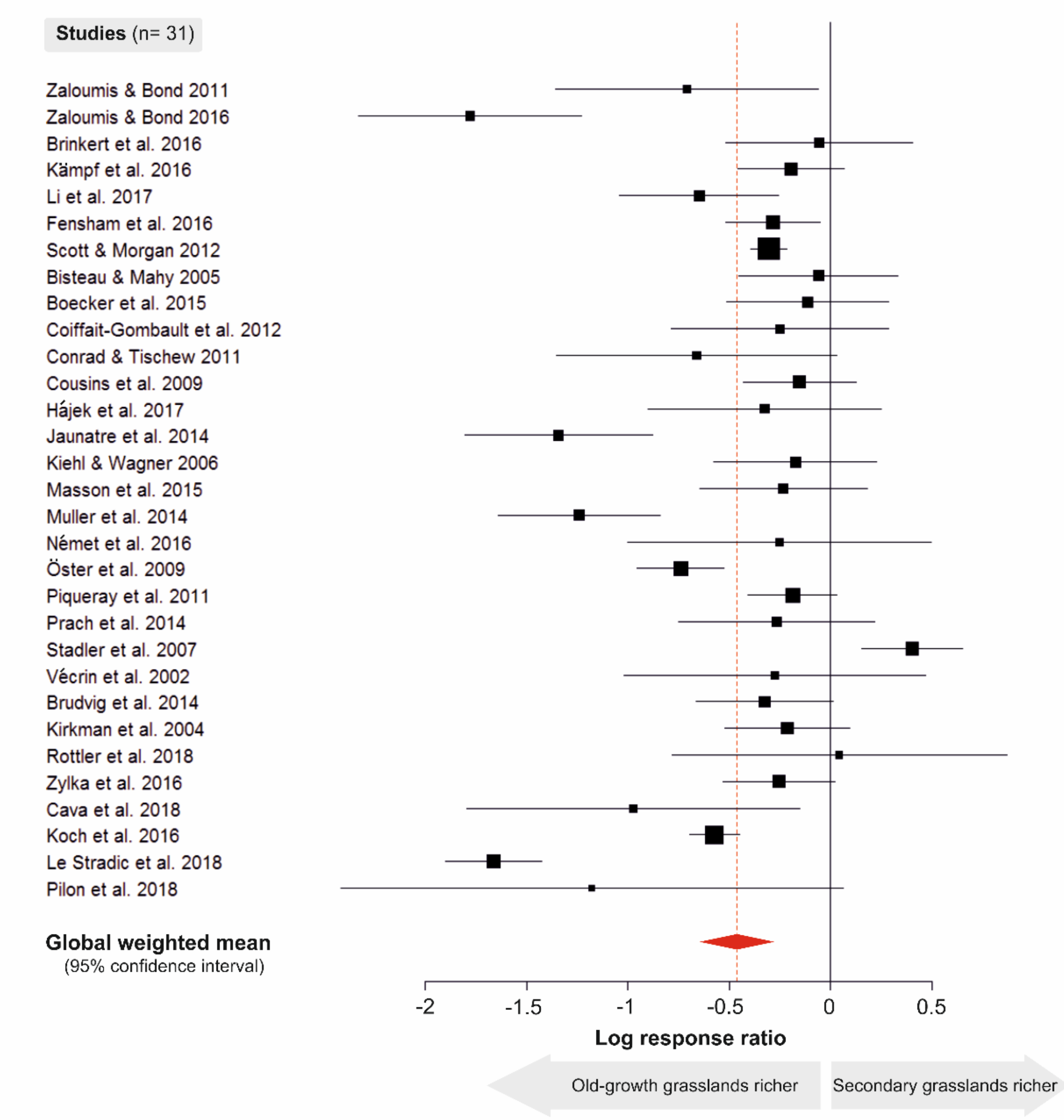

Figure S2. Sensitivity analysis using a plausible covariance of $r = 0.25$. Studies ($n = 31$) are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicates secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.46, $I^2 = 91\%$, $P < 0.0001$) equates to secondary grasslands supporting 63% of the species richness of old-growth grasslands (*SI Appendix,* Table S1).

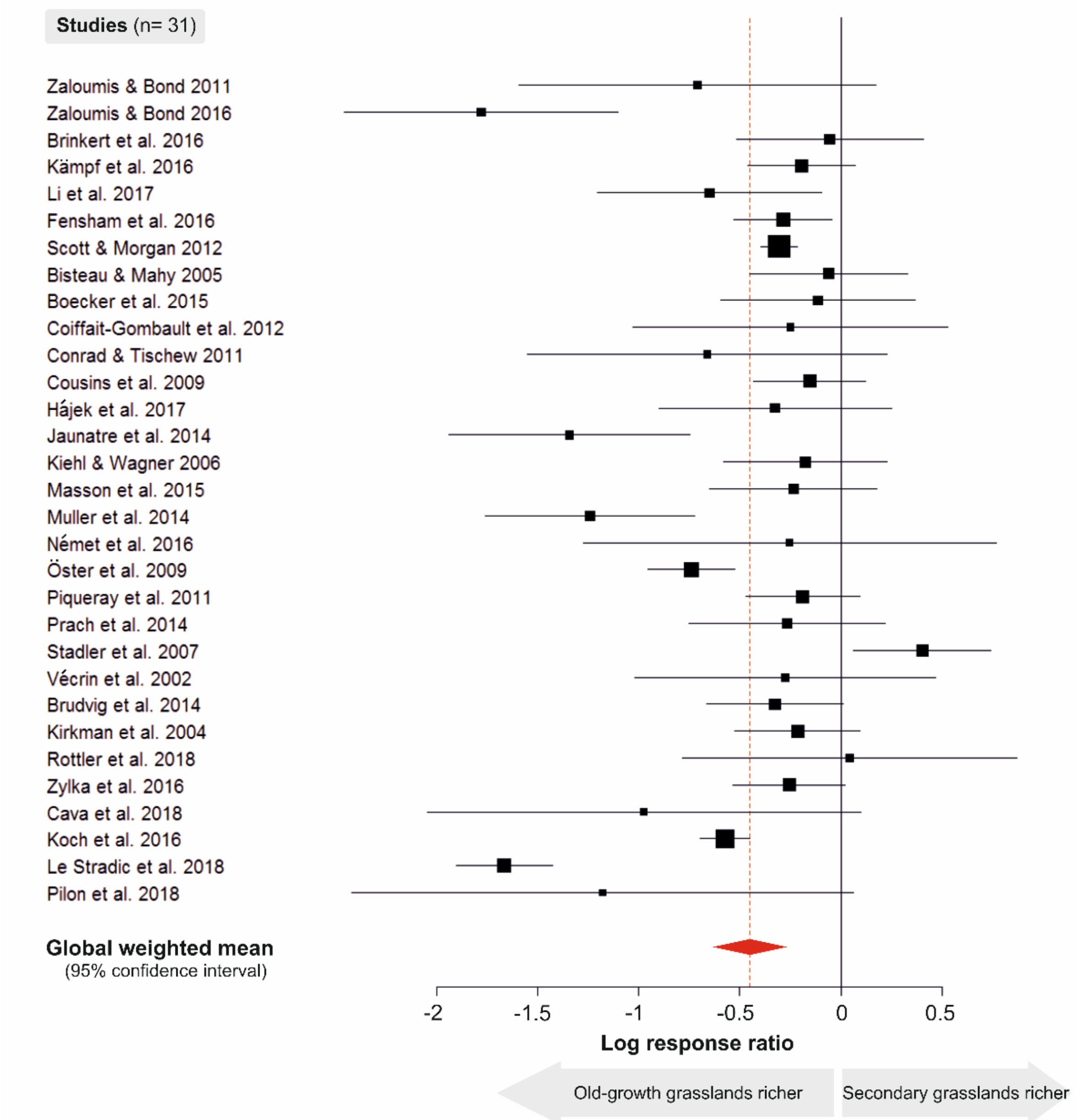

Figure S3. Sensitivity analysis using a plausible covariance of $r = 0.75$. Studies ($n = 31$) are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.45, $I^2 = 89\%$, $P < 0.0001$) equates to secondary grasslands supporting 64% of the species richness of old-growth grasslands (*SI Appendix,* Table S1).

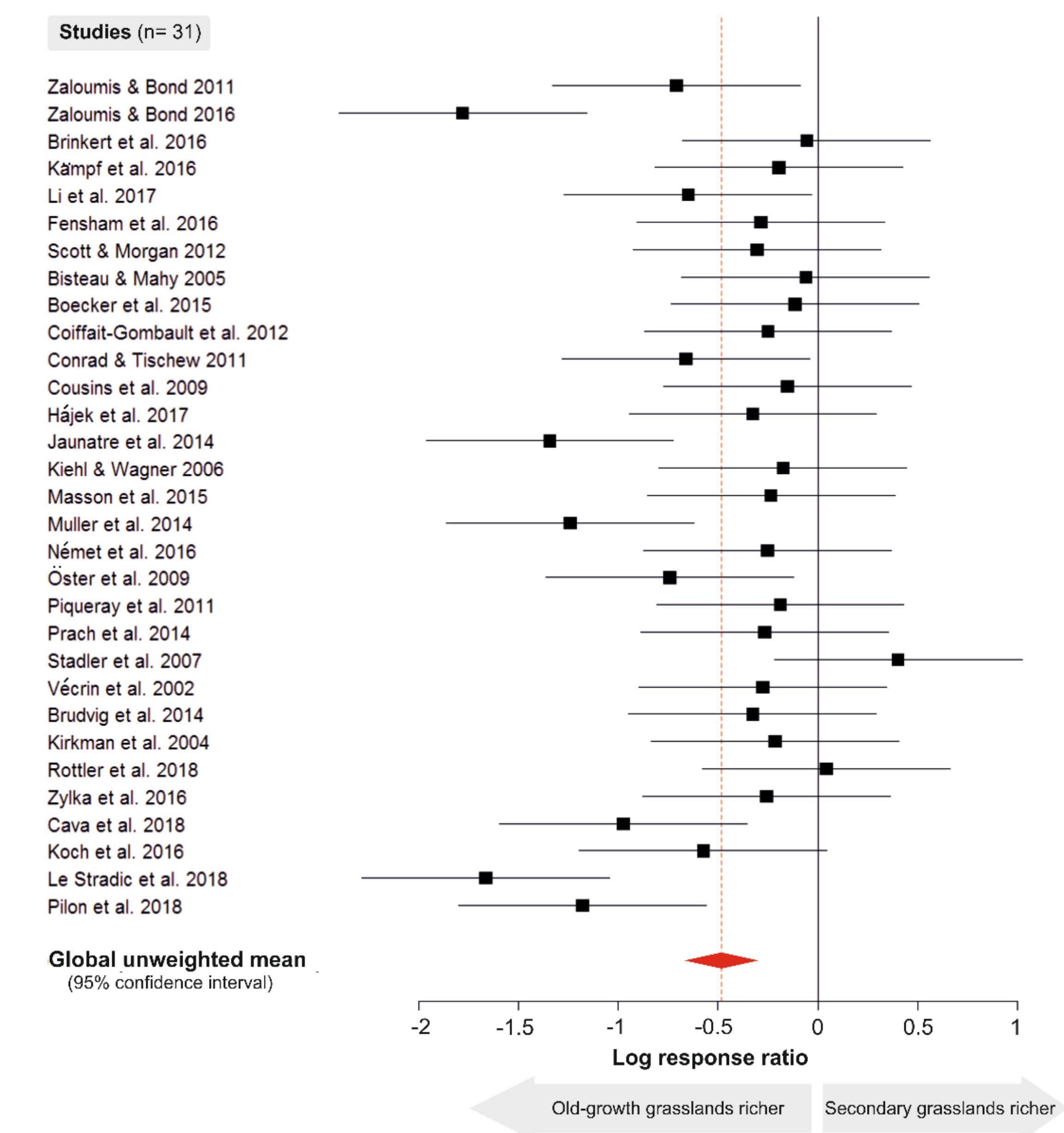

Figure S4. Sensitivity analysis with an unweighted mean lnRR. Studies (*n* = 31 are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the study weights, which are all equal for this unweighted sensitivity analysis. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global unweighted mean (lnRR = -0.48, I^2 = 61%, $P <$ 0.0001) equates to secondary grasslands supporting 62% of the species richness of old-growth grasslands (*SI Appendix,* Table S1).

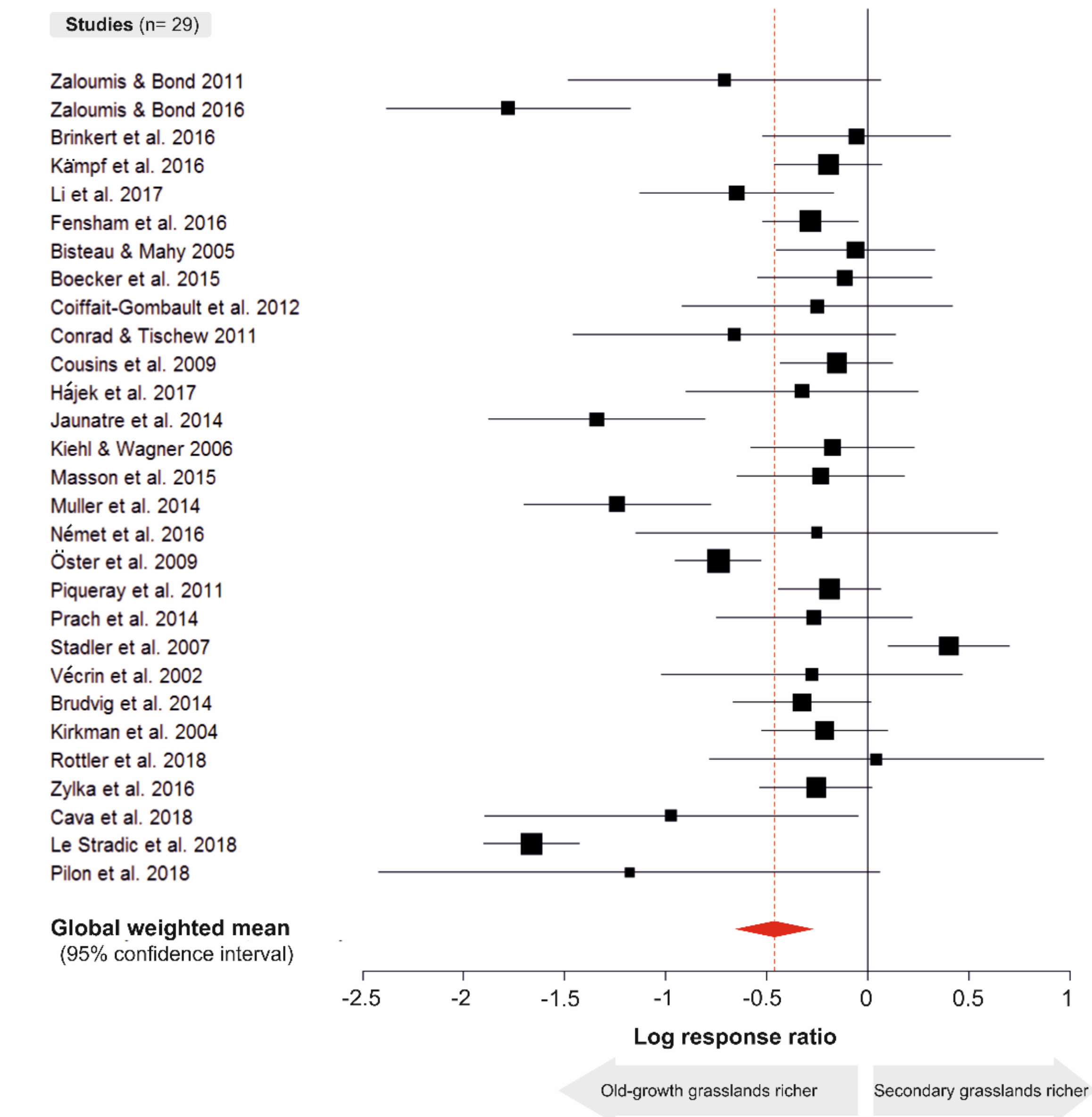

Figure S5. Sensitivity analysis with two highest-weighted studies excluded. Studies (*n* = 29 are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicates that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.46, $I^2 = 85\%$, $P < 0.0001$) equates to secondary grasslands supporting 63% of the species richness of old-growth grasslands (*SI Appendix,* Table S1)

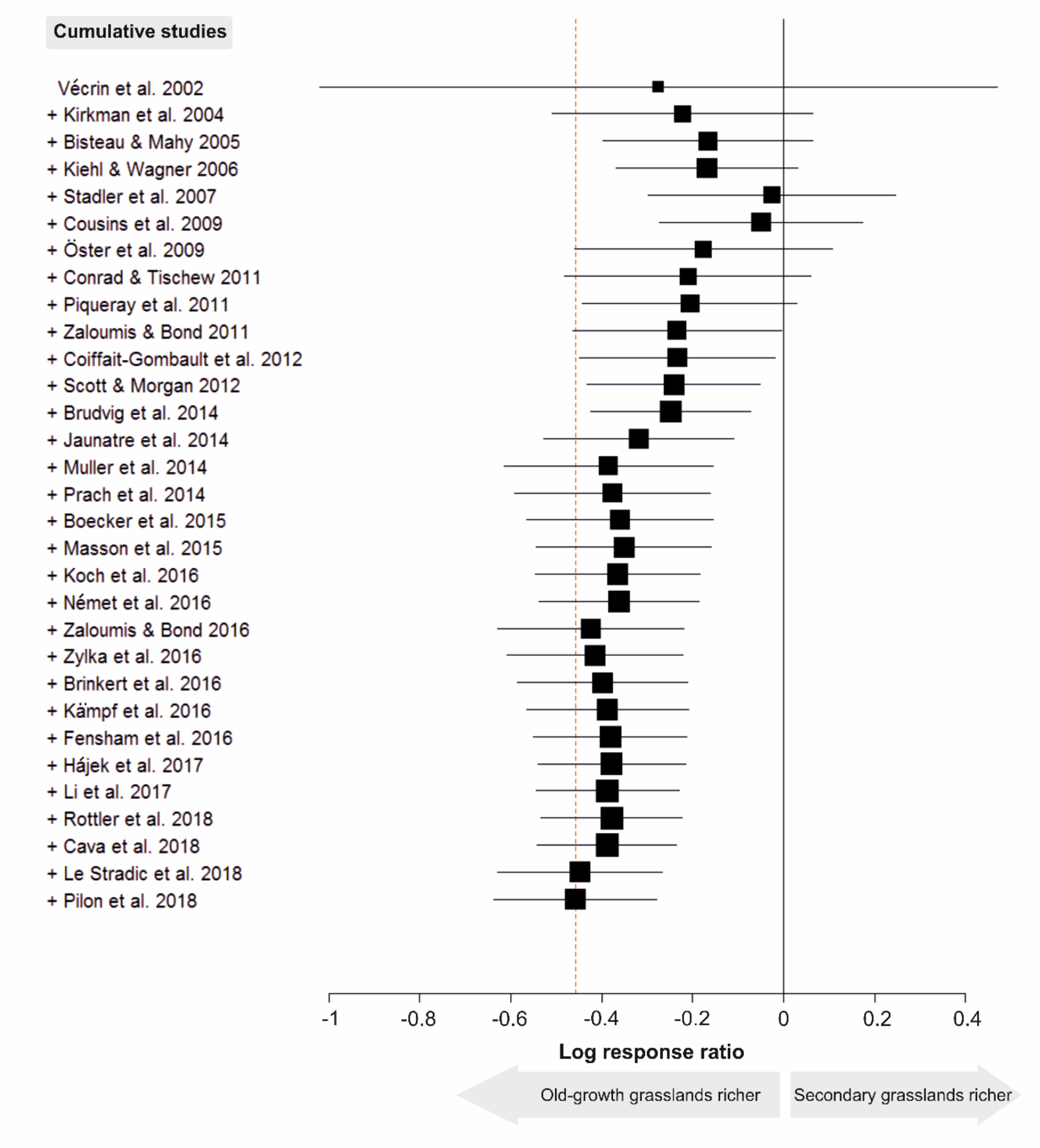

Figure S6. Cumulative meta-analysis to assess publication bias. Studies were sorted by publication year (oldest to most recent) and added one by one to the analysis. With each additional study, the effect size was recalculated using a random effects model. Boxes represent iteratively calculated effect-sizes and the bars represent 95% confidence intervals. The red dashed line represents the global mean (lnRR = -0.46, Fig. 2). Convergence of the iteratively calculated effect sizes with the global mean effect size indicates there is no publication bias.

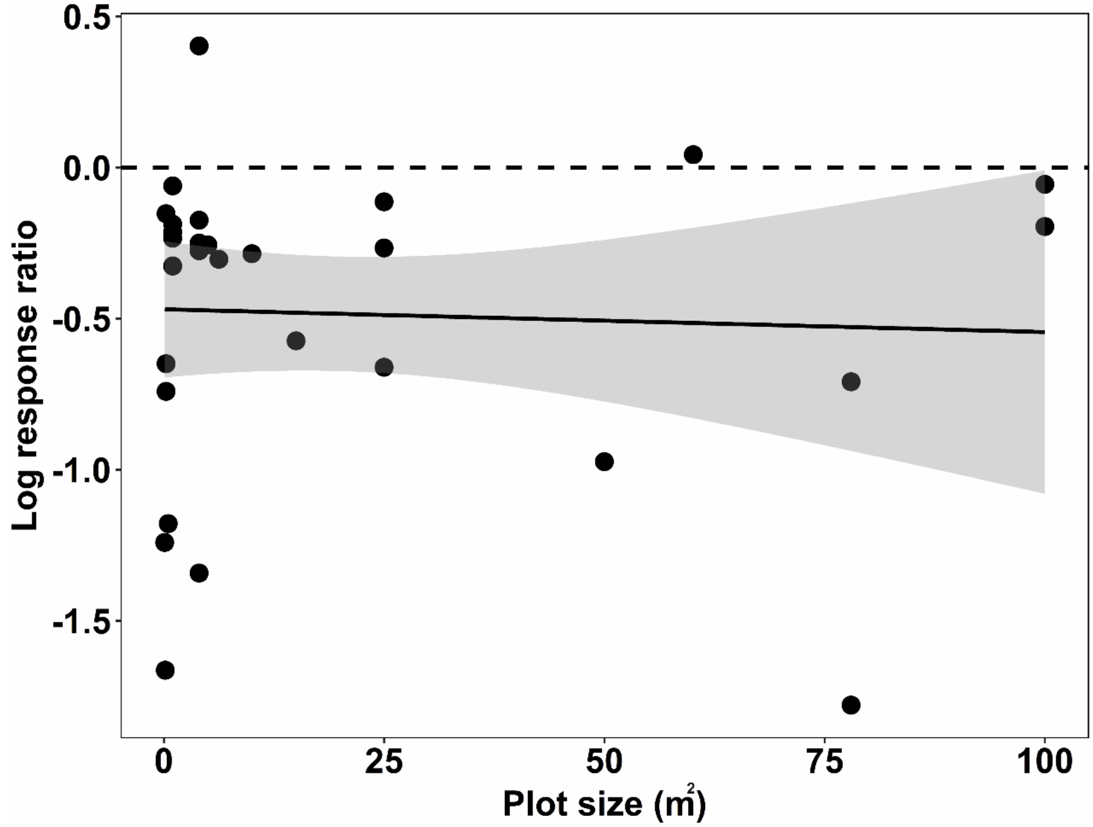

Figure S7. Relationship between plot size and log response ratio (lnRR) of secondary grassland versus old-growth grassland species richness. Because species-area relationships can differ between ecosystems, we sought to determine if variation in sample area between studies influenced lnRR. We extracted information on plot size (which ranged from 0.009 to 100 m^2) from each of the n = 31 studies and conducted a linear regression. The regression equation [lnRR = -0.00076(plot size) – 0.4688, (R^2 = 0.0021, P = 0.806)] is displayed as a solid black line; grey shading indicates the 95% confidence interval. Given that the slope is non-significant and the y-intercept (lnRR = 0.47) is very close to the global weighted mean estimates (i.e., lnRR = 0.46, Fig. 2), we conclude that variation in plot size had no influence on overall results (Fig. 2, Fig. 3).

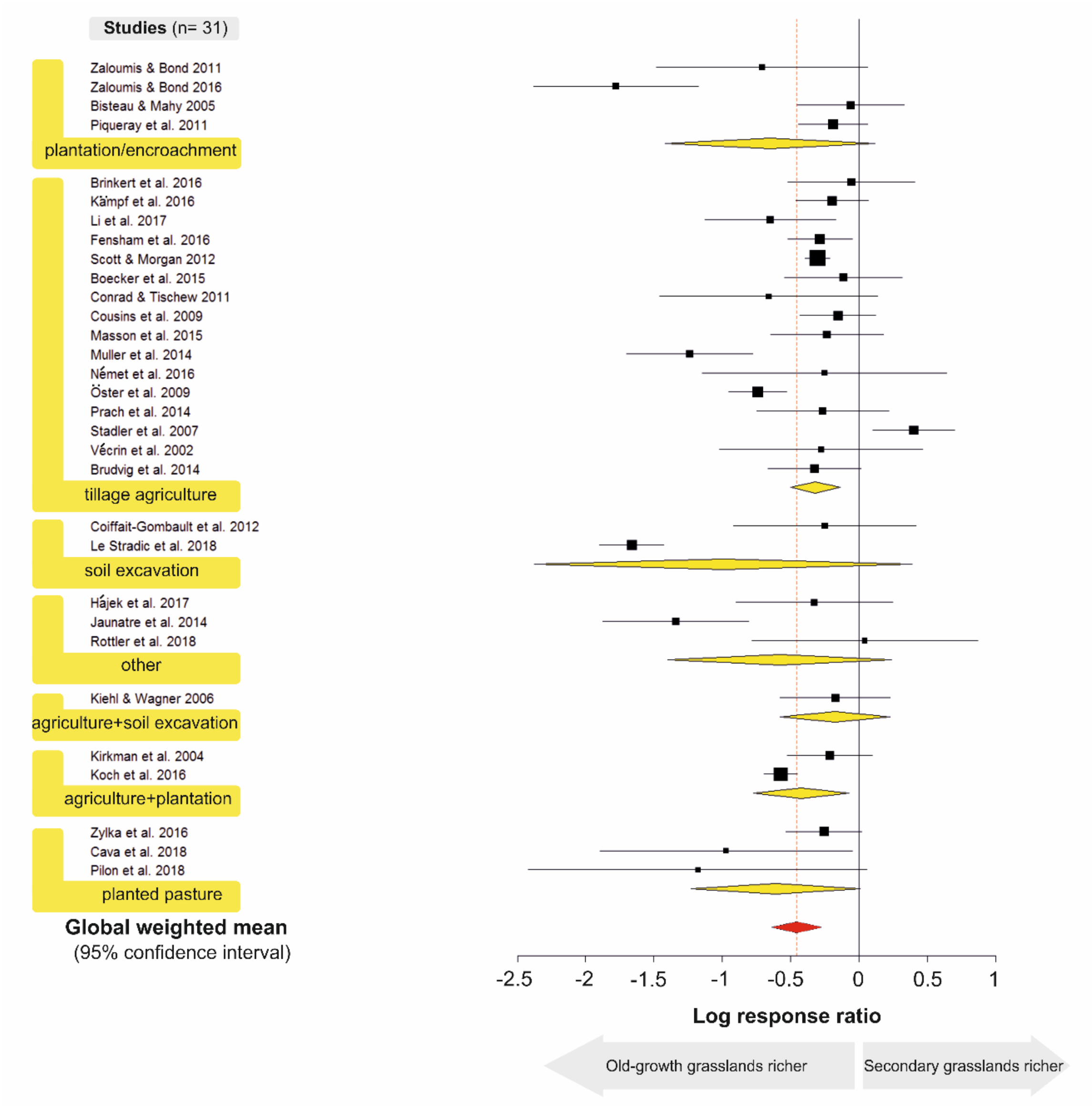

Figure S8. Comparison of old-growth grassland versus secondary grassland species richness based on type of secondary grasslands. Studies (n = 31) are listed by secondary grassland classification. Boxes and error bars display the natural logarithm of response ratio (lnRR) and 95% confidence intervals (CI), respectively. Box sizes are proportional to the weight of the study. Response ratios less than zero indicate that old-growth grasslands are more species rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Yellow diamonds represent the weighted subgroup mean and associated 95% CI. The global weighted mean is displayed as a red diamond and red vertical line. ‘Plantation/encroachment’ refers to tree plantations and woody encroachment; ‘agriculture’ refers to tillage agriculture.

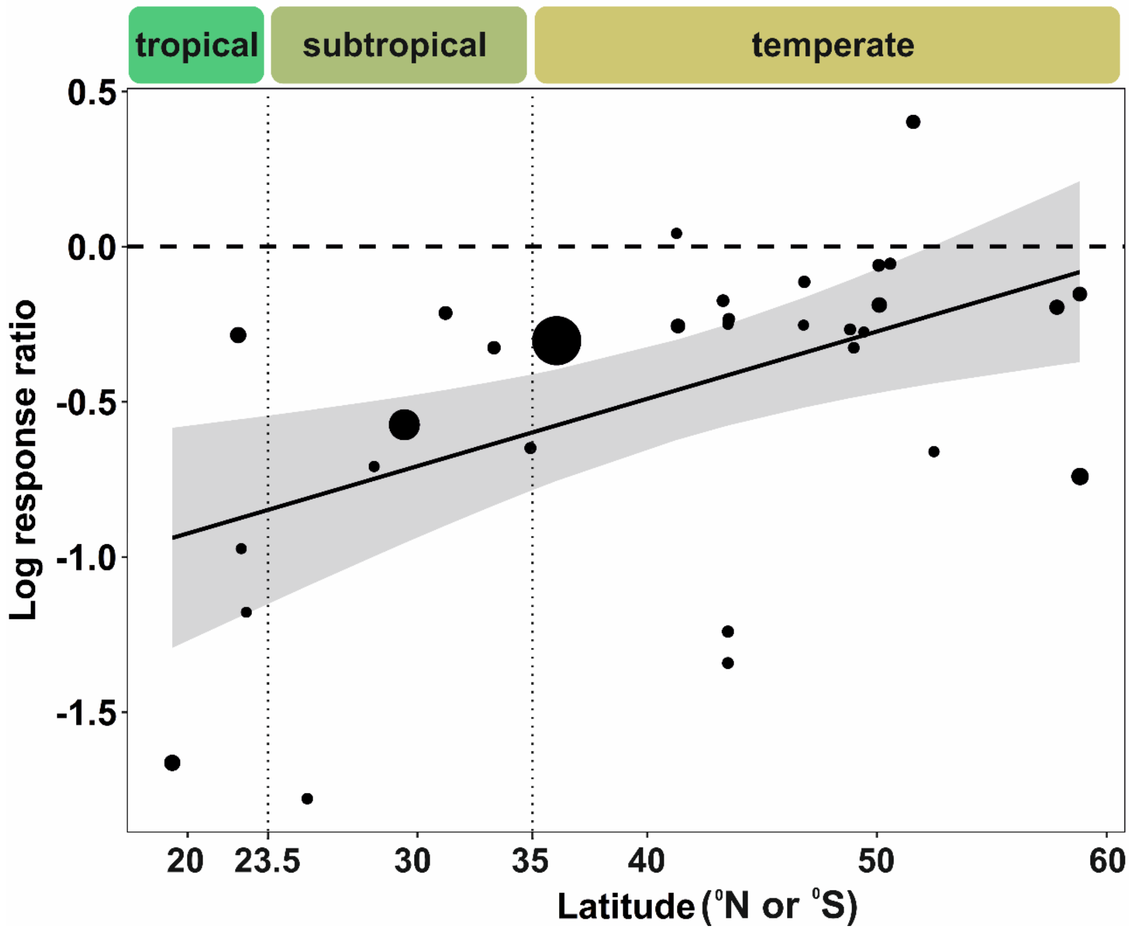

Figure S9. Relationship between the log response ratio (lnRR) of species richness and the absolute latitude of studies. Points represent data from n = 31 studies, and are scaled in proportion to their weight (see methods). The regression equation [lnRR = 0.0217(latitude) - 1.358, $R^2 = 0.24$, $P = 0.0026$], is displayed as a solid black line; grey shading indicates the 95% confidence interval. The horizontal dashed line indicates the response ratio at which secondary and old-growth grassland species richness is equal (lnRR = 0). Response ratios less than zero indicate secondary grasslands that have fewer species compared to old-growth grasslands. The labels tropical (n = 4), subtropical (n = 6), and temperate (n = 21) correspond to latitudes of < 23.5°, 23.5-35°, and > 35°, respectively.

Dataset S1 (separate file). Data used for the analyses.